CHEMICAL AND MORPHOLOGICAL VARIATION IN SOME COMMERCIALLY-AVAILABLE THYME (*T. vulgaris*).

A thesis submitted to the Faculty of Science of the University of Glasgow for the degree of Master of Science by Samantha A. L. Jackson G. I. Biol.

October, 1990.

VOLUME I

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ACKNOWLEDGEMENTS

I wish to acknowledge the financial assistance, given by Nutting and Son Ltd., Queniborough, Leicester (formerly Nutting and Speed Ltd., Long Stanton, Cambridge), without which this project would have been impossible.

I am especially grateful to my supervisor, Dr. R. K. M. Hay, who was always helpful and sympathetic when the need arose. I am also grateful to Dr. K. Svoboda, for allowing me the use of her library of papers and to Dr. W. Spoor, for useful advice at the start of the project.

Thanks to the computing and technical departments for assistance rendered. Final thanks go to my indulgent family and friends.

ABSTRACT

There were no statistically significant differences in the height, length (x dimension) or breadth (y dimension) of plants between plots but there were substantial differences between individual plants. There were also differences between plants in leaf dry weight yield/m^2, oil yield/m^2 and oil components.

A 50% increase in oil content (% leaf dry wt.) in individual plants between 1988 and 1989 was not associated with differences in the proportions of the 3 main components of the oil (thymol, ρ-cymene, γ-terpinene) and the combined proportions of these 3 compounds were very similar in all of the plants examined. There were differences in the proportion of the individual phenolic compounds between the oils of different plants.

It is suggested that the observed differences between plants may be genetic, reflecting different degrees of adaptation to the environment. Alternatively, local differences in environmental factors such as soil moisture, nutrient availability, shelter or light interception may have contributed to differences in the development of individual plants. This is to be examined in subsequent years.

The earlier flowering of hermaphrodite plants may be an adaptive feature which assists in attracting

pollinators, especially honey-bees. The larger flowers of hermaphrodite plants provide pollen and nectar, both of which are required early in the season as a food source for the bees and for rearing brood.

1. INTRODUCTION

1.1 THE IMPORTANCE OF THE LABIATES AS CULINARY AND MEDICINAL HERBS.

Herbs are known to have been used in cooking and medicine since very early times. The Chinese were the first to use herbs as medicines and their earliest writings on the subject date from more than 2.7 thousand years ago (Craker *et al.*, 1986). Although herbs are still used extensively in folk medicine in the less developed countries of the world, and despite the fact that plant materials have yielded morphine (*Papaver somniferum* L.), digitalis (*Digitalis purpurea* L.) and atropine (*Atropa belladona* L.), the scientific study of the chemistry and pharmacology of herbs has been very limited up to the present day (Craker *et al.*, 1976).

The *Labiatae* (the mint family) contains around 200 genera and approximately 3000 species, most of which are shrubs or herbaceous plants. This family is amongst the ten largest families of flowering plants. They are found worldwide, in temperate and sub-tropical regions, but their greatest concentration is in the Mediterranenan region. It is a highly evolved family and is closely related to the *Verbenaceae*, members of which are more likely to be found in tropical regions (Encyclopaedia Britannica, 1974).

This large family is of major economic importance, being an important source of oils used in perfumes, foods, beverages and medicine. Many species have characteristic glandular hairs which store aromatic volatile (essential) oils (terpenoid compounds). The oils are thought, in some species, to give protection against disease or predators, and may inhibit the growth of other species (allelopathy). For example, it is known that grasses will not grow near *Salvia leucophylla L.*, possibly due to the presence of volatile terpenes exuded from the leaves of this species into the surrounding air (Heywood, 1978). Oils used in perfumery include lavender *(Lavandula)*, rosemary *(Rosmarinus)* and thyme *(Thymus)*. An essential oil used medicinally is obtained from horehound *(Marrubium vulgare L.)* and oils used in food include spearmint *(Mentha spicata L.)* and peppermint *(M. piperita L.)*. Sage *(Salvia officinalis L.)*, thyme *(Thymus vulgaris L.)* and basil *(Ocimum basilicum L.)* are amongst the Labiates used extensively as culinary herbs/spices. Some of these species are also used as ornamentals.

The approximate annual consumption of Labiate herbs in the major industrial countries of the west [excluding France and Italy] and Japan is twice that of non-Labiates (Table 1). Labiates make up 8.6% (20 species) of the total plants named in the British Herbal Pharmacopoeia (B.H.P.) (Appendix 1) and half of

Author: JACKSON, Samantha A L

Title: Chemical and morphological variation in some commercialy-available thyme (T. Vulgaris)

Date of deposit: 10.6.91 M.Sc.

these are natives of the British Isles (Appendix 4) (Ross-Craig, 1967). The only family with more species in the B.H.P. is the *Compositae* with 25 species (10.8%). These may be compared to the *Rosaceae* (12 species) and the *Umbelliferae* (14 species).

Table 1: Annual Consumption Of The Major Culinary Herbs [EXCLUDING PARSLEY] by "first world" countries [excluding France & Italy] (Greenhalgh, 1979a).

Family	Species	Consumption (t.p.a.) (dry matter)
LABIATAE	Basil	745 - 915
"	Sweet marjoram	1030 - 1685
"	Peppermint	850 - 2700
"	Oregano	2290 - 2820
"	Rosemary	490 - 720
"	Sage	1850 - 1945
"	Savoury	249 - 336
"	Thyme	1010 - 1170
Total tonnage per annum		**8514 - 12291**
LAURACEA	Bay	870 - 1160
UMBELLIFERAE	Celery seed	2045 - 2620
"	Chervil	15 - 45
"	Dill herb	225 - 230
"	Dill seed	580 - 780
COMPOSITAE	Tarragon	99 - 184
Total tonnage per annum		**3834 - 5019**

1.2 THYME

Thymus vulgaris is native to Western Mediterranean and Southern Italy and is found up to 2400m above sea level (Stuart, 1979). It is

cultivated and collected from the wild in Mediterranean areas, cultivated in Central and Eastern Europe and the U.S.A. (Leung, 1980), and is believed to have been brought to Britain by the Romans (Stuart, 1979). Most thyme produced commercially is *T. vulgaris* (common thyme) or in Spain *T. zygis* L., although thyme collected from the wild may be a mixture of species. The species are similar in habit but the latter has purple, rather than pink or white, flowers (although white on the Balearic Islands [Grieve, 1971]) and large white hairs at the base of the leaves.

Spain is the greatest exporter of thyme, with a mean annual total export figure of 1000 tonnes (Table 2) (Greenhalgh, 1979a). U.S.A and France import more than four hundred, and eight hundred tonnes, respectively, each year (1967 - 1976) (Greenhalgh, 1979a). It is thought to be the most important herb consumed in the United Kingdom after sage, parsley and the mints. In the Netherlands, the pharmaceutical and food processing industries use large quantities of thyme (Greenhalgh, 1979a).

Table 2: Thyme Exports (Greenhalgh, 1979a).

Country	Mean of 1968-76 tonnes (S.D.)*	
Spain	1032 (244)	
France	298 (73)	
Turkey	106 (99)	
Syria	95 (83)	
Lebanon	326 (45)	[1968-73]
Morocco	395 (119)	

* Standard deviation of variation between years.

1.3. USES OF THYME

1.3.1 Culinary Uses

Thyme is used in meat and savoury dishes, stuffings and bouquet garnis, in mixed herbs and is often combined with marjoram. It is used to flavour soups, sauces, cheese, liver, pork sausages, fish, vegetables (including aubergines, mushrooms, onions, beetroot and courgettes), game and poultry dishes (used in the traditional dish, jugged hare), pizzas and Italian style sauces (Simon *et al.*, 1984; Greenhalgh, 1979a; Grieve, 1971). The oil is used in ice cream, sweets, baking and chewing gum (Furia and Bellanca, 1975). Thyme is an ingredient of Benedictine liqueur, and Greek honey from wild thyme has a unique flavour. Thyme has anti-oxidant properties and the essential oil and oleoresin, as well as the whole herb, are used in the food industry (Simon *et al*,

1984). Lemon thyme, a cross between *T. vulgaris* and *T. pulegioides* L. is also used as a culinary herb (Hemphill, 1982). Thyme is used extensively in Creole cooking (Farrell, 1985).

1.3.2 Orthodox Medicine In the British Herbal Pharmacopoeia the actions given for thyme are: carminative, spasmolytic, anti-tussive, expectorant, bactericidal, anthelmintic and astringent (glossary of terms, p 7-8). This authority also states that thyme may be used in dyspepsia, chronic gastritis, bronchitis, pertussis, asthma, diarrhoea and enuresis in children, laryngitis and tonsilitis (as a gargle). The thymol in thyme is known to be a powerful anti-bacterial and disinfecting agent (Greenhalgh, 1979b; Valnet, 1982; Stuart, 1979). An aqueous solution of peroxidised essence of thyme kills both the typhus and Shiga's bacilli in two minutes (*S. shigae*, otherwise *S. dysenteriae*, causes bacilliary dysentry), *Escherichia coli* in two to eight minutes, *Streptococcus* and diphtheria bacillus in four minutes, *Staphylococcus* in four to eight minutes and the tuberculosis bacillus in thirty to sixty minutes (Valnet, 1982). At 0.1% in dilute soapy water it kills microbes in the mouth in three minutes (Valent, 1982). Thymol was used extensively during the First World War on dressings for its antiseptic and anaesthetic properties (Grieve, 1971) and probably

saved many lives before the discovery of the sulphonamides and penicillins. Its antiseptic properties are twenty times greater than those of phenol and it is much safer to use (Wheelwright, 1974). Thymol is also known to inhibit the growth of yeasts and moulds (Sticher, 1977).

1.3.3. Folk Remedies In folk medicine thyme has a reputedly large number of theraputic properties: it has been used against asthma, arteriosclerosis, colic, bronchitis, diarrhoea, rheumatism, and to promote perspiration (Farrell, 1985; Simon *et al.*, 1984; Mabey, 1988). Used as an infusion (28 g to 0.5 l of boiling water) it can be taken, in wineglassfuls, frequently as a tonic, expectorant, emmenagogue, antispasmodic and antiseptic (Wren, 1975; Simon *et al.*, 1984; Greenhalgh, 1979a). It was thought to strengthen the lungs and to cure whooping cough (Wren, 1975), has been used as a rubifacient (Simon *et al.*, 1984; Greenhalgh, 1979a; Grieve, 1971) and was said to make childbirth safer and faster (Wren, 1975). An ointment made from thyme was said to reduce hot swellings, warts and to help sciatica and poor eyesight (Wren, 1975).

GLOSSARY OF SOME TERMS USED (Shorter O. E. Dictionary, 1978)

ANTHELMINTHIC - of use against intestinal worms
ANTI-SPASMODIC - good against spasms

ASTRINGENT	- having the power to contract organic tissue, styptic
CARMINATIVE	- expels wind from the stomach or bowels
EMMENAGOGUE	- promoting menstruation
ENURESIS	- incontinence of urine
EXPECTORANT	- promotes secretion of phlegm from the lungs
PERTUSSIS	- whooping cough
RUBIFACIENT	- causes to heat up or become red.

1.3.4. Other Uses

The oil is used as flavouring, antiseptic and rubifacient in toothpaste, mouthwashes and cough medicines and is also used in perfumes and cosmetics (Simon *et al.*, 1984; Greenhalgh, 1979b). The flowering tops are used in sachets (Simon *et al.*, 1984). Some species are grown as ornamentals in rock gardens and along paths and borders (Simon *et al.*, 1984). Thyme is very attractive to bees and bee hives were, at one time, rubbed with thyme (Hemphill, 1982). In earlier times, it was used as a strewing herb and was said to dispel anguish when taken as a tea (Ceres, 1976). The oil is added to some anti-mildew preparations because of its fungicidal properties (Greenhalgh, 1979b). Fresh thyme can be used as a conditioner on all hair types (Mabey, 1988). The flowers have been used in the same way as lavender to keep insects away from clothes (Furia and Bellanca, 1975). It was burned in ancient Greek temples and Pliny called it a fumigant.

2.1 BIOLOGY OF THE LABIATAE

2.1.1 LABIATAE (Jones, 1939; Wren, 1975; Heywood, 1978; Encyclopaedia Britannica, 1974)

Labiates are shrubs or herbaceous plants with square stems and opposite simple leaves which are decussate. The flowers form whorls and are usually in cymes in the axils of the upper leaves. They are bisexual but some species may contain a proportion of plants which have no stamens or very reduced stamens. These flowers are male sterile or functionally female and are usually smaller and paler in colour than the hermaphrodite flowers. The tubular calyx has 5 teeth (5 fused sepals) and the corolla is tubular (5 fused petals) usually forming 2 lips. There are either 2 or 4 stamens, the latter in two pairs of 2 (one pair usually longer). The superior ovary has 4 lobes, each of which contains one nutlet, and the pistil rises from the centre of the ovary (gynobasic) with a vertical split at the tip. The anthers open longitudinally. At the base of the ovary is a disc which secretes nectar. Some species propagate vegetatively, by stolons. Some are xerophytic with small, hairy reflexed leaves. Most species have volatile oils in a glandular epidermis.

2.1.2 *Thymus Vulgaris* (Polunin, 1978; Farrell, 1985)

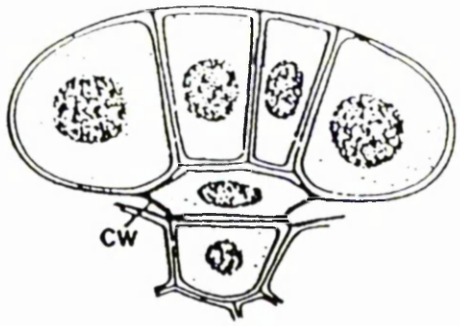
This is a small bushy compact sub-shrub, 20 - 30 cm high. The grey-green leaves are short stalked, narrow lance-shaped, 5 - 9 mm long and 2 - 3 mm wide with reflexed edges. The flowers are 4 - 6 mm long and form numerous whorls around the stem. Lower whorls become separated from those above and below. The calyx has bristly hairs. Flowers are pink or whitish and typically labiate. They may be female or hermaphrodite (stamens in two pairs of 2, with crimson anthers). Only one type of flower is found on a single plant (Valdeyron *et al.*,1977). The plant tastes and smells aromatic.

Plate 1: A young *T. vulgaris* plant (x 0.4).

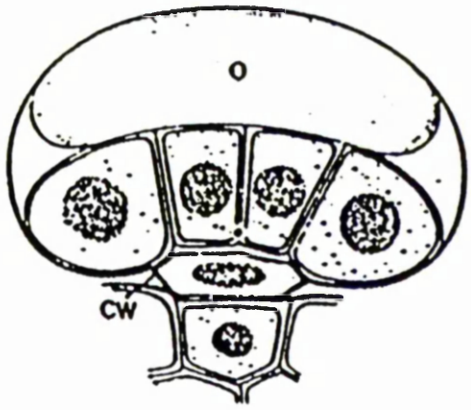
2.2.1 OIL GLANDULAR TRICHOMES (HAIRS)

Leaf glandular structures are found in species from various families including the Labiatae, Solanaceae and Compositae (Loomis and Croteau, 1980). For example *Capsicum* (Solanaceae) has oil ducts in the pericarp, tarragon (Compositae) has oil ducts in leaves and stems, and in peppermint, basil and thyme leaves (all Labiates), there are glandular hairs which secrete oils (Hardman, 1977). These glandular trichomes are usually characteristic for a species and can be used in identification.

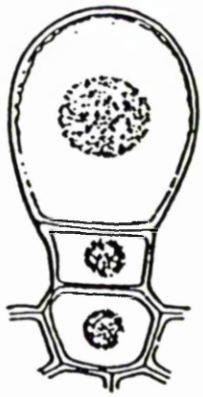
Mentha piperita leaves have 2 types of glandular hair (Loomis and Croteau, 1980). The first has a basal cell, a stalk cell and a secretory head cell (capitate short-stalked) [Fig. 1c]. The second type also has basal and stalk cells but has 8 secretory cells in the head (peltate) [Fig. 1a & b]. In many species where the latter type occurs they are sunk in pits in the epidermis. Each type arises from the modification of a single epidermal cell which is different from surrounding epidermal cells in having a larger nucleus, more dense cytoplasm and a smaller vacuole. Secretory cells in peltate hairs have numerous mitochondria in the cytoplasm and lining the apical plasmalemma (Bosabalidos and Tsekos, 1982). It is suggested that the asymmetry of the apical plasmalemma

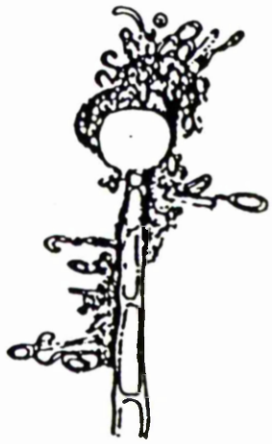


a - young peltate hair.
CW , cutinized wall



b - older peltate hair with oil drop (O) between secretory cells and apical cuticle





c - short stalked capitate glandular hair

d - long stalked capitate glandular hair

Fig 3: a - c: *Mentha piperita* glandular hairs
d: *Salvia glutinosa* glandular hair
(Loomis and Croteau, 1980)

Glands magnified approximately 4000 times.

seen by Bosabalidis and Tsekos (1982) in secretory cells of *Origanum dictamnus L.* is associated with movement of the oil molecules through the membrane, and structural changes are thought to occur in the oil as it passes through the plasmalemma to the sub-cuticular space. In peltate hairs, oil is stored in this sub-cuticular space as long as the thickened cuticle is undamaged, but in capitate hairs, oil can be secreted through pores in the cuticle. In some Labiate species examined by Werker *et al.* (1984), the head cells of peltate hairs were arranged in 2 concentric circles with 4 cells on the inside and 8 - 14 on the outside depending on the species. In *Salvia glutinosa* and *S. pratensis* there are both of the above types of gland on the leaves but the sepals have long stalked (4 - 6 cells) capitate glands which secrete a viscous oil through the cuticle on to the head and upper stalk (Fig. 1d). The upper stalk cell is completely cutinized as is the stalk cell of the peltate gland (Schnepf, 1969). Werker *et al.* (1984), however, found that, in the species they examined (*Majorana syriaca L., Melissa officinalis L., Micromeria fruticosa L., Rosmarinus officinalis L., Salvia fruticosa Mill., S. officinalis L., Satureja thymbra L. and Thymus capitatus (L) Hoff. & Link.*), the stalk cells of capitate glands were cutinized only in *Rosmarinus officinalis*.

The 'glandular scales' on leaves of *Origanum*

dictamnus have 1 foot cell, 1 stalk cell and 12 head cells (Bosabalidis and Tsekos, 1982) and would more usually be called peltate. Bosabalidis & Tsekos (1982) propose, from work on *Origanum dictamnus* leaves, that biosynthesis of terpenes takes place in the cytoplasm of the secretory cells (Loomis and Croteau, 1980) before being passed to the sub-cuticular space via the plasmalemma, situated on the apical surface of the head cells. They suggest that this would require a specialised type of plasmalemma and enzymes to enable large quantities of oil to pass out of the cells without damaging the membranes; furthermore, movement of the oil droplets towards the apex of the gland and into the sub-cuticular space is thought to be due to cell polarity and a possible membrane pump mechanism. As in *Mentha piperita* (Loomis and Croteau, 1980), the secretory cells disintegrate after secretion is complete. The oil is maintained in the space formed between the head cells and the cuticle, as there are no pores in the cuticle and it is covered with a thin layer of wax. The side walls of the stalk cells of peltate hairs are also cutinized (Loomis and Croteau, 1980). It is suggested that some oil fractions may pass through the cuticle but most are retained until the cuticle is damaged (Loomis and Croteau, 1980). Loomis and Croteau (1980) found that, in peltate hairs, synthesis of monoterpenes goes on

after the leaves reach full size and continues until the plants flower. They suggest that the breakdown of cells allows free enzymes to carry out anaerobic changes in the essential oils present to give monoterpenes as end products.

In the species they examined, Werker *et al.* (1984) found that peltate and capitate hairs were present on both leaf surfaces but that capitate hairs were more numerous over veins. Secretion in peltate hairs was as described for other species which have been studied (Loomis and Croteau, 1980; Schnepf, 1969). Secreted material was mostly essential oil but traces of polysaccharides were found, and these were also found in the inner head cells. In capitate hairs, much more polysaccharide was secreted (Werker *et al.*, 1984). Short-stalked capitate hairs on *Salvia glutinosa* and *S. pratensis* leaves secrete mainly mucilage and/or water whilst peltate glands secrete mostly oils (Schnepf, 1969). Werker *et al.* (1984) found that secretion in capitate hairs stopped when the leaves were still young. At the same stage of leaf development the peltate hairs had not reached their maximum number of head cells. These continued secreting oil until the leaf was mature (Werker *et al.*, 1984).

In *Satureja thymbra*, Bosabalidis (1980) showed that the peltate glands consisted of a foot and a stalk cell with twelve head cells. The four central

head cells were surrounded by eight larger cells. These glands were shown to be around seventeen times larger than capitate glands, in surface view. The peltate glands occupied around 6% of the leaf surface and the theoretical oil yield of the dry leaves was calculated at 3.5% by calculating the number and volume of peltate glands present.

The amount of monoterpenes produced in five-day old *Thymus vulgaris* seedlings was closely associated with the number of peltate glands present on the cotyledons (Tanaka *et al.*, 1988). Nine days after germination, almost all of the thymol and γ-terpinene in the leaves were shown to be present in the peltate glands. Irradiation with red and far-red light showed that phytochrome was involved both in monoterpene biosynthesis and in formation of peltate glands in these seedlings (Tanaka *et al.*, 1988).

No information was available on the morphology of *T. vulgaris* glands. However, the author was able to obtain slides, prepared for Auchincruive by Andrew Syred of Photomicroscopix in Powys, showing peltate and capitate glands from this species, magnified between 100 to 1000 times. These are presented as plates which show the glands at a final magnification of 400 to 4000 times (Plates 2 - 9).

Plate 2: Glandular hairs on leaf surface of fresh *T. vulgaris* leaf. Incident illumination (x400).

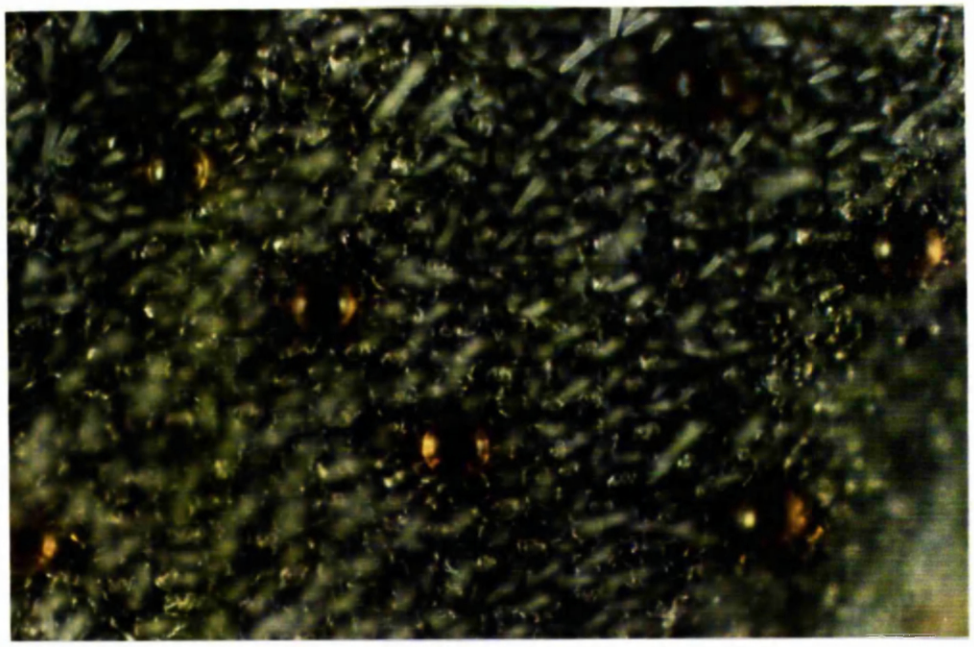


Plate 3: Glandular hairs on surface of fresh *T. vulgaris* leaf. Rheinberg illumination (x400).

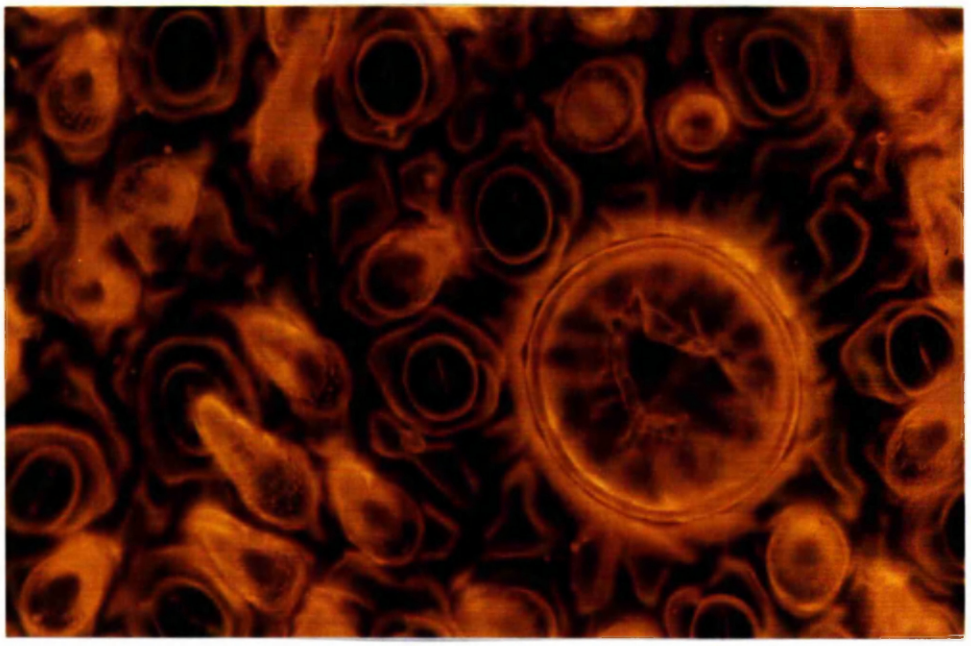


Plate 4: Non-secretory trichomes (capitate glands) on fresh *T. v.* leaf. Bright field illumination (x1600).

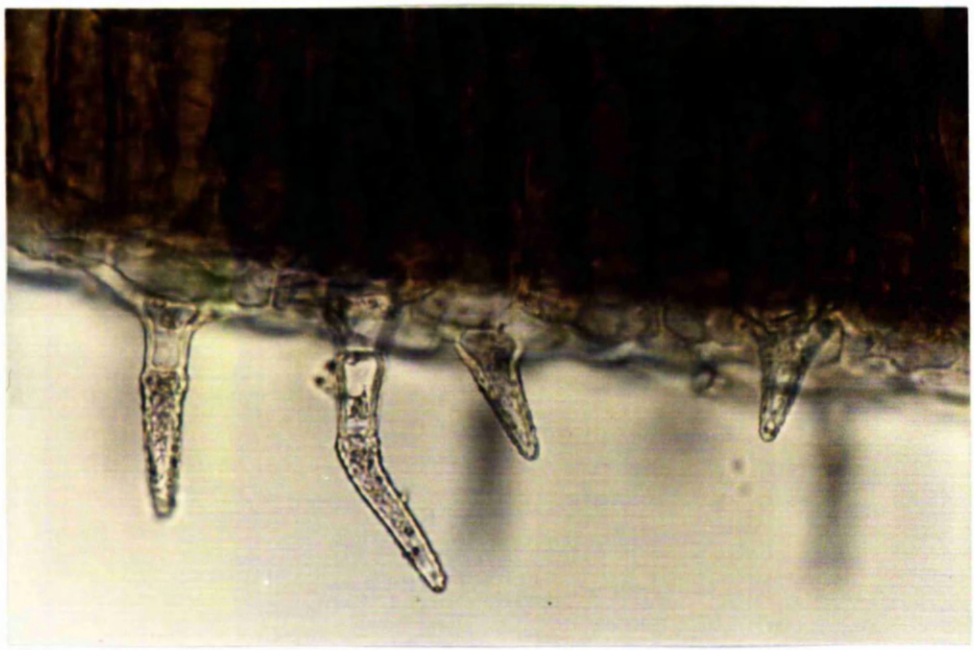


Plate 5: Capitate glandular hair from fresh *T. v.* leaf. Bright field illumination (x4000).

Plate 6: Peltate glandular hair from fresh *T. v.* leaf. Bright field illumination (x1600).

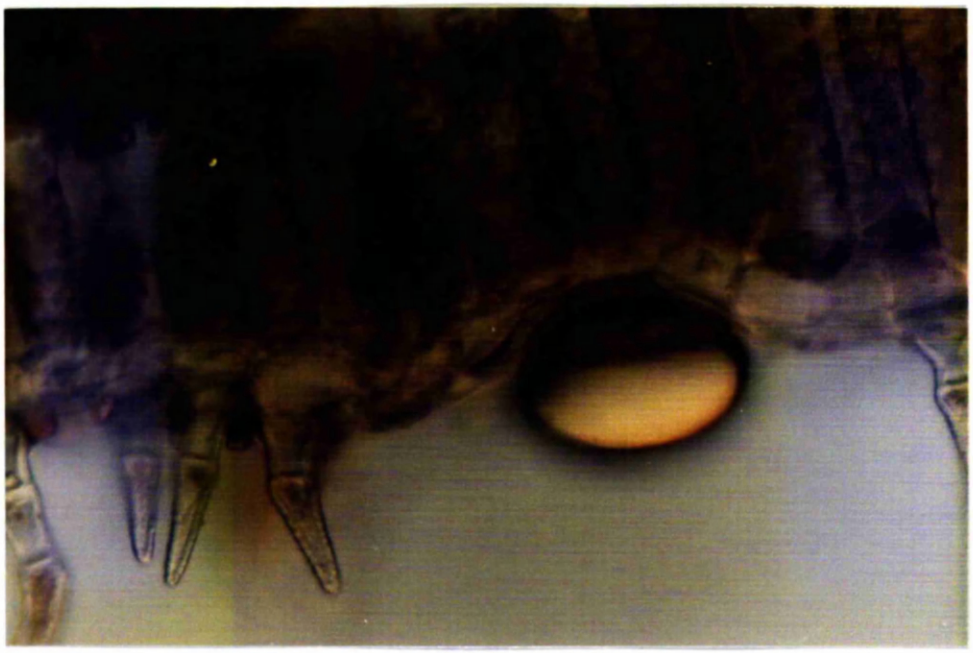


Plate 7: Peltate glandular hair from fresh *T. v.* leaf. Bright field, stained (x4000).

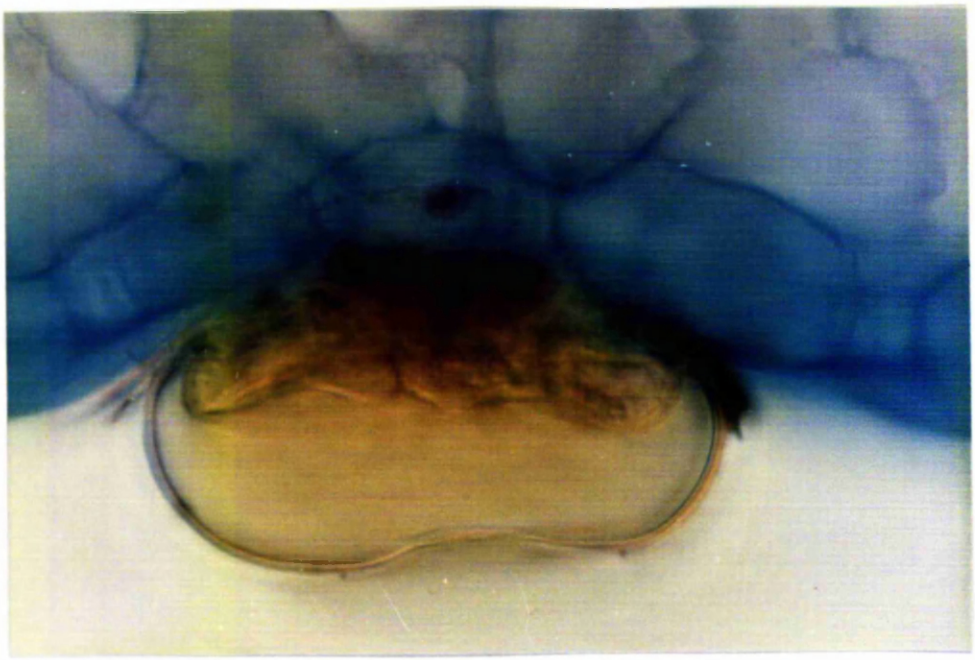


Plate 8: Fresh leaf section from *T. v.* leaf showing a peltate glandular hair. Bright field (x1600).

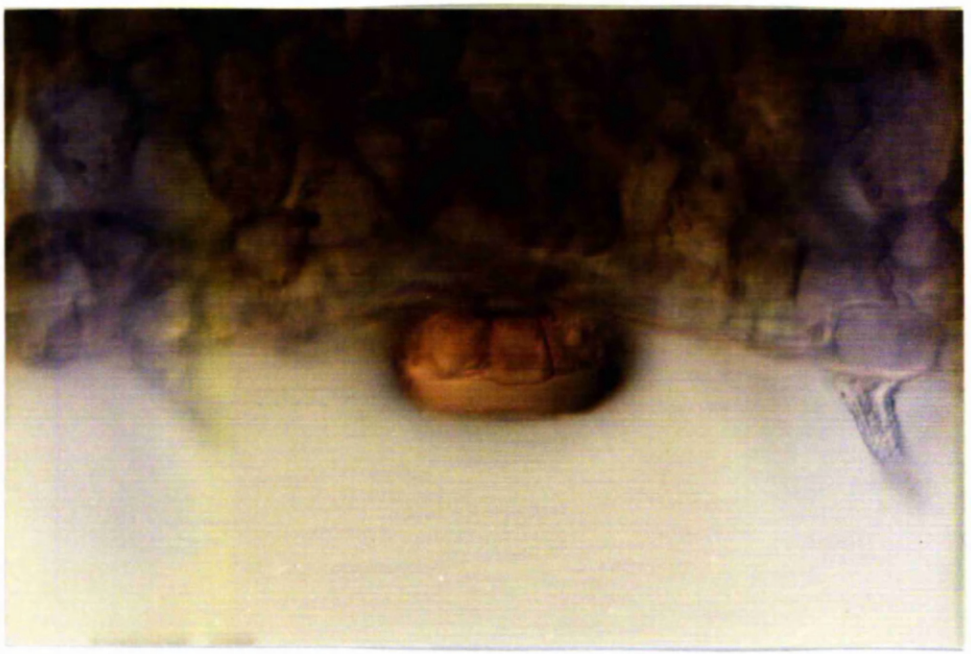
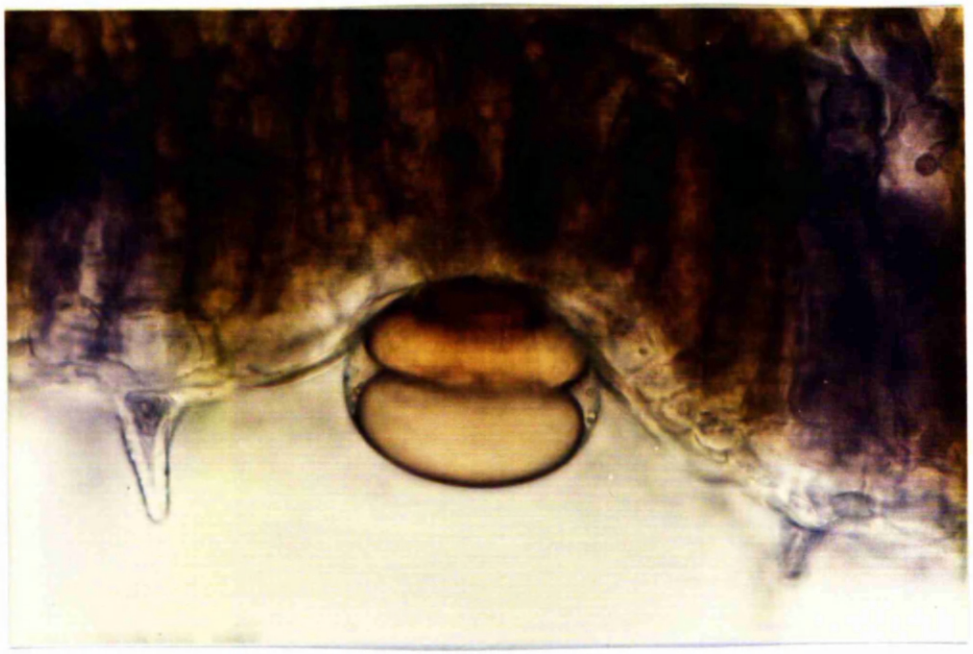


Plate 9: Fresh leaf section from *T. v.* leaf showing a peltate glandular hair (head cells breaking down). Bright field (x1600).



2.2.2 TERPENOID BIOSYNTHESIS

Plant volatile (essential) oils and resins are lipophilic and consist, primarily, of terpenoid compounds (Croteau and Johnson, 1984). These form the largest, and structurally most varied, group of natural secondary metabolic products known (Geishmann and Crout, 1969). The essential oils are classed as volatile, low molecular weight terpenoids and are mostly monoterpenes (C10) (b.p. 140 - 180C), with some sesquiterpenes (C15) (b.p. > 200C) [Harborne and Turner, 1984]. Resins may contain volatile and non-volatile terpenoids but are mainly made up of diterpenes (C20) (Croteau and Johnson, 1984). Most terpenoids are uncombined but some are found in the form of esters of organic acids, glucosides or combined with proteins (Geishmann and Crout, 1969).

Glandular trichomes (oil glands) are the primary sites of terpene accumulation in labiate leaves. The quantity and quality of oil varies between individual glands of the same type and between glands of different type. Since these cells lack chlorophyll, it is thought that the energy and structural precursors must come from adjacent cells. Young leaves are more capable of synthesizing terpenes from exogenous precursors than are fully expanded leaves but terpenes continue to accumulate in mature leaves, which suggests that they are synthesized from stored

material when exogenous material is no longer available (Croteau and Johnson, 1984).

Historically, isoprene was found as a product of the pyrolytic decomposition of terpenes but has only recently been found to occur naturally. The term 'isoprenoid' precursor is used for the basic 5 carbon unit, from which the terpenoids are built by adding units head to tail (Fig. 2). This is called the 'isoprene rule', although not all terpenoids appear to follow this rule.

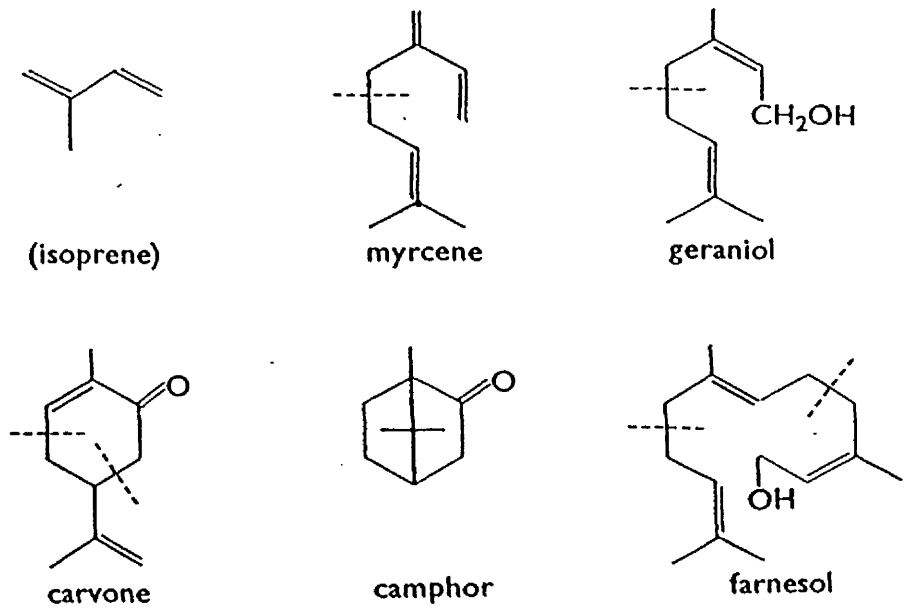


Fig 2: Isoprene and some isoprenoid compounds (Geishmann and Crout, 1969)

The irregular types can be shown to have undergone some rearrangement of the basic isoprene units during

synthesis, for example, during or after ring closure or by loss of some of the original carbon atoms (Geishmann and Crout, 1969).

Precursors of the terpenoids are the acyclic pyrophosphate esters geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). The pathway begins with the two carbon intermediate, acetyl coenzyme A, formed from ethanoic (acetic) acid and coenzyme A. Condensation of two molecules of acetyl coenzyme A gives acetoacetyl coenzyme A (4C). This is condensed with a further molecule of acetyl coenzyme A to give β-hydroxy-β-methyl glutarate coenzyme A (6C) which is reduced to mevalonic acid (6C) (Fig. 3).

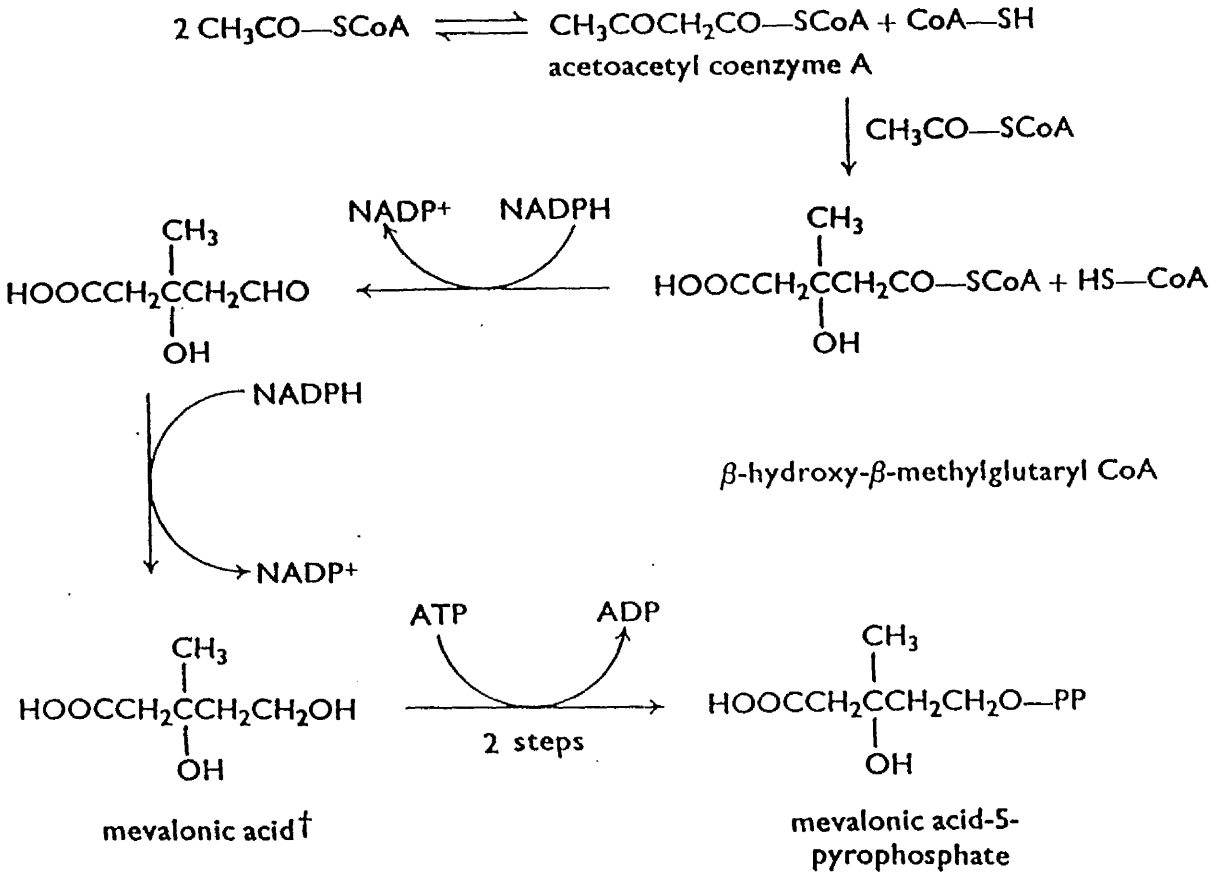


Fig 3: Biosynthesis of mevalonic acid-5-pyrophosphate (Geishmann and Crout, 1969)

Pyrophosphorylation of the primary alcohol group gives mevalonic acid pyrophosphate, decarboxylation of which results in an equilibrium mixture of the hemiterpene, isopentenyl pyrophosphate (IPP) (5C) and its isomer dimethylallyl pyrophosphate (DMAPP) (Fig. 4) (Geishmann and Crout, 1969). GPP is formed by enzyme-induced polymerisation of IPP and DMAPP. There is

divergence in the common pathway resulting in different end products (Fig. 5). These reactions are catalysed by prenyl transferases (Croteau and Johnson, 1984). GPP can form monoterpenes or C15 intermediates.

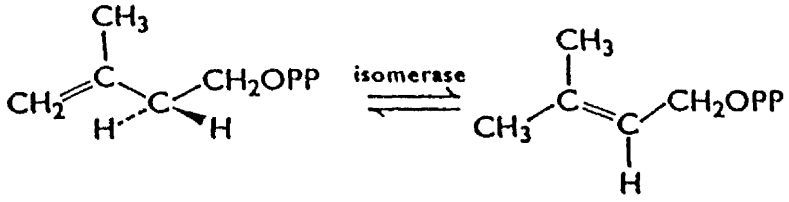


Fig 4: Isomers of isopentenyl pyrophosphate (Geishmann and Crout, 1969)

FPP can form sesquiterpenes, triterpenes on dimerization, or undergo further elongation. GGPP can form diterpenes, tetraterpenes after dimerization, or be further elongated to give polyprenols (Croteau and Johnson, 1984).

The gibberellins, plant growth hormones, are diterpenes (C20) or, in some cases (GA3), degraded diterpenes (C19) where a carbon atom has been lost (Goodwin, 1967; Berk, 1976). Squalene, a triterpene hydrocarbon derived from tail to tail linkage of two farnesyl pyrophosphate molecules, is a precursor of the steroids (cholesterol) (Porter and Spingeon, 1981;

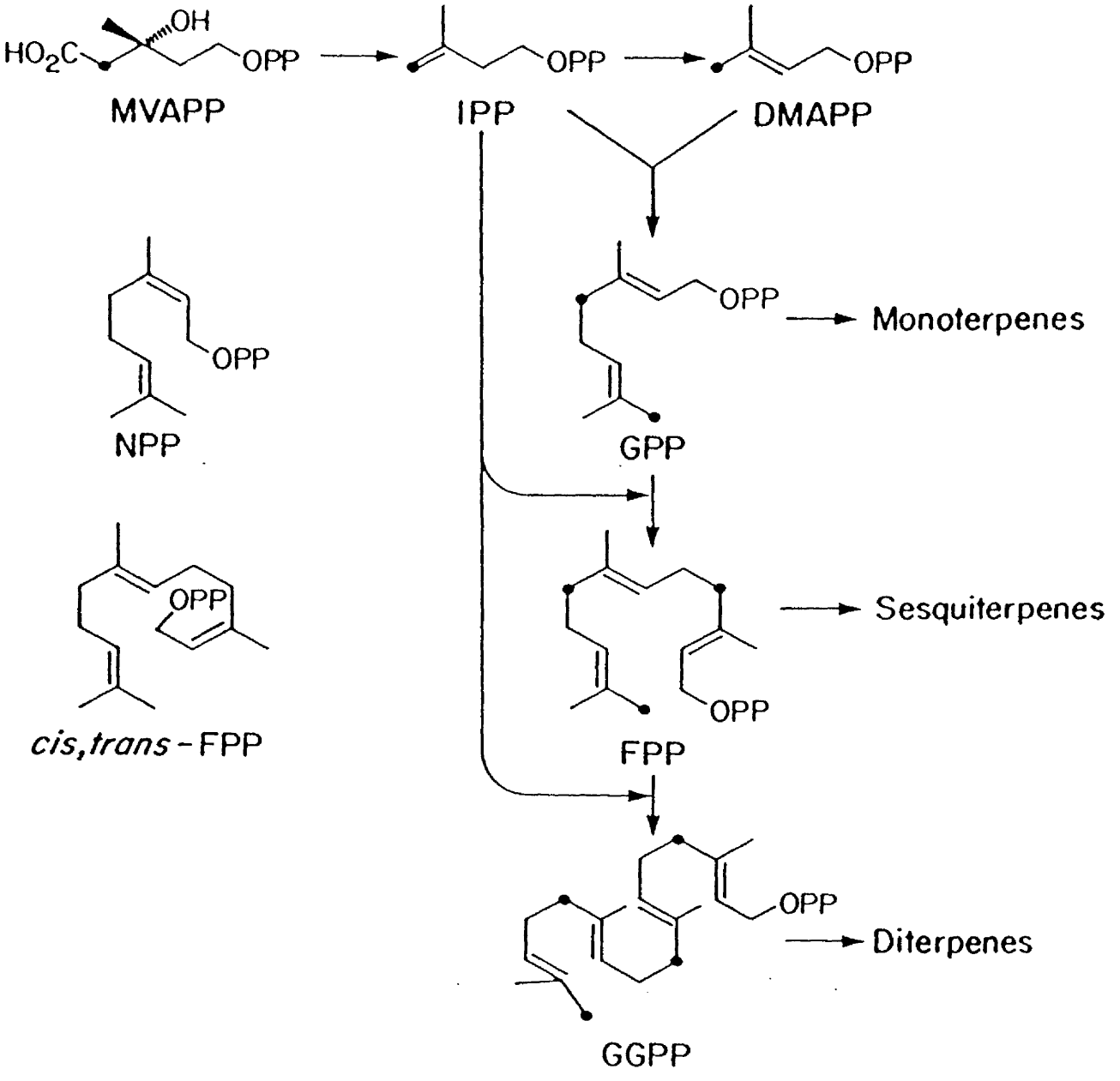


Fig. 5: Biosynthesis of acyclic terpenoid precursors from mevalonic acid-5-pyrophosphate (Croteau and Johnson, 1984).

Vickery and Vickery, 1981). Phytol, which is an isoprenoid alcohol, is an essential part of the chlorophyll molecule. It forms a side chain and makes the molecule lipid soluble. It is also partly responsible for the biological activity of chlorophyll (Berk, 1976; Vickery and Vickery, 1981). Phytol is also a precursor of vitamins E and K (Vickery and Vickery, 1981). The carotenoids (e.g. fucoxanthin, capsanthin) are tetraterpenes with a skeleton consisting of two geranyl geranyl pyrophosphate molecules attached tail to tail. Although the main skeleton does not form a ring, each end of the molecule may do so (β-carotene) (Porter and Spingeon, 1981). Rubber, obtained from *Hevea brasiliensis*, is a polyterpenoid compound derived from isopentenyl pyrophosphate (Trease and Evans, 1983).

A few of the natural monoterpenes are acyclic although the majority are of the cyclohexanoid type and may be mono- or bi-cyclic (Fig. 6). They may be unsaturated hydrocarbons (α-pinene, β-pinene), alcohols (α-terpineol, thymol) or aldehydes and ketones (carvone, thujone, citral) for example (Geishmann and Crout, 1969). Both geometric and stereoisomers may be found. Geometric isomers are possible with unsaturated acyclic monoterpenes such as geraniol (the trans- isomer) and nerol (the cis- isomer) (Croteau and Johnson, 1984). These may be present in different proportions in oil from a single

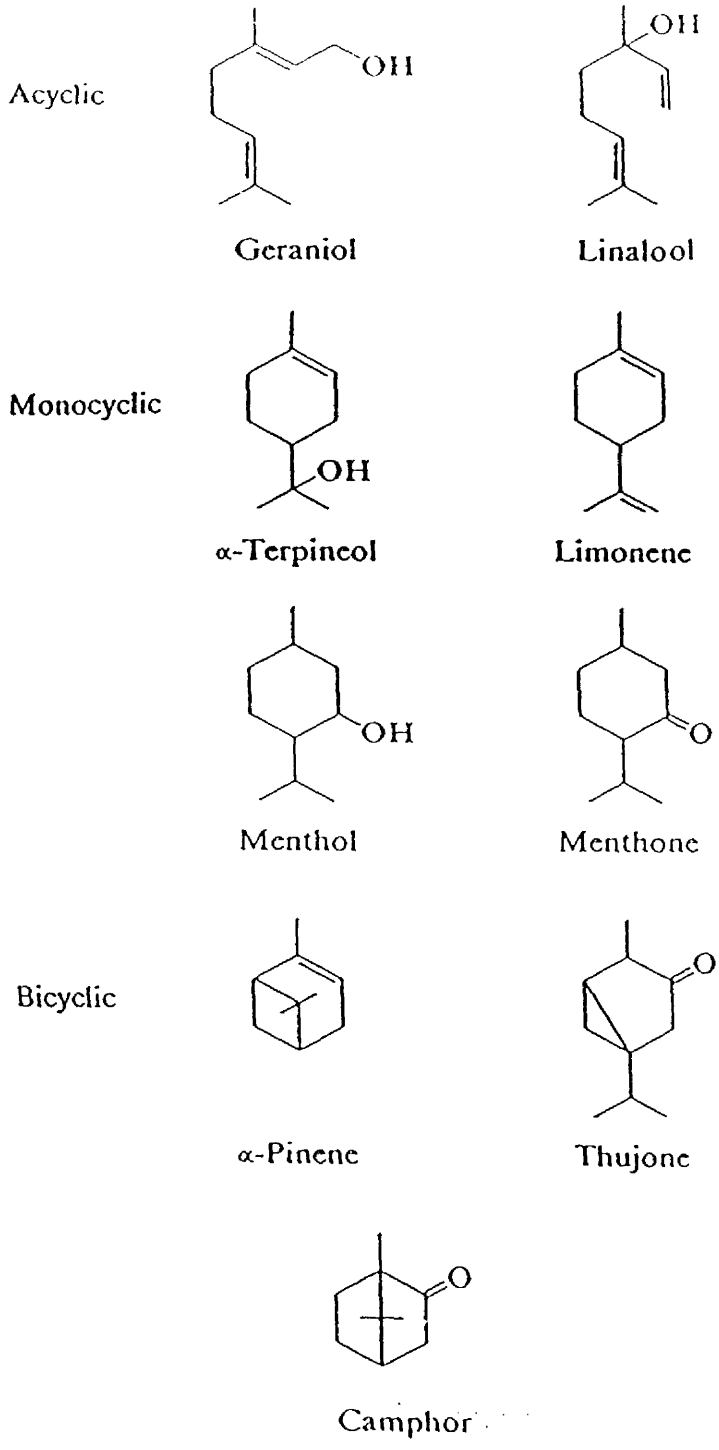


Fig 6: Typical plant monoterpenes (Croteau and Johnson, 1984)

source. Terpenoids with asymmetrical carbon atoms may be found in the optically active d- and l- forms. These rotate plane-polarised light in different directions but are chemically identical molecules. For example, limonene is usually present in the l- form but the d- form also occurs occasionally, alone or mixed with the l- form (Harborne and Turner, 1984). It is thought that such enantiomers are found where enzymes are involved very little in the cyclisation process (Croteau and Johnson, 1984).

SESQUITERPENES

All sesquiterpenes originate from a single precursor in one of its three isomeric forms. Farnesyl pyrophosphate is formed by addition of IPP to GPP. FPP exists as cis- and trans- isomers, corresponding to geraniol and nerol. The third isomer, nerolidol pyrophosphate arises from the tertiary alcohol configuration and corresponds to linalool (Fig. 8). Both farnesol and nerolidol occur widely in essential oils. Cyclisation of FPP leads to a large number of possible products which depend on how the isomers are orientated prior to ring closure (Geishmann and Crout, 1969). Like the monoterpenes they may be acyclic, monocyclic or bicyclic (Fig. 7) (Harborne and Turner, 1984). It is thought that only a few cyclase enzymes give the basic skeletal structures but that many secondary enzymes perform the transformations which give the diversity of derivatives found in nature, and that these modifications determine the biological activity of the final compounds (Croteau and Johnson, 1984). Almost all common monoterpenes have sesquiterpene analogs (Fig. 8) but it is thought that different cyclase enzymes synthesise the two analogs, although the mechanisms involved may be similar (Croteau and Johnson, 1984).

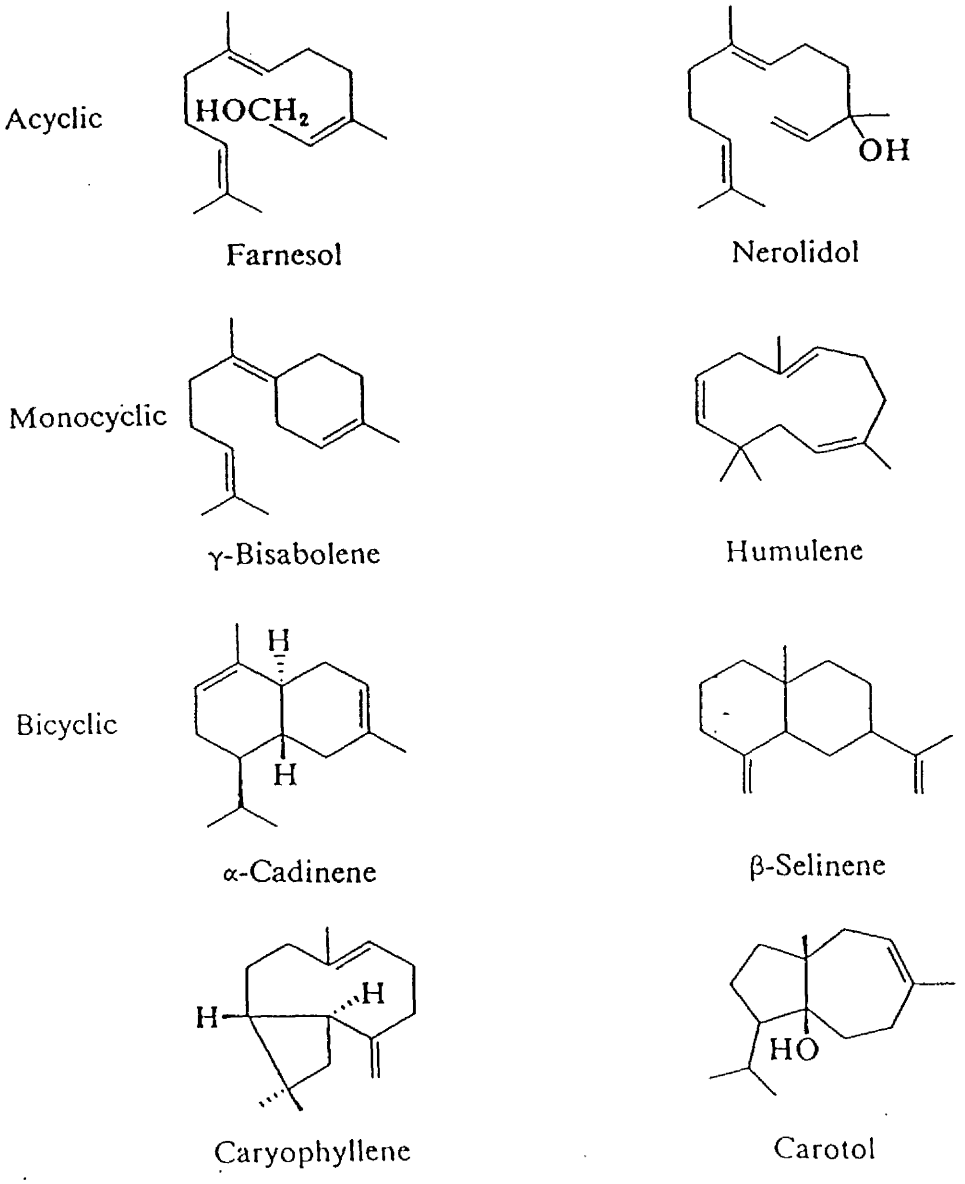


Fig 7: Typical plant sesquiterpenes (Croteau and Johnson, 1984).

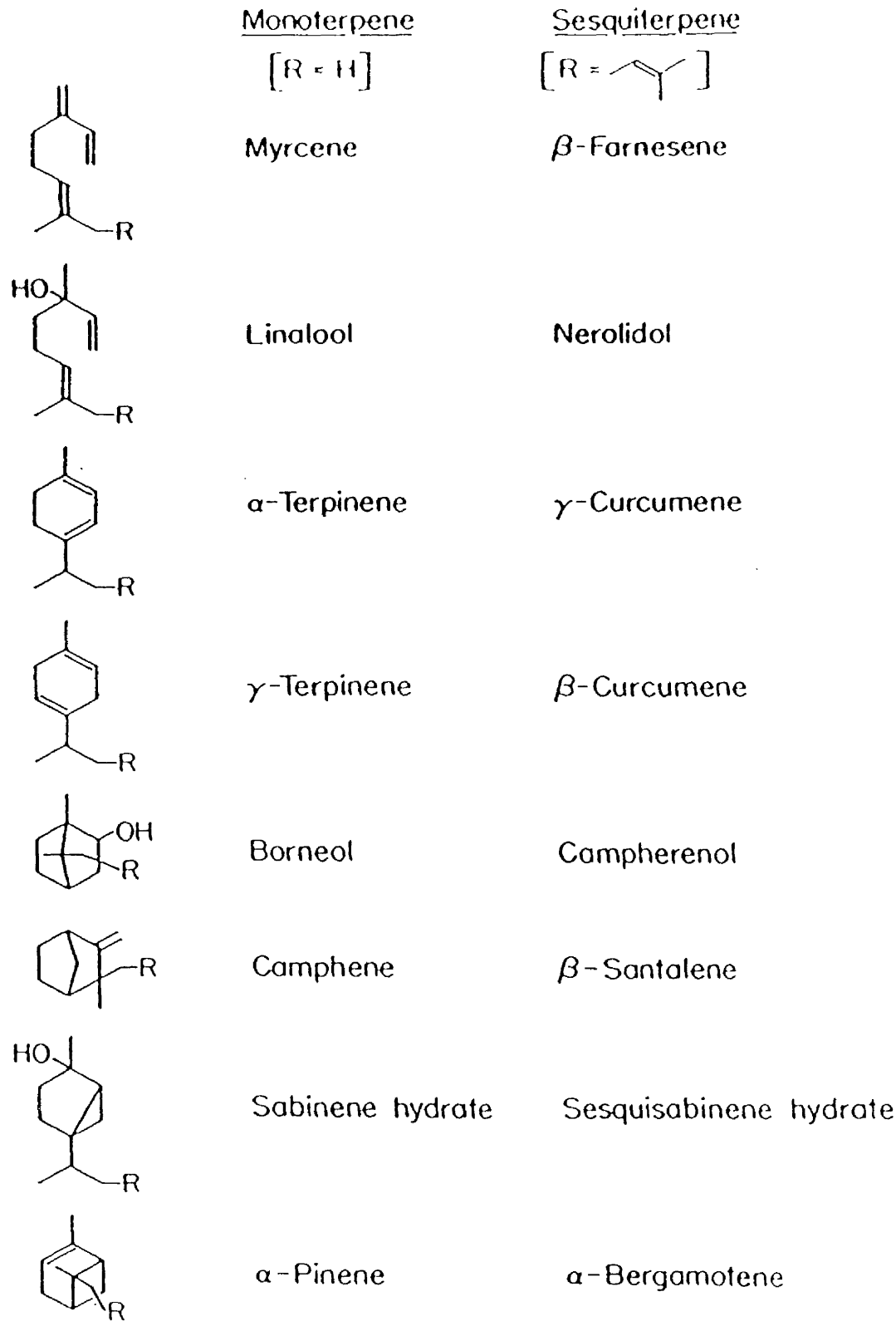


Fig 8: Some monoterpenes and their sesquiterpene analogs (Croteau and Johnson, 1984).

Gershenzon *et al.* (1988) working on spearmint *(Mentha spicata)* showed that the primary monoterpene found in spearmint, (-)carvone, is synthesized in the leaf glandular hairs by cyclisation of GPP to give (-)limonene. Hydroxylation of this compound gives (-)trans-carveol which on dehydration gives (-)carvone (Fig. 9).

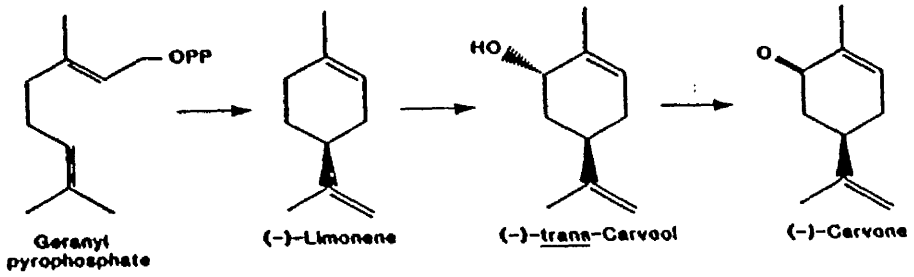


Fig 9: Biosynthesis of carvone from GPP in spearmint (Gershenzon *et al.*, 1988)

It is thought that the full terpenoid biosynthetic pathway is not present at all terpene biosynthetic sites in the secretory tissue and that control can come about by compartmentalisation of prenyl transferases, cyclases and modifying enzymes to give specific products at a given site (Martinkus and Croteau, 1981; Kjonaas *et al.*, 1982). Work by Martinkus and Croteau (1981), on peppermint, showed that l-menthone is specifically converted to l-menthol and l-menthol acetate and to d-neomenthol and d-neomenthol-β-D-glucoside (Fig. 10a). This selectivity

is brought about by the compartmentalisation of specific enzymes (menthol dehydrogenase with acetyl transferase and neomenthol dehydrogenase with glucosyl transferase). In vitro studies have shown that other products would be formed in similar quantities with those above if the enzymes were not compartmentalised from the menthone reduction step (Kjonaas *et al.*, 1982). The two dehydrogenase enzymes are different stereo-chemically but are otherwise very similar.

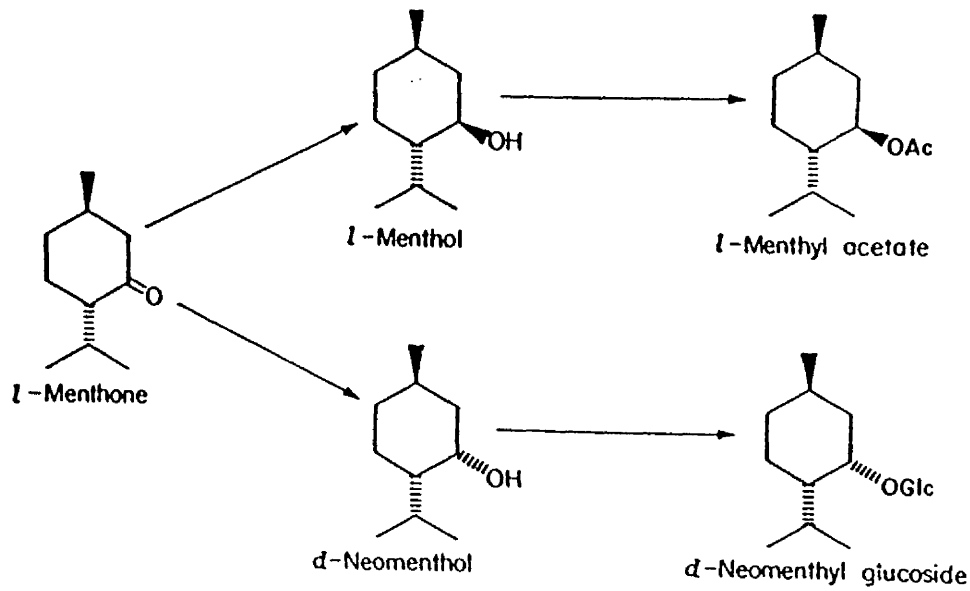


Fig 10a: Metabolism of l-menthone in peppermint leaves (Croteau *et al.*, 1984)

Evidence shows that the terpenes are not stored in the glands over long periods. Quantities of

specific products may change from day to day, or over a longer period, and plants are able to convert terpenes to primary metabolites (Croteau *et al.*, 1984; Croteau *et al.*, 1987). This appears to depend on the environment, physiology and development of the plant and may be controlled by the balance between rate of photosynthesis and use of photosynthate or by the balance between growth and differentiation (Croteau and Johnson, 1984). In peppermint *(M. piperita)*, for example, the rate of catabolism increased near the start of flowering and coincided with the conversion of menthone to menthol and, to a lesser extent, menthyl acetate and neomenthol. The neomenthol produced was converted to the water soluble glucoside and transported to the roots where it was reconverted to menthone (Fig. 10b) (Croteau et al., 1984; Croteau and Martinkus, 1979). This has also been shown to occur in flowering sage plants (Croteau *et al.*, 1987). Modified β-oxidation gives oxidised acetyl Co-A and reduced pyridine nucleotides and it is suggested that this metabolic turnover of monoterpenes is a mechanism for recycling carbon and energy from mature leaves to give metabolites for use by the rhizome (Croteau and Sood, 1985).

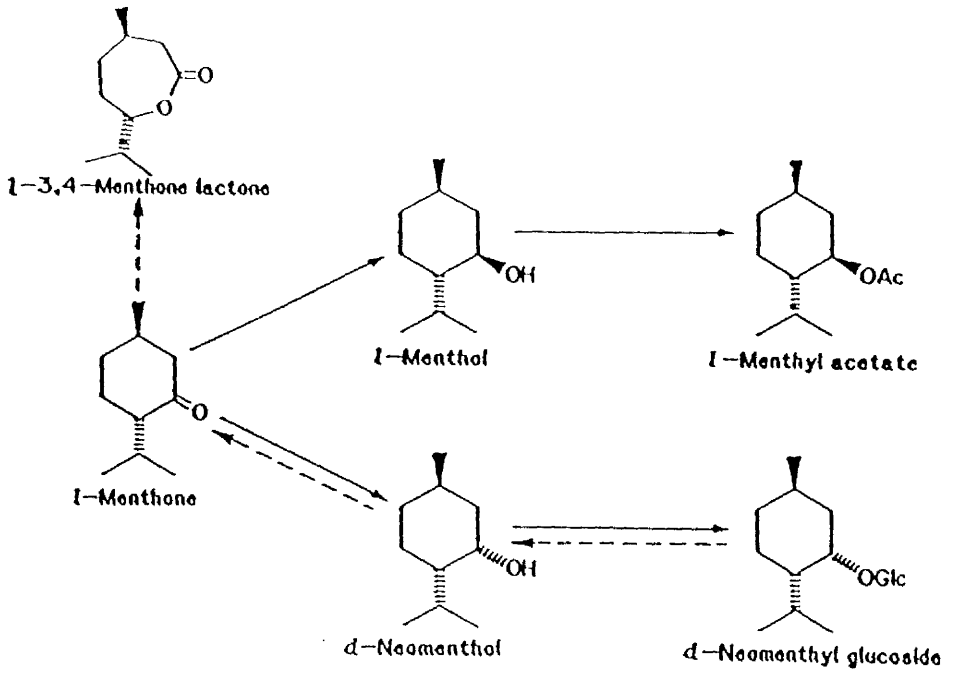


Fig 10-b: l-menthone metabolism in peppermint leaves (solid lines) and d-neomenthyl-β-D-glucoside metabolism in peppermint rhizomes (broken lines) [Croteau and Sood, 1985]

2.3. THYME OIL

2.3.1 Thymus vulgaris

It has been known for many years that *T. vulgaris* exists as several chemotypes (Clapham *et al.*, 1962) and Gouyon *et al.* (1986) showed that this species, when found around Montpellier, in France, had 6 chemotypes, controlled by epistatic genes (non-allelic genes which may suppress or mask the effect of other genes [Encyclopaedia Britannica, 1974]). G (geraniol), A (∝-terpineol), U (thuyan-4-ol) and L (linalool) chemotypes carry dominant allelles which prevent production of phenols. However, since they may be heterozygous, recombination of allelles on crossing can give rise to phenolic individuals, T (thymol) and C (carvacrol) (Boursot and Gouyon, 1983). These six chemotypes were found by Gouyon *et al.* (1986) to have fairly specific preferences in terms of soil type, soil moisture and temperature (which were all related). Progeny produced on selfing each type showed differences in genetic diversity: progeny from 'thymol' types all produced thymol as the major oil component. Genetic diversity of the progeny from the remaining 5 types varied as follows:

CARVACROL < LINALOOL < THUYAN-4-OL < ∝-TERPINEOL < GERANIOL

That is, the geraniol progeny showed the greatest genetic diversity.

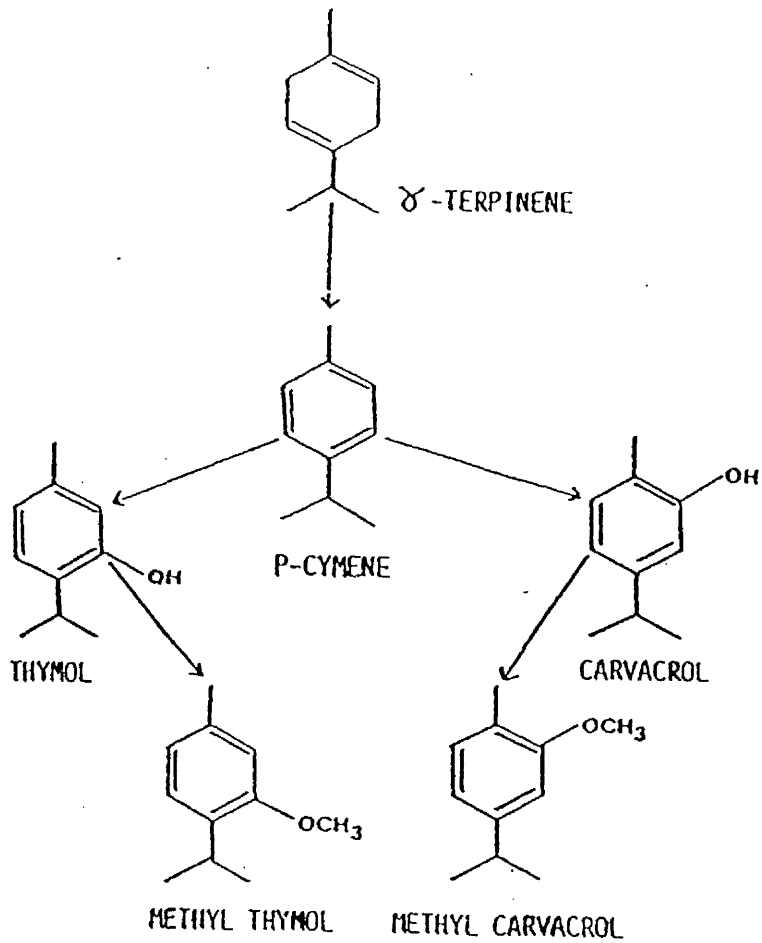


Fig. 11: Part of the metabolic pathway in the production of thymol and carvacrol in plant essential oils (Lawrence, 1984).

Thymol and carvacrol types were found on the driest, shallowest soil and α-terpineol types on the deepest and wettest. Thymol types were found on slightly damper soils than carvacrol and in somewhat colder areas. The remaining three types ranged between these extreme habitats. In the area of changeover of soil types there was also a gradual change from one chemotype to another. There was a good correlation between soil type and chemotype of the thyme population (Gouyon *et al.*, 1986).

There appears to be a further distinct chemotype of *T vulgaris* in which thymol and carvacrol occur together. Lamy (1983) using a French clone of this type showed that there was variation in oil components during a single season. During the course of growth from bud to the end of flowering there was an increase in thymol and carvacrol content and a decrease in γ-terpinene and ρ-cymene (both precursors of thymol and carvacrol - Fig. 11) (Lawrence, 1984). Differences in quantity of thymol and carvacrol have also been found in plants from a single clone grown under different conditions. For example, a German clone of *T vulgaris* grown in Turkey had four to five times more carvacrol in the oil than when grown in Germany (Lawrence, 1984). Phenol production is thought, by some, to depend on variations in soil or climatic conditions (Gouyon *et al.*, 1986).

It seems possible that *T. zygis* and *T. serpyllum*

also exist as a number of chemotypes.

2.3.2 Thymus Zygis

Table 3a shows the major components in oil from seven individual *T. zygis* plants collected by Garcia Martin and Garcia Vallejo (Lawrence, 1984) in various regions of Spain. Clearly, five of the six chemotypes given for *T. vulgaris* are present. There is also an example of a thymol/carvacrol type. The seventh plant is more difficult to define. They also found some hybrid species which gave mixed chemotypes from single plants. For example, a 1,8-cineole/linalool type which they suggest may be a hybrid of *T. zygis* and *T. mastichina* L.. The oils in Table 3a were obtained by steam distillation and analysed using GLC & IR.

Table 3a: Oil of *T. zygis* plants collected in different parts of Spain, subspecies not specified (peaks > 5%) (Lawrence, 1984).

Compound	1	2	3	4	5	6	7
myrcene + myrcenol	-	-	-	-	-	-	28.6
trans-thuyanol	-	-	-	-	-	-	6.9
terpinen-4-ol	-	-	-	-	-	-	10.0
α-terpineol + borneol	-	-	-	-	-	13.3	6.9
α-terpinyl acetate	-	-	-	-	-	70.3	-
linalool	-	-	-	79.0	-	-	-
linalyl acetate	-	-	-	8.6	-	-	-
geranyl acetate	-	-	-	-	68.6	-	-
geraniol	-	-	-	-	16.1	-	-
γ-terpinene	9.9	11.9	5.8	-	-	-	-
ρ-cymene	18.9	20.8	20.8	-	-	-	-
thymol	49.8	-	25.9	-	-	-	-
carvacrol	-	43.9	26.3	-	-	-	-
Total phenols	49.8	43.9	52.2	0	0	0	0

Table 3b was compiled by Velasco and Perez (1984). The *T. zygis* subsp. *zygis* is known to have come from a large sample and may have mixed types present although it is known that linalool types produce thymol or carvarol for the first three months, depending on which gene is present at the PH (phenolic) locus. All of the other samples. B - F (except E), appear to be from mixed stands (see also *T. zygis*, Table 4).

Again, Table 3c , from work done by Velasco and Perez (1984), was compiled from large samples (200g)

rather than from individual plants and appears to be mixed chemotypes (except 2 & 3).

Table 3b: Oil of 3 subspecies of *T. zygis*. Compiled by Velasco and Perez, 1984).

	Source					
Compound	A	B	C	D	E	F
myrcene	-	-	-	5.2	-	-
linalool	32.8	-	-	-	-	-
1,8-cineol	-	-	14.2	14.4	-	-
α-thuyene	-	-	-	6.6	-	-
α-pinene	-	-	5.4	8.0	-	-
camphor	-	5.6	11.3	9.0	-	5.0
borneol	-	-	-	-	-	7.5
γ-terpinene	-	6.2	6.9	8.6	-	13.0
ρ-cymene	17.9	15.2	9.1	11.0	18.0	22.4
thymol	15.1	46.8	27.5	20.9	61.1	11.7
carvacrol	-	-	-	-	-	20.6
Phenols	15.1	46.8	27.5	20.9	61.1	32.3

A - subsp. *zygis* from Guadalajara
B - " *sylvestris* from Viacia Madrid
C - " " Arganda
D - " " C de Cresa
E - " *gracilis* Granada
F - " " "

Table 3c: Essential oil components of *T. zygis* subsp. *sylvestris* from different regions of Spain (Velasco and Perez, 1984).

	Regions				
Compound	1	2	3	4	5
linalool	-	-	-	11.4	-
borneol	-	-	-	5.4	10.2
γ-terpinene	5.2	8.1	7.9	9.6	5.4
ρ-cymene	17.3	11.8	12.1	10.1	11.5
thymol	45.1	53.2	57.7	37.4	49.1
carvacrol	6.8	-	-	-	-
Total phenols	51.8	53.2	57.7	37.4	49.1

REGIONS (Table 3c):
1 - Cerros de Aranjuel, Madrid
2 - Ontigola, Madrid
3 - Cuesta de la Reina, Madrid
4 - Herencia, Ciudad Real
5 - Yebenes, Toledo

Table 4: A comparison of the oil from 2 *Thymus* species grown in Spain (Lawrence, 1984).

Compound	Spanish *T. Vulgaris*	Spanish *T. Zygis*
camphene	8.1 - 10.9	-
limonene + 1,8-cineole	35.7 - 44.4	-
linalool	3.3 - 6.3	32.8
camphor	11.6 - 16.3	-
∝-terpineol + borneol + bornyl acetate	7.8 - 8.9	-
ρ-cymene	-	17.9
geraniol	-	5.8
geranyl acetate	-	12.9
thymol	-	15.1
Total phenols	0	15.1

2.3.3 *Thymus Serpyllum* L.

Table 5 gives the major components (>5%) of five *T. serpyllum* plants. Oils from 1, 2, 4 and 5 have been obtained from 'taxonomically authenticated' plants (Lawrence, 1980). It can be seen that each of these plants has caryophyllene as a main component, unlike *T. vulgaris* and *T. zygis*.

Plant number 3 in Table 5 was originally identified using GLC and TLC but when the oil was run at a later time using GLC/MS it was found to contain 60.0% of thymol and caryophyllene was also given as a component (Lawrence, 1981).

Table 5: Oil from *Thymus serpyllum* plants (peaks > 5%) (Lawrence, 1980; Lawrence, 1981).

Compound	1	2	3	4	5
caryophyllene	9.0	12.0	-	6.0	7.0
β-bisabolene	-	5.3	-	7.6	-
linalool	-	-	-	-	25.2
linalyl acetate	-	-	-	-	8.3
α-terpinyl acetate	-	-	-	-	9.6
10-(α)-cadinol	-	-	-	-	5.9
geraniol	-	-	5.9	-	-
γ-terpinene	16.8	18.5	6.1	-	-
ρ-cymene	15.8	17.2	13.5	5.7	-
methyl thymol	-	-	-	7.5	-
thymol	-	-	48.0	16.7	-
carvacrol	36.9	21.2	*	27.8	-
methyl carvacrol	-	12.0	-	6.0	-
Total phenols	36.9	21.2	48.0	44.5	0

* = Thymol + Carvacrol

2.3.4 THYME OIL

Thyme oil is produced commercially from *T. vulgaris*, *T. zygis* and *T. zygis* var. *gracilis* (Uphof, 1959; Clapham *et al.*, 1962). Table 6a gives the composition of commercial thyme oils from various sources. Oil contents given for *T. vulgaris* vary but are usually between 0.5 - 2.8% (v/w leaf dry matter) (Technologii, 1982; Leung, 1980).

The importance placed on phenolic components (esp. thymol) shows in the lack of non-phenolic components in these oils. It appears that plants have been selected for phenolic content and, since the phenolic genes are recessive (Boursot and Gouyon, 1983), the commercially selected stands can remain pure.

Table 6a: 'Thyme oil' from various sources (Lawrence, 1980; Lawrence, 1983; Lawrence, 1984).

	Country of origin of oil					
Compound	A	B	C	D	E	F
∝-pinene	7.0	-	-	-	-	-
ρ-cymene	13.0	41.0	27.3-42.8	-	4.1-22.8	15.1-35.3
ɣ-terp.*	10.0	-	3.2-7.6	9.5	0.5-14.9	1.2-11.7
thymol	58.0	-	30.0-43.6	48.9	15.1-61.5	32.0-62.9
carvacrol	-	43.0	3.5-5.4	-	2.2-44.3	1.2-5.0
Phenols	58.0	43.0	33.5-49.0	48.9	17.3-105.8	33.2-67.9

* = ɣ-terpinene

COUNTRY OF ORIGIN OF THE OIL:

A - Kenya B - Chile C - commercial (1) [country not known]
D - Spanish E - commercial (2) [country not known]
F - Spanish

Since the work on individual *T. vulgaris* and *T. zygis* plants by (Boursot and Gouyon, 1983) and Garcia Martin (Lawrence, 1984) shows quite distinct chemotypes, and there is no camphor present in any of these, it can be assumed that the *T. vulgaris* samples presented by Mateo (Table 6b - source I) (Lawrence, 1982) and Garcia Martin (Table 4) (Lawrence, 1984) were obtained not only from a mixed stand (e.g. including *T. carnosus* Boiss., *T. cilicicus* or *T. revolutus*) but that there may also have been other species present in the sample. It is known that *T.*

vulgaris can produce hybrids and these may occur between sections (see Appendix III) or ploidy levels (Clapham *et al.*, 1972). Tables 6b and 7 show the composition of commercial thyme oils from various countries including France, Spain and Italy. In all cases it is difficult to identify a definite chemotype which suggests that these oils may also have been produced from a mixture of chemotypes if not species/genera.

Table 6b: 'Thyme oil' from various sources (peaks ¢ 5%) (Lawrence, 1982;Lawrence, 1984).

	Source			
Compound	G	H	I	J
camphene	-	12.8	11.4	-
limonene + 1,8-cineole	-	7.9	33.0*	5.6*
α-pinene	-	-	5.1	-
myrcene	-	-	5.4	-
camphor	-	-	14.5	-
limonene + β-bourbonene	-	-	-	8.0
α-terpinyl acetate	-	-	-	5.7
ρ-cymene	27.4	19.4	6.8	22.8
γ-terpinene	10.7	-	6.1	5.6
thymol	5.0	7.3	-	15.9
carvacrol	41.8	6.4	-	-
Total phenols	46.8	13.7	0	15.9

* = 1,8-CINEOLE only.

SOURCE: G - France H - commercial (Polunin, 1978)

I - Spain J - commercial (Ross-Craig, 1967)

Table 7: 'Thyme oils' from various parts of Italy (peaks > 5%) (Lawrence, 1984).

Source	Compound						
	1	2	3	4	5	6	7
1	-	30.0	-	-	7.0-10.5	-	-
2	8.0	40.0	7.0	10.4-16.3	5.6-8.8	-	-
3	15.0	50.0	13.0	8.0-20.0	-	8.0	-
4	5.0	20.0	6.0	-	40.0-50.0	-	-
5	-	15.0	8.0	-	40.0-60.	-	6.0
6	-	46.0	8.5	13.0	13.0	6.0	6.0

COMPOUND IDENTIFICATION (Table 7)

1 = camphene + pinene
2 = ρ-cymene
3 = linalool
4 = carvacrol
5 = thymol
6 = borneol
7 = linalyl acetate

2.3.5 OTHER SPECIES

Many other thyme species have been analysed in recent years, especially in countries where *T. vulgaris* and *T. zygis* are not endemic. Vokou and Margaris (1986) did an ontogenetical study of *T. capitatus* Hoff. and Link., a seasonally-dimorphic species (summer leaves are smaller which reduces the transpiration surface), growing at 350m on the limestone slopes of Mount Hymettus, in Greece. Dry flowers/fruiting tops and leaves were steam distilled for 3 hours and analysed and it was found that the flowers contained more oil than the leaves. The oil concentration of the flowering/fruiting tops increased from May (7.2% v/w) to a maximum in June (7.6% v/w) and declined in July (6.7% v/w). Leaf oil content was lowest in January (1.8% v/w) when winter leaves were present and maximum in May and June (4.6% v/w) when both summer and winter leaves were present. Winter leaves fell in July.

T. marshallianus Willd. (*T. pannonicus* All.) plants growing at 2000 m and 1000 m on the steppes of Russia showed no difference in oil quality and very little difference in oil quantity at the two sites (Dembitskii *et al.*, 1985). This was unlike the results of work done by Cabo *et al.* (1987) where they found that the oil yield from *T. zygis* plants increased with increasing altitude above sea level (600m and 800m

resp.). The main component they found in the oil of plants grown at the lower level was ρ-cymene (a precursor of thymol, Fig. 11) (30.3%) but thymol (36.0%) was the main component in those from the higher level. There was also more carvacrol (22.2%) in the former than in the latter (3.9%). Bellomaria *et al.* (1981) grew two varieties of *T. longicaulis*, *T. longiculis longicaulis* and *T. longicaulis subisophyllus* at various altitudes. They found that oil yield from both varieties increased with increasing height above sea level to a maximum at 900m. Dembitskii *et al.* (1985) identified 35 of the 49 components present in the *T. marshallianus* oil using GLC and IR and compared it to other species grown in Russia and from elsewhere (Tables 8a, 8b). The oil which was from fresh, flowering plants was yellow-green in colour and smelled of thymol and linalool.

Table 8a: Components of oils from various *Thymus* species (peaks >5%) (Dembitskii *et al.*, 1985).

	Species			
Compound	1	2	3	4
α-pinene	-	6.5	-	-
limonene	-	6.8	-	-
caryophyllene oxide	-	-	-	5.7
linalool + linaly acetate	9.7	-	15.7	-
methyl ether of thymol	-	8.1	-	-
terpinen-4-ol	9.4	8.1	-	-
geraniol + geraniol acetate	16.9	-	11.5	-
γ-terpinene	-	8.2	-	19.3
ρ-cymene	-	10.9	-	22.4
thymol	-	15.8	22.0	20.0
carvacrol	-	15.8	-	-
Total phenols	0	31.6	22.0	20.0

Table 8b: Components of oils from various *Thymus* species (Dembitskii *et al.*, 1985) (peaks > 5%)

	Species					
Compound	4	5	6	7	8	9
camphene	-	-	-	-	-	6.0
δ-3-carene	-	-	-	5.1	-	-
1,8-cineole	-	-	-	10.0	-	-
caryophyllene oxide	5.7	-	-	-	-	-
linalool + l acetate	-	-	-	18-21	-	-
terpinen-4-ol	-	-	-	-	-	9.0
γ-terpinene	19.3	-	-	11-18	41.0	8.9
ρ-cymene	22.4	-	-	-	-	30.3
thymol	20.0	9.8	56.1	10-20	-	-
carvacrol	-	67.0	8.3	8-14.5	43.0	22.2
Total phenols	20.0	76.8	64.4	18-34.5	43.0	22.2

1 = *T. trautvetter* Klok. et Schost. (from Azerbaidzhan)
2 = *T. fominll* (")
3 = *T. eriphorus* Ronn. (")
4 = *T. marschallianus* Willd. (from Kazakhstan)
5 = *T. capitatus* (from Greece)
6 = *T. quinquecostatus* Celak (from Japan)
7 = *T. vulgaris* (from Egypt)
8 = *T. vulgaris* (from Chile - see Table 6a)
9 = *T. zygis* (from Spain)

T. praecox subsp. *arcticus* (E. Durand) Jalas, collected from several parts of Iceland by Stahl (1982) was analysed using GLC/MS. The fresh herb samples were steam distilled and were all found to have linalool as the main component of the oil. This is a widely distributed species which grows on all

types of soil and is common on both high and low ground. 6 chemotypes were found according to the quantity of 3 compounds present (or absent): nerolidol, elemol (not definitely identified) and a further unidentified compound of molecular weight 200 a.m.u. There was no apparent correlation between chemotype and location, climate or edaphic factors.

Marhuenda and Menendez (1988), using TLC, GC, GC-MS and IR spectroscopy found that the oil from *T. carnosus* Boiss. contained only trace amounts of thymol and carvacrol. The main component was borneol. Fresh flowering material was used and the oil yield was 2.2% (v/w). 41 of 46 components were identified. Table 9a lists all of the components present over 5% of the total.

Table 9a: Components of the oil of T. carnosus (peaks ¢ 5%) (Marhuenda and Menendez, 1988).

Compound	% Oil
camphene	10.7
camphor	6.1
borneol	43.7
terpinen-4-ol	8.0
bornyl acetate	8.6

Miquel *et al.* (1976) analysed dried samples of *T. satureioides* (*T. brousonetti* Boiss.) using GLC, UV, IR and physical measurements and found a high percentage

of borneol in the oil (Table 9b).

Table 9b: Components of the oil of *T. satureioides* (peaks >5%) (Miquel *et al.*, 1976).

Compound	% Oil
camphene	5.4
β-caryophyllene	6.4
α-terpineol	12.2
borneol	26.2
thymol	19.2

Although there was only 2.6% of camphor present, it was noticeable in the odour. From the literature available to them they were unable to find any other thyme species with more than 8% of camphor in the oil and the level was more usually between 2 - 3%, when found.

However, *T. hyemalis* Lange., collected on the Sierra de Alfarcar, in the Granada region of Spain, was found to have a mean quantity of 15.4% of camphor in the oil when sampled monthly during the course of a year (Cabo *et al.*, 1987). Although the total oil produced varied from 0.15% in December to 0.58% in July the maximum variation in the amount of camphor present was from 10.9% in July to 20.9% in September. This was one of the main components in the oil along with 1,8-cineole and myrcene (Table 10). The fresh

plant material was steam distilled for, unusually, 5 hours and analysed using GLC and GLC/MS. The maximum quantity of essential oil was found in July (0.58% v/w), but the maximum 1,8-cineole level was found in August.

Table 10: Mean values of the components present in *T. hyemalis* oil during one year (peaks > 5%)(Cabo *et al.*, 1987).

Compound	Mean value (all samples)
myrcene	18.4 (7.4)
citronellol	7.0 (1.8)
limonene	5.4 (1.3)
1,8-cineole	20.0 (3.6)
α-pinene	5.7 (1.3)
camphene	9.5 (2.0)
camphor	15.4 (3.4)

T. transcaucasicus Ronn. and *T. eriphorus* Ronn. from the wild in Azerbaidzhan, were analysed by Kasumov (1983) at three different growth stages: during flowering, at the end of flowering and he analysed the oil content of the seeds. The oil content of both species was greatest during flowering (Table 11b). GLC and physical measurements were used to identify the compounds (Table 11a). 51 components were found in *T. transcaucasicus* and 69 in *T. eriphorus*.

T. kotschyanus Boiss. et Hohen, another U.S.S.R.

species was investigated in Azerbaidzhan, by Guseinov *et al.* (1987) (Tables 11a, 11b). The oil concentration of this species was much higher than in the two preceding species. 52 components were found , the main ones being given in Table 11a.

Table 11a: Major oil components of 3 Russian *Thymus* species (peaks > 5%) (Guseinov *et al.*, 1987).

	Species		
Compound	*T. transcau-casicus*	*T. eriphorus*	*T. kotsch-yanus*
caryophyllene	5.4	-	6.3
terpinolene	9.6	-	6.7
linalool	-	12.2	-
γ-terpinene	-	-	7.7
borneol	-	8.7	-
thymol	46.8	7.0	10.0
carvacrol	10.8	-	13.7
carvacrol acetate	-	7.0	-
Total phenols	57.6	7.0	13.7

Table 11b: % oil in 3 *Thymus* species at different ontogenetical stages (% v/w of dry weight).

	Species		
	T. transcau-casicus	*T. eriphorus*	*T. kotsch-yanus*
Budding	-	-	0.89
Flowering	0.37	0.48	1.33-1.46
End of flowering	0.24	0.27	-
Seeds	0.18	0.20	0.72-0.80

Kasumov (1986) also investigated *T. karamarianucus* Klok. et Schost. collected initially from the dry hilly slopes near Karamar'yan in the Geok-chai region of the U.S.S.R.. Some of these plants were planted in the Botanical Garden at Baku and some at the Center of the Zakataly Institute of Botany of the Az.S.S.R. Academy of Sciences. Plants were selected for greater oil yield. This was achieved at both of the introduced sites to different degrees which the authors attribute to height above sea level (Zakataly - 500 m, Baku - 100 m). As well as differences in the oil yield there were differences in the yield of certain components of the oil at the three sites, sampled during flowering (Table 12). For example, the amount of citral was doubled at Baku and almost three times greater at Zakataly than at the 'wild site' and

the amount of thymol was three times greater at both of the introduced sites. (The oil, which was yellow and smelled of lemon was analysed using GLC and the retention times of known compounds.)

Table 12: Oil from *T. karamarianucus* grown at 3 different sites in Azerbaidzhan (peaks > 5%) (Kasumov, 1986).

	Site		
Compound	Geok-Chai	Zakataly	Baku
caryophyllene	7.9	5.1	-
citral	10.3	28.6	20.4
linalool	7.4	5.4	-
limonene	-	-	5.4
∝-terpineol	18.2	14.9	12.4
∝-pinene	6.0	-	-
thymol	9.1	26.3	26.3
carvacrol	9.1	10.0	14.8
Total phenols	18.2	36.3	41.1
Essential oil	0.15-0.25	0.26-0.40	0.38-0.74

HEIGHT ABOVE SEA LEVEL : Geok-chai - 200 to 250 metres
Zakataly - 500 metres
Baku - 100 metres

NUMBER OF OIL COMPONENTS IDENTIFIED: Geok-chai - 13
Zakataly - 14
Baku - 15

The main aim of Lundgren and Stenhagen (1982) was to investigate pest and disease resistance in five *Thymus* species and their method of obtaining the oil

from the leaves was different from that of the other papers quoted here. They cut fresh leaves (from single plants) into strips and adsorbed the volatile oils onto a porous polymer (TENAX GC 35-60) in glass tubes. This was then analysed using GLC and GLC/MS (Table 13). Values of oil components are given for only two species. The second *T. vulgaris* sample (*T. vulgaris* 2) in Table 13 shows oil from a steam distilled sample (Lundgren and Stenhagen, 1982). The *T. serpyllum* was grown from seed and *T. vulgaris* was obtained from a nursery. They suggest that this *T. serpyllum* corresponds to the subspecies *tanaensis* from the North of England.

Table 13: Components of the oil of 2 *Thymus* species, obtained by direct volatilisation and one by steam distillation (*T. vulgaris* 2) (Lundgren and Stenhagen, 1982).

Compound	*T. serpyllum*	*T. v.* 1	*T. v.* 2
myrcene	14.1	-	-
linalool	17.3	-	-
β-caryophyllene	6.7	-	-
cis-3-hexen-1-ol	5.9	-	-
γ-terpinene	15.3	31.6	14.8
ρ-cymene	-	22.8	25.2
thymol	-	6.4	37.7
Total phenols	0	6.4	37.7
Total peaks identified	97.9	99.3	

2.4.1 LIFE CYCLE

Thymus vulgaris has many small flowers which are arranged in false whorls (verticillaster) about the stem and originate in the leaf axils. They have short corolla tubes which allow pollination by bees. Plants are gynodioecious: some have flowers which are bisexual whilst others have only female flowers (no stamens or non-functional ones). The style is gynobasic (originates in the base of the ovary). Female flowers are usually smaller and paler in colour than bisexual ones. In the family Labiatae, species are often protandrous: the anthers maturing before the stigmas. In the wild, this species is reproduced mainly by seed. These are dispersed over short distances (usually less than one metre from the parent plant [Valdeyron *et al.*, 1977]) (Encyclopaedia Britannica, 1974) and are capable of germinating immediately when shed, if the conditions are suitable. Around 60% germination is usual. Under some conditions adventitious roots may be produced; for example, where soil is blown over branches resulting in vegetative propagation by layering. Thyme is a perennial and plants are known to live for up to 15 years.

2.4.2 REPRODUCTIVE SYSTEM

Thymus vulgaris plants are gynodioecious: hermaphrodite plants have functional stamens and ovaries but male sterile plants either have no stamens or imperfectly developed ones. In a nine year study, in Montpellier, France, Valdeyron *et al.* (1977) found no change in the reproductive type of individual plants with time or habitat although a higher proportion of hermaphrodite plants than male sterile plants were found in more stable environments (e.g. rocky sites with no access for sheep) compared with a high proportion of male sterile plants in less stable, more disturbed environments (old grass fields which are occasionally reploughed and where sheep are able to graze). Male sterile plants are more heterozygous (obligate outbreeders) than hermaphrodite and in disturbed situations this may give them a selective advantage (Gouyon and Vernet, 1982).

The main pollinator of *Thymus vulgaris* is the honey bee *Apis mellifera L.*. Assouad *et al.* (1978) showed that 96% of the insects visiting five different populations of thyme were honey-bees. The remainder were solitary bees. They also showed that bees spend more time on hermaphrodite flowers, although there was no difference in the average number of inflorescences on hermaphrodite and male sterile plants. However

male sterile (MS) stigmas retained more pollen after a single pollination than hermaphrodite (MF) stigmas did and individual male sterile plants produced more seeds. The ratio of MS:MF seed production varied within and between populations. Populations of large plants and populations of high density gave a higher MS:MF seed ratio. Hermaphrodite flowers and styles are longer than those of male sterile plants but the stigmas are receptive for a relatively short period: male sterile flowers are receptive as soon as they open (Assouad *et al.*, 1978). They suggest that the difference in pollen retention may explain the difference in seed set between the two types. Differences in seed set disappear with repeated pollination of the hermaphrodite flowers (Assouad *et al.*, 1978).

Out of a possible four seeds per flower, Assouad *et al.* (1978) showed that seed set in hermaphrodite flowers was never greater than 2 whilst MF x MS crosses gave more than 2 seeds in each of ten cases. There were large differences in numbers of flowers giving no seeds on selfing and crossing: 45% of self-pollinations gave no seeds, MF x MF gave no seeds in 20% of cases and only 6% of MF x MS pollinations resulted in no seeds being produced.

Under controlled conditions Assouad *et al.* (1978) found that the largest numbers of viable seeds were produced from MS x MF crosses and the smallest numbers

from self-pollination of hermaphrodite plants. In the field, the number of seedlings found around male sterile plants was four times the number around hermaphrodite plants. Open pollinated hermaphrodite plants gave almost exclusively hermaphrodite progeny whilst male sterile plants gave an average of 70 - 75% male sterile progeny (Dommee and Jacquard, 1978) Couvet *et al.* (1986) also found that male sterile plants produced three to nine times more viable seeds than did hermaphrodite plants but that there was no difference in the rate of survival of seedlings. Furthermore there was no correlation between relative fertility of male sterile plants and the number of these in a population but that the frequency of male sterile plants in the progeny of both types of plant was significantly correlated with the frequency of male sterile plants in the parental population. There was no difference in the vigour or ability to compete between progeny of the two sexual forms. They believe that interactions between cytoplasmic and nuclear genes which determine male sterility explain the differences in segregation ratio and differences in this ratio among populations can explain why there are different frequencies of females. Assouad *et al.* (1978) suggest that the difference in viability between selfed hermaphrodite and MF x MF crosses may be due to partial self-incompatibility or zygotic

lethality of the progeny from selfing.

Mean rates of self-fertilisation in wild populations of thyme tested by Valdeyron *et al.* (1977) were 36%, 35% and 49% with a variation of 0 - 79% in individual plants. This was thought to be due mainly to differences in density of male fertile plants around those being tested: where density was high self-pollination was lower.

3.1.1 THE NEED FOR IMPROVED SELECTION OF THYME

Much of the thyme used commercially is collected from the wild but where it is to be grown on a commercial scale it is desirable for harvesting to be carried out with machinery. This requires a much more uniform crop than is produced from the seed stocks presently available. Clonal production can give uniform plants but is more labour intensive and, therefore, more expensive than producing the plants from seed.

By selecting plants with the desired characteristics and keeping them in isolation from other thyme plants it may be possible, by making further selections from the progeny of the group (thus narrowing the genetic base) to produce a more uniform crop from seed than has been possible before.

3.1.2 BREEDING TO PRODUCE A MORE UNIFORM COMMERCIAL STAND

Although *Thymus vulgaris* can be grown clonally it is known to be a very variable species when grown from seed. The aim of the present project, sponsored by Nutting and Son Limited was to see whether plant selection could result in seed lots which, in turn, gave less variation in morphology and time of flowering. A stock of ten plants was selected in

1988, under the guidance of Mr Christopher Nye, at that time a director of the seed firm Nutting and Son Limited, Cambridgeshire, from over 900 plants. These were chosen for upright, bushy habit, large oval leaves and later flowering. They were grown in isolation from other thyme plants during 1989 and seedlings produced were potted and kept in the glasshouse over winter and planted out in the Temple field in the summer of 1990. Measurements of these plants will be made to compare their variability with that of the original plants and of other non-selected plants. Seeds from the 10 selected plants will be released to the sponsors during the autumn of 1990. It is, however, too early to say if there has been a reduction of variability in the seed produced from the selected plants.

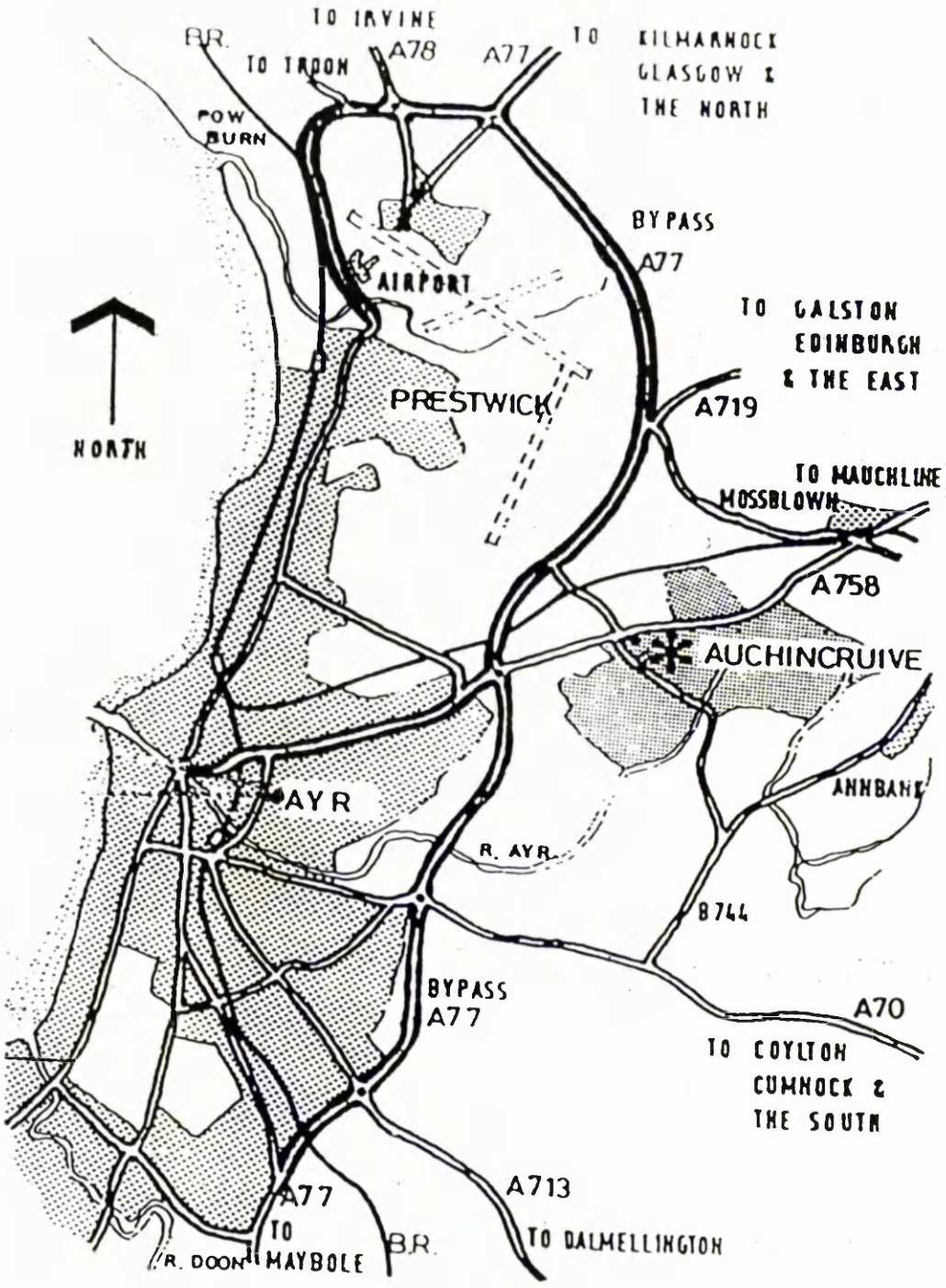
4 MATERIALS & METHODS

4.1 SOWING & PLANTING

Eight thyme seed lots, supplied by Nutting & Son Ltd., Queniborough, Leicester (formerly Nutting & Speed Ltd., Long Stanton, Cambridge), were sown into No 3.5 Hassey modules filled with Fisons F1 seed compost on 5/4/88. Two seeds were sown into each cell and these were thinned to a single seedling, when germination was complete, on 12/4/88. By 5/5/88 there were 3 - 4 pairs of true leaves present. On 25/5/88 the plants were moved to a cooler area within the glasshouse to harden off. The eight lots were planted out in their permanent positions (Figs. 12 & 13, Appendix VII), on the following dates:

PLOT A	(Claus - 120 plants)	- 14/6/88
B	(Lot 5524 - 120 plants)	- 16/6/88
C	(Lot 6093 - 120 plants)	- 16/6/88
D	(Spegi - 120 plants)	- 14/6/88
E	(Vikima - 121 plants)	- 3/6/88
F	(Arco - 121 plants)	- 6/6/88
G	(Morris Moran - 119 plants)	- 2/6/88
H	(Lot 7263 - 117 plants)	- 15/6/88

The soils were well-drained, sandy clay loams with pH adjusted to 6.5 by liming. The plots received no additional fertiliser during the experiment.



SCALE: 1cm to 1km.

Fig. 12: Local map of Ayr and district (supplied by Buildings Dept, Auchincruive.

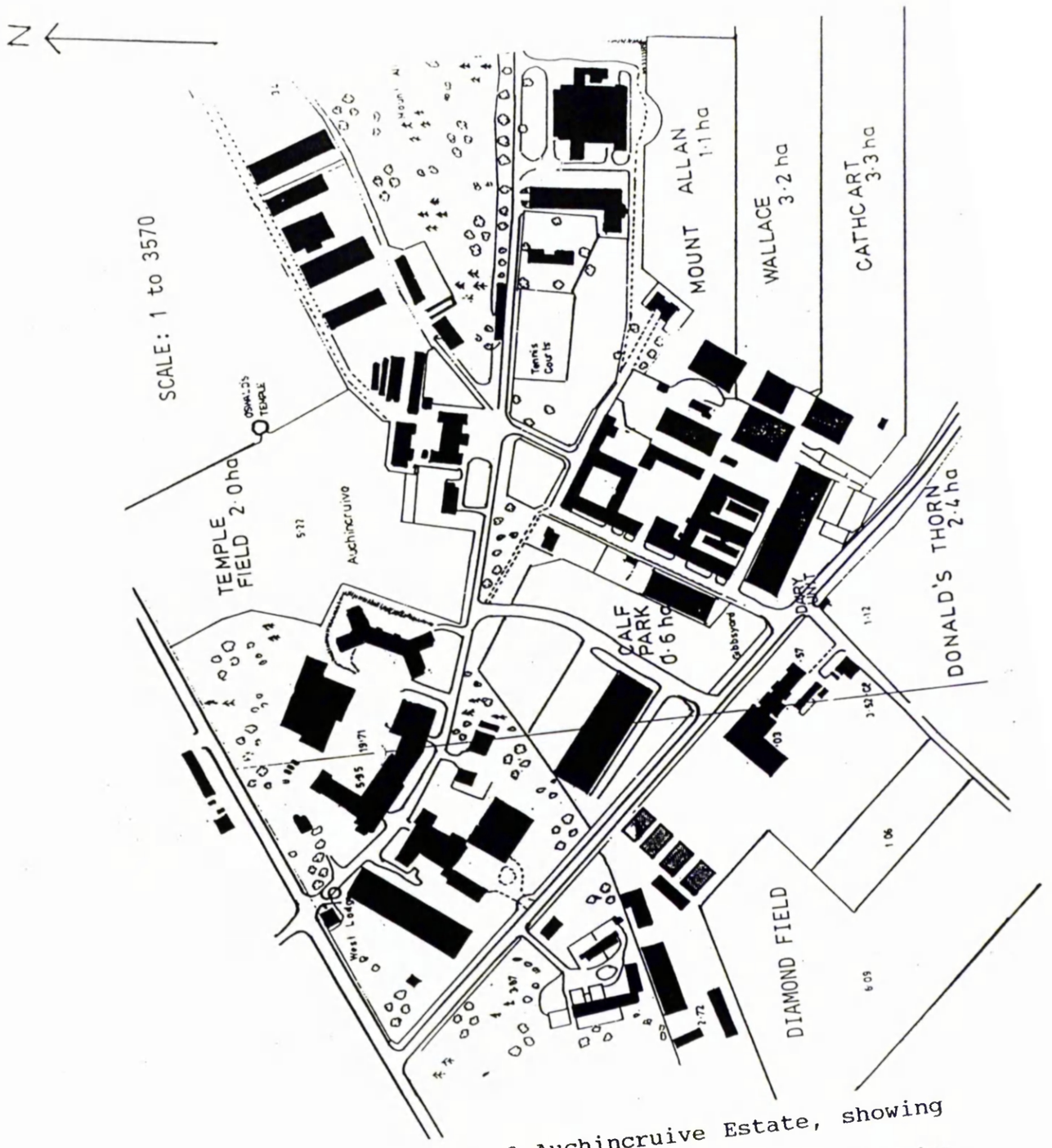


Fig. 13: Map of part of Auchincruive Estate, showing Templefield and Diamondfield (supplied by Buildings Dept., Auchincruive).

Spacing between plants was 60 cm by 45 cm, and 20 m between lots, to avoid cross-pollination between lots. Plots A - D and E - H were planted in separate fields in order to achieve this spacing. Due to the very sunny weather during May and June it was necessary to irrigate the plots on some days in June, especially plots A - D, which were planted in the middle of the month, during a prolonged dry spell (Appendix VIII). These conditions of high evaporative demand appeared to inhibit the growth of the plants in plots A to D and H. During April, 1989, high winds (which may have carried salt spray) and dry weather at the start of active growth caused extensive damage to some apparently healthy plants, especially those in plots E and G.

Table 14: Wind damage in thyme plots April, 1989.

Plot	Mean % damage	Standard deviation
A	16.4	16.0
B	21.0	22.2
C	39.0	23.5
D	21.9	14.8
E	47.4	33.5
F	17.5	20.8
G	66.0	31.7
H	3.3	9.9
H*	6.8	17.9

* Most of the damage in plot H was to plants directly under the leaves of kale plants. This figure includes the values for those plants. The other value for H excludes those plants.

This damage was concentrated in the quarter of each plant facing the prevailing south west wind (Table 14). These plants never fully recovered and it was decided to abandon both plots.

A black polythene mulch was laid between the plants in plots A - D on 5th June, 1989 to avoid the need for continual weeding between the plants. Plots F & H continued to be weeded but this was necessary less often since the weed seed bank in these plots appeared to be much smaller and the plants were somewhat larger.

4.2 STAND MEASUREMENTS

Between 11th and 17th August, 1988 the following measurements were made on the plants in plots A, B and E to H:

1 - Height

2 - Breadth (measured across the middle of the plant at right angles to each other and called x and y dimensions).

3 - Size and shape of leaves.

4 - Growth habit.

Plots C and D were measured, as above, on the 29th November, 1988. Plots A to D and F & H were again measured (height and width between 27th June and 3rd July, 1989).

4.3 FLOWER AND LEAF LENGTH AND INTERNODE DISTANCE

The dimensions of flowers, leaves and internodes were measured by placing randomly-selected branches on a clear plastic ruler and comparing against mm gradations under a binocular microscope (x10).
Samples of branches were also photocopied, at actual size, as a permanent record. Mean leaf lengths and widths, mean flower lengths and mean internode distances per branch were calculated, and comparisons were made between branches from a single plant and between plants.

4.4 FLOWER COLOUR AND REPRODUCTIVE TYPE

Flower colour was checked in May - June, 1990, as the plants began to flower. ICI 'Dulux Matchmaker' colour cards were used to identify flower colour. Card numbers 1 - 5, 13, 32, 34 and 36 were used. It was intended to convert the Dulux colours to international codes but I.C.I. refused to supply the appropriate information.

Reproductive type was checked when flower colour was determined. A 76mm (3") hand held magnifying lens was used, as required, to check if stamens with functional anthers were present. It was necessary to re-check some plants.

Three categories of flower type were found:

1. hermaphrodite - functional anthers present (cerise in colour)
2. an intermediate type - short stamens with white anthers
3. male sterile - no stamens, pistils with no anthers (very few of these were found) or short stamens with small brown anthers.

4.5 TIME OF FLOWERING

Flowering was recorded approximately every 10 days from 4th May, 1989 to 23rd July, 1989 and again in 1990. On each date budding and flowering plants were noted and, at the later dates, those plants whose flowers were senescing (when approximately 50% of the florets were over).

4.6 OIL SAMPLING

A few stems were taken from randomly selected plants in plots E - H on 30th September, 1988. These were dried for 24 - 48 hours at 40 C in a fan-assisted oven. The dried samples were rubbed to remove the stems and the remaining leaves and flowers (where present) were steam distilled for 2 hours. The volume of oil was recorded and then collected in glass bottles (7ml), the caps of which were then sealed with

Parafilm. These were stored at 4 C until required for analysis by GLC.

Further samples from the same plants, in plots F and H, were collected on 2nd October, 1989. All plants were cut back to the old wood during the last week in July, 1989, so that the new growth could be harvested at approximately the same ontogenetical stage as in 1988. The new growth from half of each plant was collected in order to obtain accurate measurements of dry leaf and oil yields per square meter of available ground. As in 1988, the dried leaves were distilled and the oil collected for analysis by GLC. Yields per square meter were calculated using the initial plant spacing of 45cm x 60cm, equivalent to 1 plant per 0.27 m^2. Since actual yields were from half of each plant, they were multiplied by 2 to give the potential yield per plant. This figure was then multiplied by the inverse of 0.27 to give the yield per m^2. Samples were collected in the same way and on the same date from plots A - D.

4.7 DISTILLATION

A Clevenger type distillation apparatus was used (Fig 14) with the dimensions as specified in the European Pharmacopoeia (Svoboda, 1988 p 70). The dried leaves were distilled for 2 hours in 400 ml of distilled water. The volume of oil obtained was recorded before being collected in glass vials which were then sealed with screw caps and Parafilm and stored at 4 C until analysed by GLC.

4.8 GAS-LIQUID-CHROMATOGRAPH

The GLC was a United Technologies Packard model 439 operated as below:

INJECTOR TEMPERATURE	250 C
DETECTOR TEMPERATURE	250 C
OVEN INITIAL TEMPERATURE	50 C
OVEN FINAL TEMPERATURE	200 C
INITIAL TEMPERATURE HELD FOR	10 min
FINAL TEMPERATURE HELD FOR	12 min
TEMPERATURE INCREASE RATE	5 C min^{-1}
NITROGEN FLOW RATE	30 ml min^{-1}
HYDROGEN FLOW RATE	50 ml min^{-1}
AIR FLOW RATE	300 ml min^{-1}
SAMPLE SIZE	0.1 l
SPLIT:INJECTION	1:100

All samples were run in duplicate. Peak area was

calculated on a Hewlett Packard model 3390A, on integrator mode. No correction factor was used and chart speed was 0.5 cm min^{-1}.

The capillary column used was a Carbowax 20 M. The column was 25 m long with internal diameter of 0,22 mm. Film thickness was 0.1 and maximum operating temperature was 250C.

4.9 GLC-MASS SPECTROMETER

GC-MS sampling was carried out, to confirm the identification of the major components of the oil, at the Phytochemistry Research Laboratory, Department of Pharmacy, Strathclyde University, using a Hewlett Packard model 5890/5988A connected to a 1000 E-series data processing system. The column was a Carbowax 20 M with column length of 25 m and width of 0.22 mm. The carrier gas was helium. Injector temperature was 170 C, initial ovem temperature was 50 C, rising in 5 C min^{-1} increments and final oven temperature was 170C.

4.10 STATISTICAL METHODS

Values used in height, length and breadth histograms and histograms of leaf length and breadth were calculated using the statistical package Minitab Release 6.1.1. Chi-squared, analysis of variance and

Student's t-tests were also carried out using this package. All other calculations were made using a hand held, scientific pocket calculator.

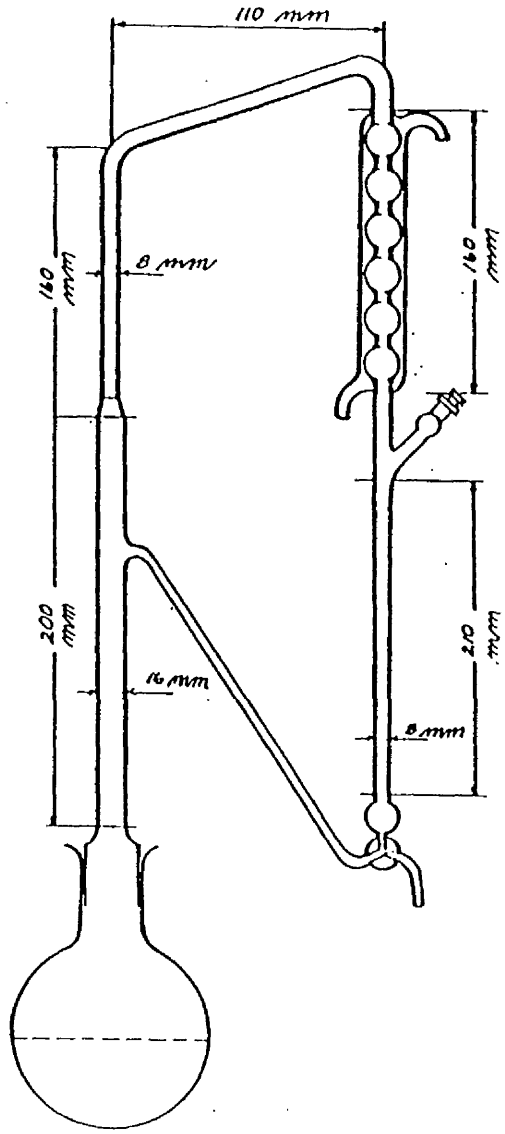


Fig. 14: European Pharmacopoeia distillation apparatus.

5. RESULTS

VARIATION IN PLANT CHARACTERISTICS (means with standard deviations presented on each figure)

5.1 PLANT SIZE (height, length (x dimension), breadth (y dimension)).

There was inter-plant and inter-plot variation in these characteristics which, in general, followed a slightly-skewed, normal distribution.

In the establishment year, 1988, the mean values for plots A - H (Figs. 15 - 22; 23 - 30; 31 - 38) were lowest in plot B (Figs. 16, 24, 32) and highest in plot F (Figs. 19, 27, 35).

There was no significant difference in plant height between the plots in this year (Figs. 15 - 22), but mean plant length and mean plant breadth were significantly lower in plots A and B than in plot F (Figs. 23, 24, 27; 31, 32, 35). There was no significant difference in these characteristics between the other plots (Figs. 25, 26, 28 - 30; 33, 34, 36 - 38).

Plots C and D were measured later in the year (Nov, 1988) than the other plots. Plots E - H were grown in Templefield (Figs. 12 & 13) and plots A - D in Diamondfield.

In 1989, plots E and G were not measured because of wind damage sustained during April of 1989 (Table

14). All other plants were measured at the same time (during June). Plants were, generally, larger in this year (Figs. 39 - 56) although not yet in physical contact with each other, with lowest mean values for height, length and breadth in plot C (Figs. 41, 47, 53) and highest in plot H (Figs. 44, 50, 56). Occasionally plants > 60cm across were found in 1989. This small number of exceptionally large plants were often found next to small plants or where plants were missing. Plot C was the most severely damaged by the wind in the spring of this year (Table 14) and plot H the least severely. There was, however, no significant difference in height (Figs. 39 - 44), length (Figs. 45 - 50) and breadth (Figs. 51 - 56) therefore no apparent difference due to plot site.

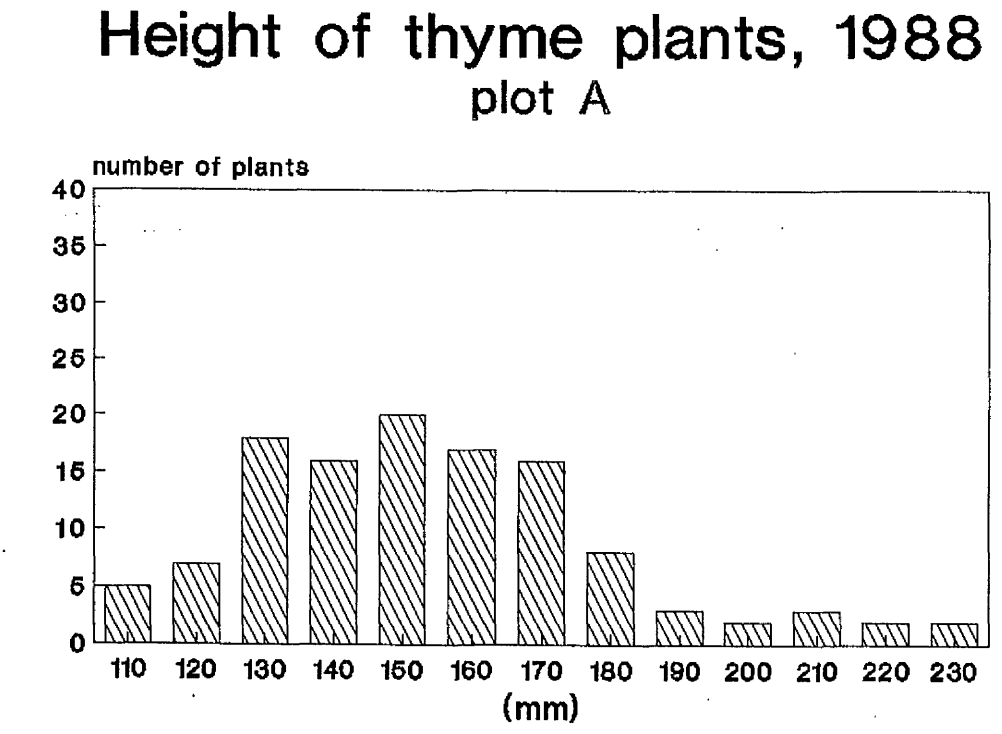

Height of thyme plants, 1988
plot A
number of plants
40
35
30
25
20
15
10
5
0
110 120 130 140 150 160 170 180 190 200 210 220 230
(mm)
mean = 151.6; SD = 25.8

Fig. 15

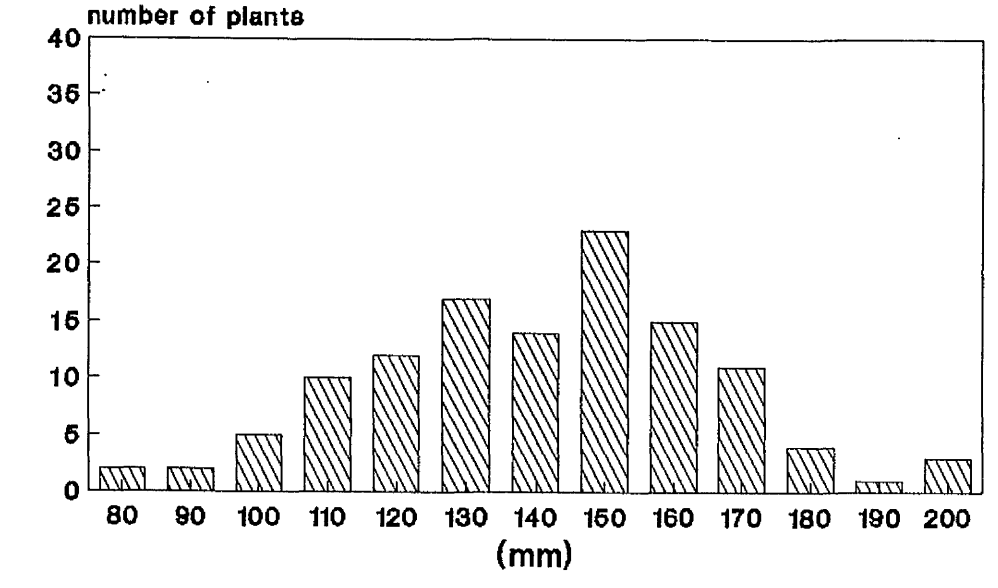
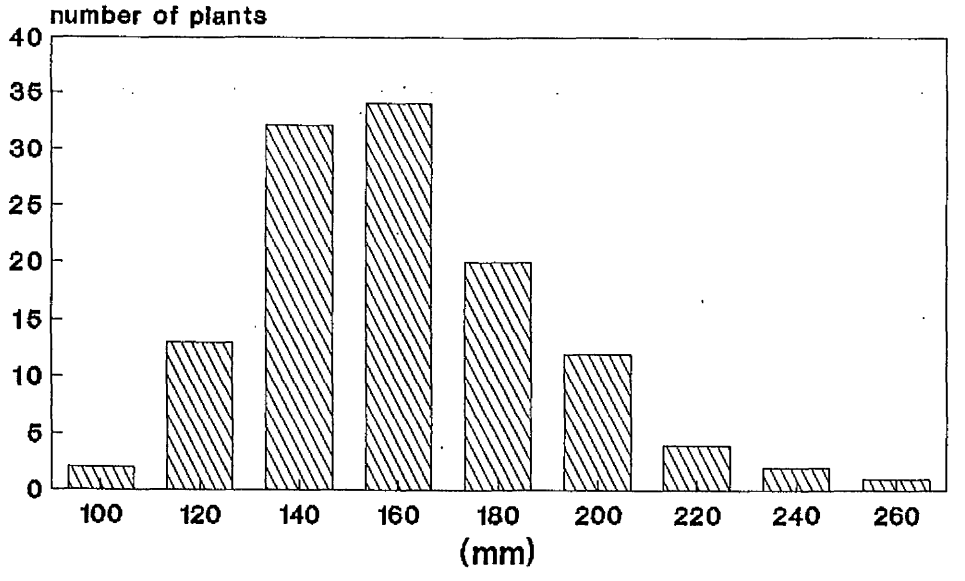

plot B
number of plants
40
35
30
25
20
15
10
5
0
80 90 100 110 120 130 140 150 160 170 180 190 200
(mm)
mean = 138.5; SD = 25.1

Fig. 16

Height of thyme plants, 1988

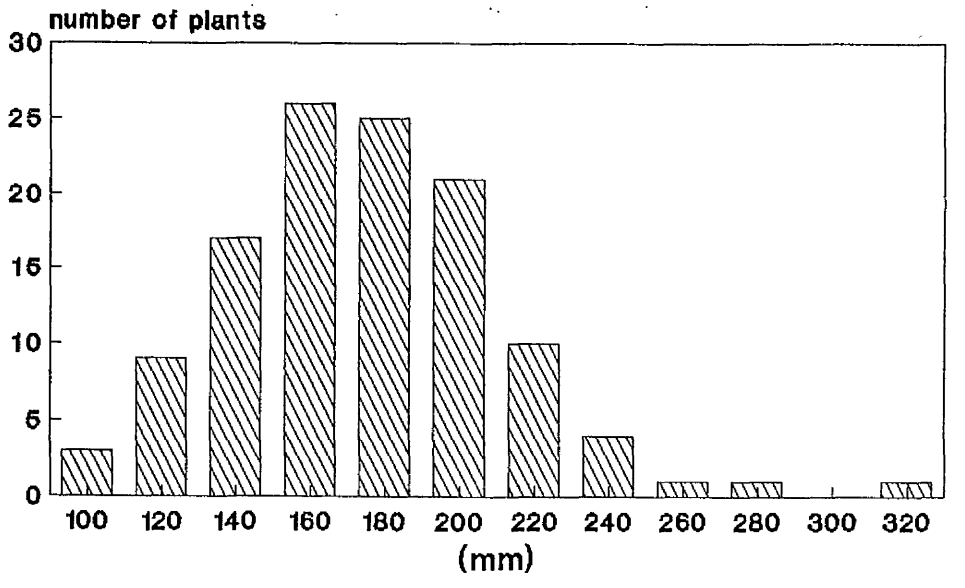
plot C



mean = 157.67; SD = 28.6

Fig. 17

plot D

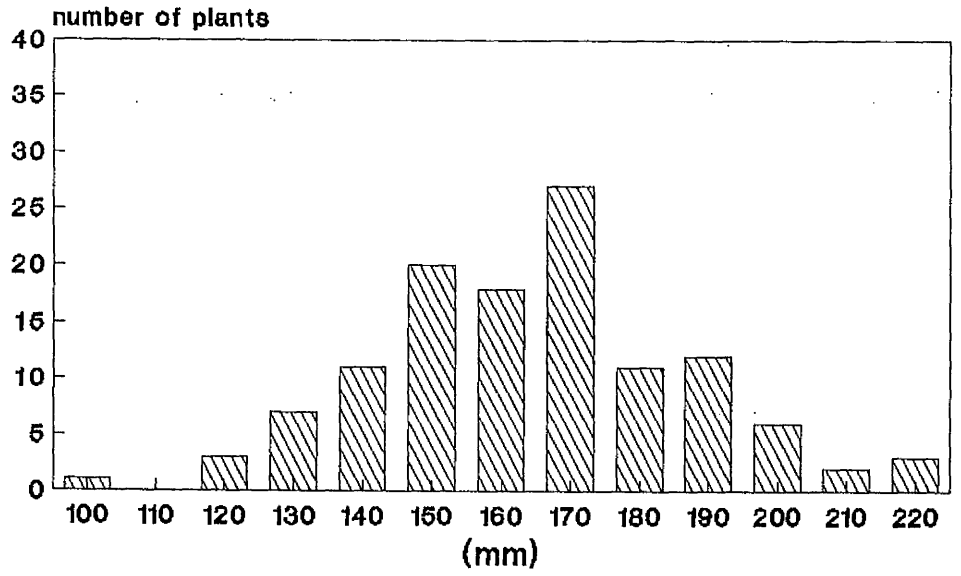


mwn = 173.09; SD = 36.54

Fig. 18

Height of thyme plants, 1988

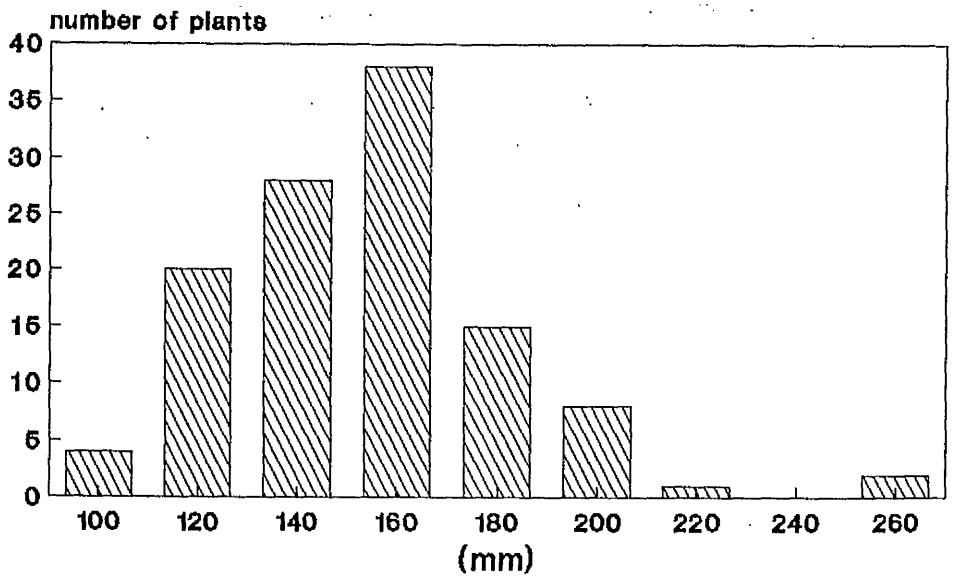
plot F



mean = 162.0; SD = 22.9

Fig. 19

plot H

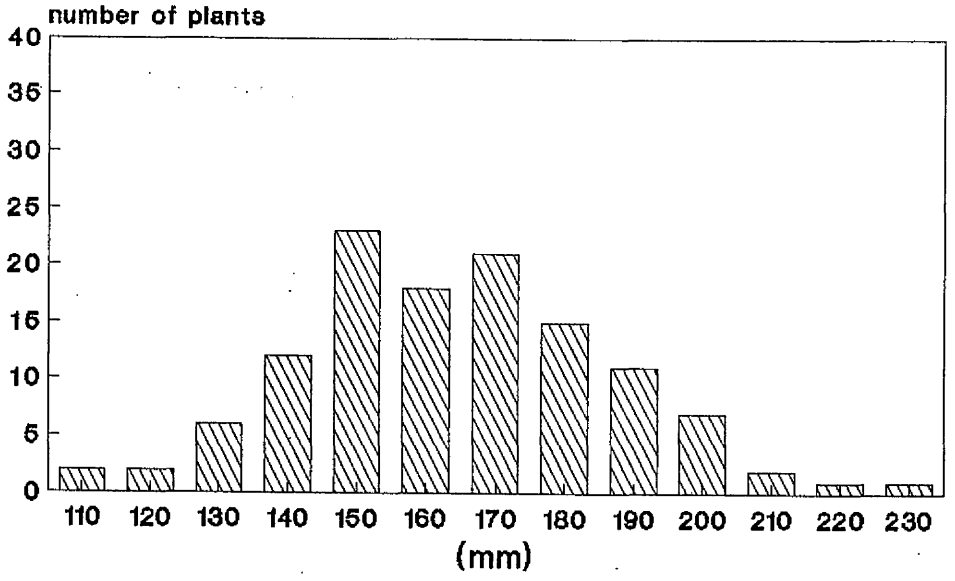


mean = 150.8; SD = 29.1

Fig. 20

Height of thyme plants, 1988

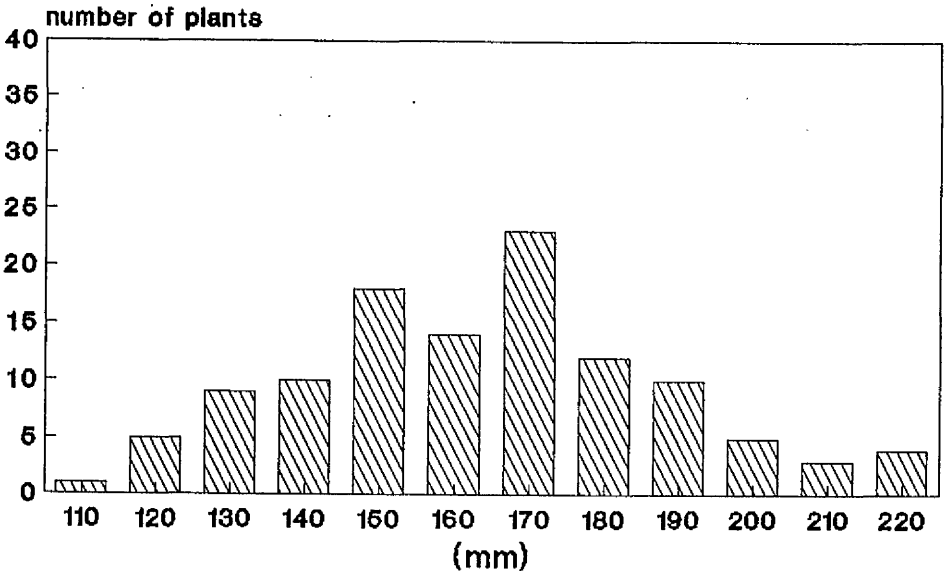
plot E



mean = 161.4; SD = 22.3

Fig. 21

plot G



mean = 161.2; SD = 24.7

Fig. 22

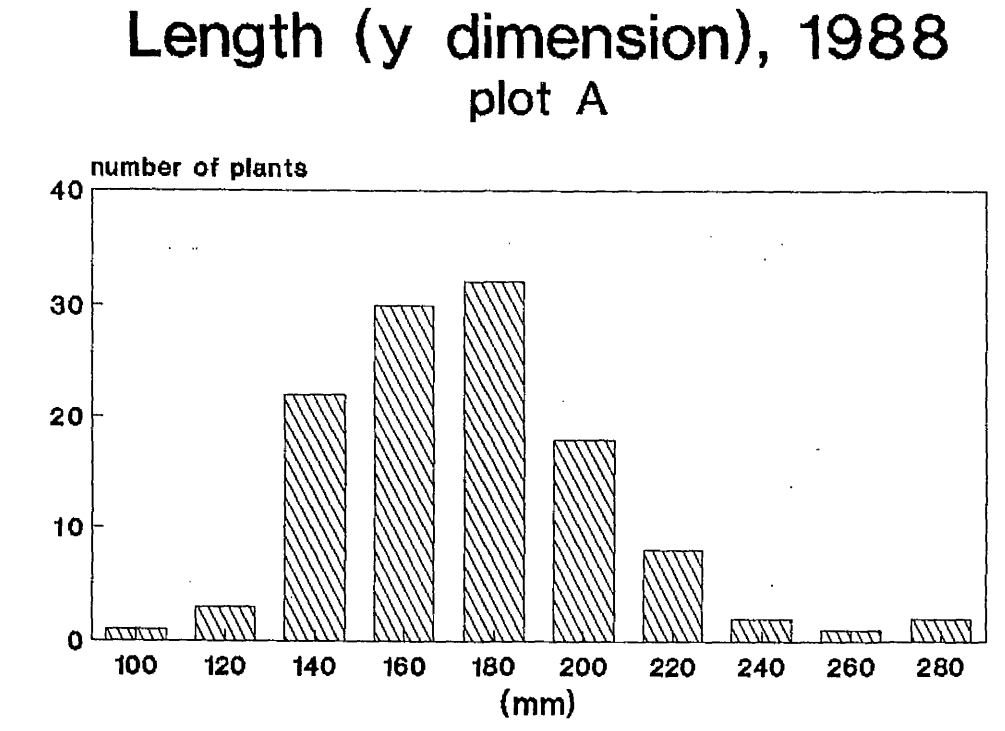

Length (y dimension), 1988
plot A
number of plants
40
30
20
10
0
100
120
140
160
180
200
220
240
260
280
(mm)
mean = 172.2; SD = 30.7

Fig. 23

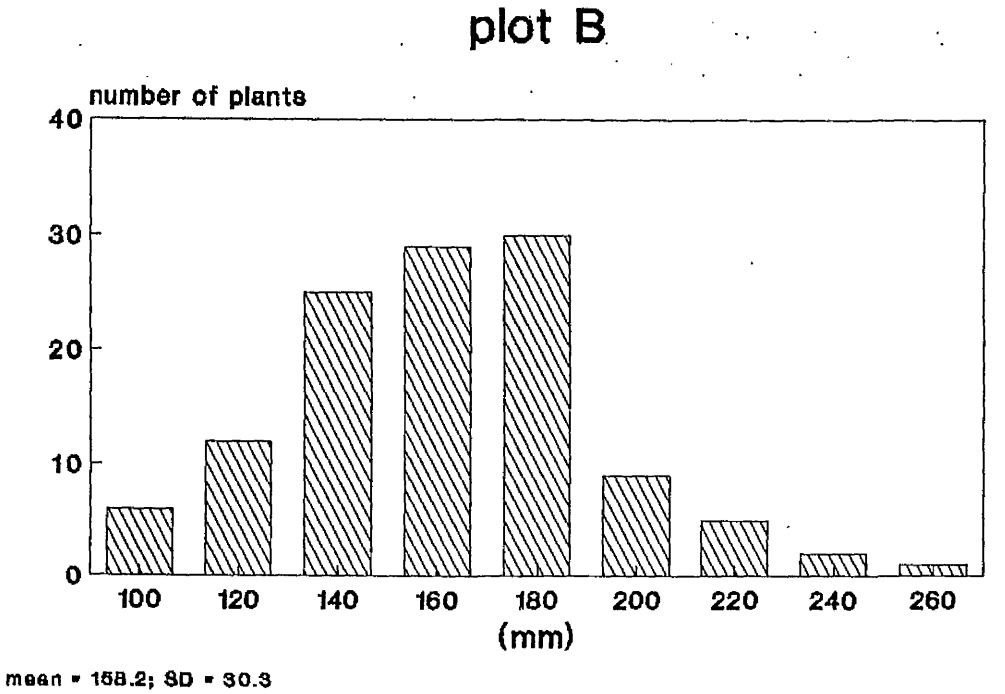
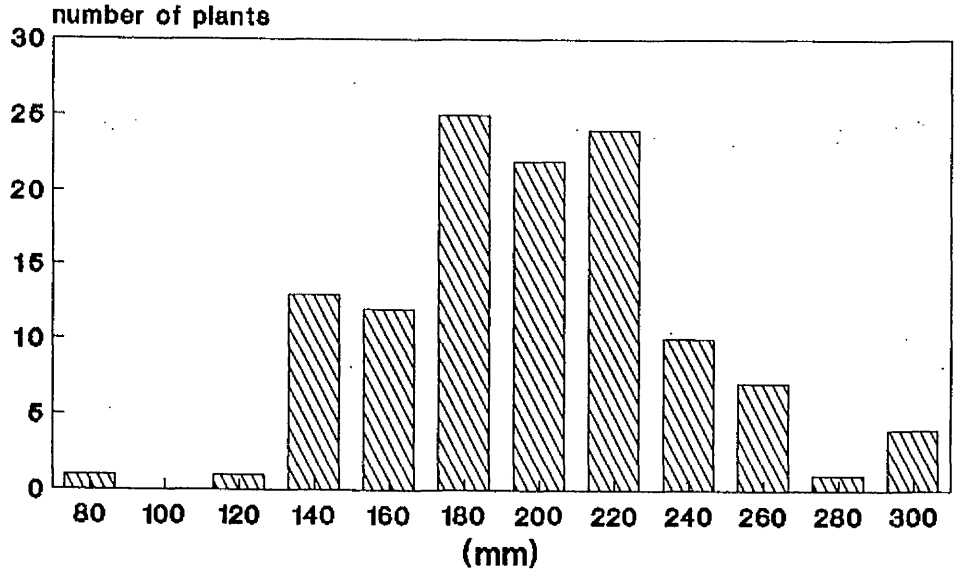

plot B
number of plants
40
30
20
10
0
100
120
140
160
180
200
220
240
260
(mm)
mean = 158.2; SD = 30.3

Fig. 24

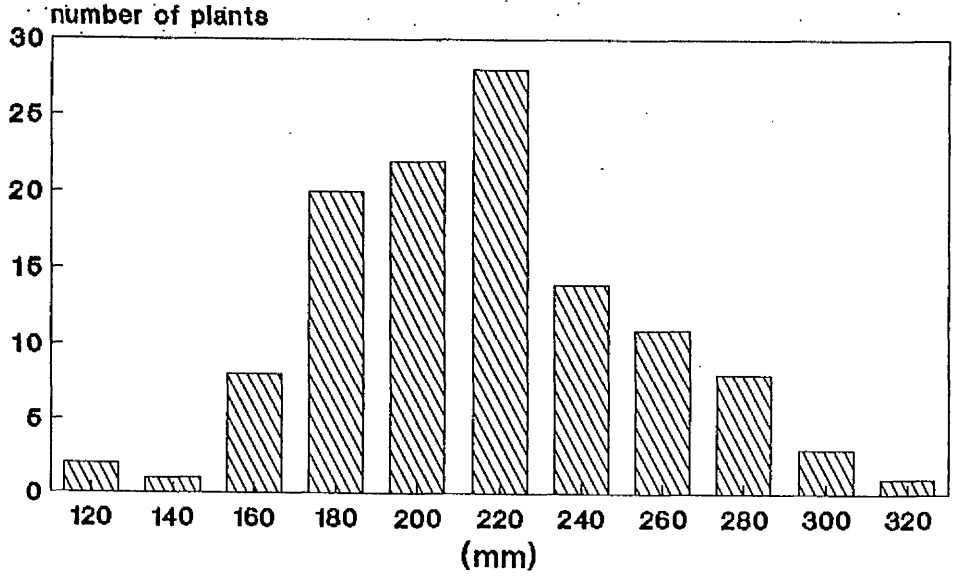
Length (y dimension), 1988
plot C



mean = 194.92; SD = 39.51

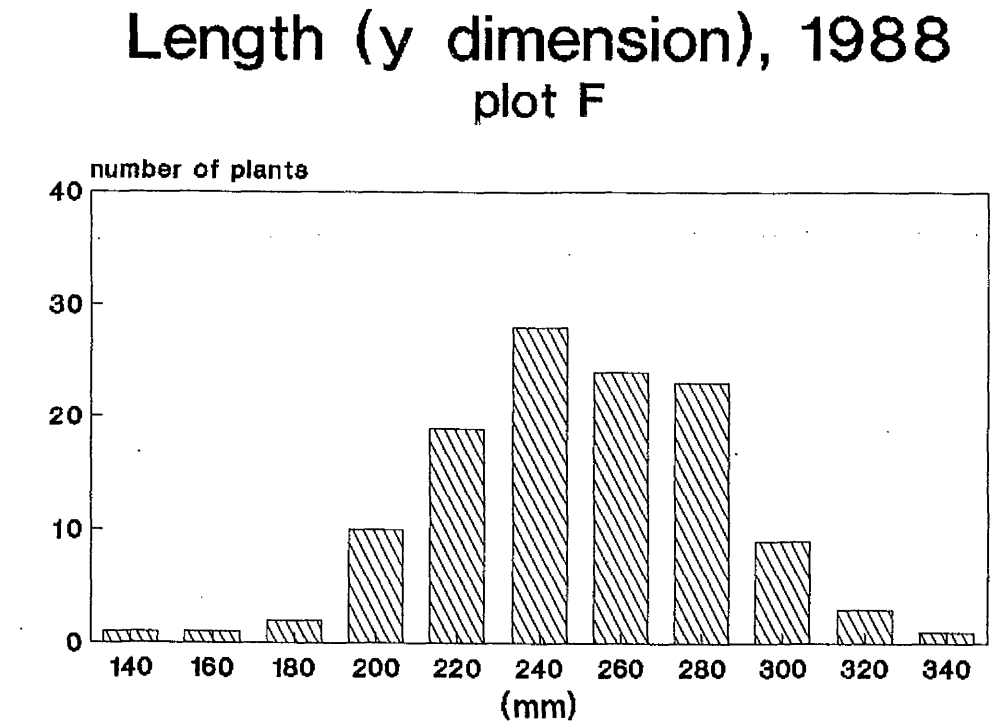
Fig. 25

plot D



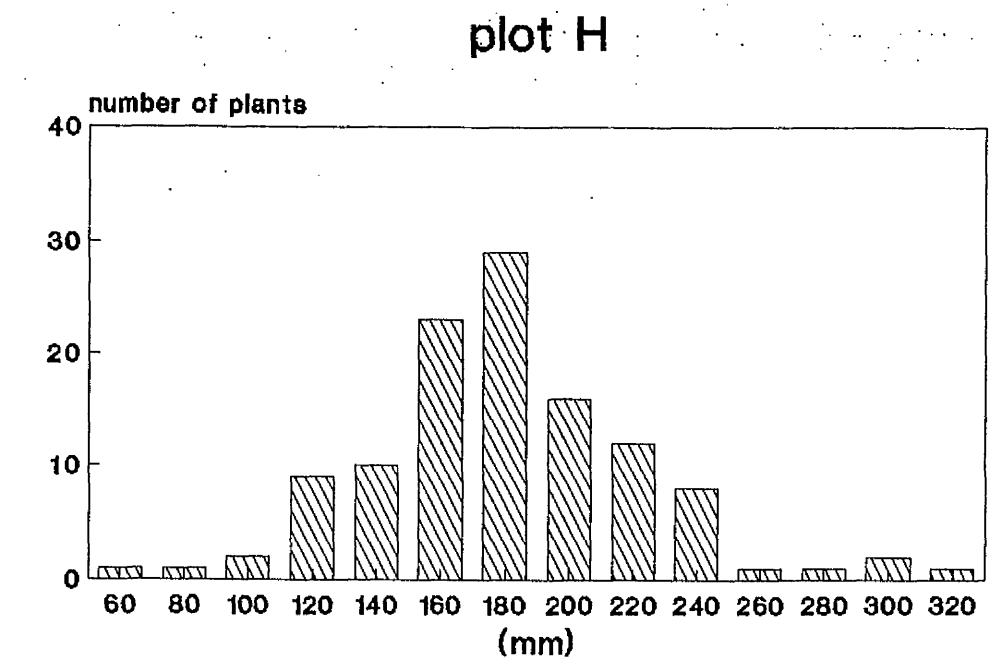
mean = 213.39; SD = 38.34

Fig. 26



mean = 244.7; SD = 34.7

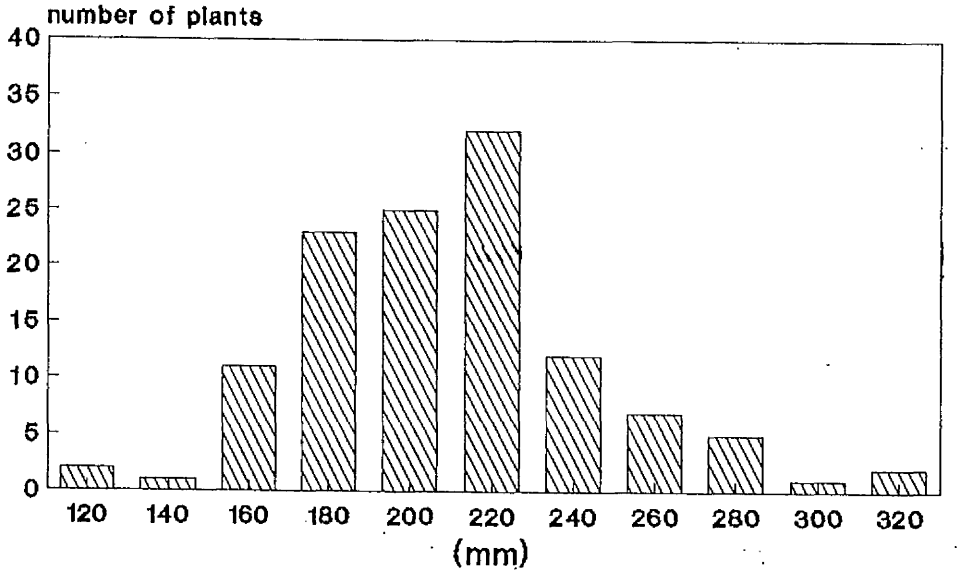
Fig. 27



mean = 178.2; SD = 42.5

Fig. 28

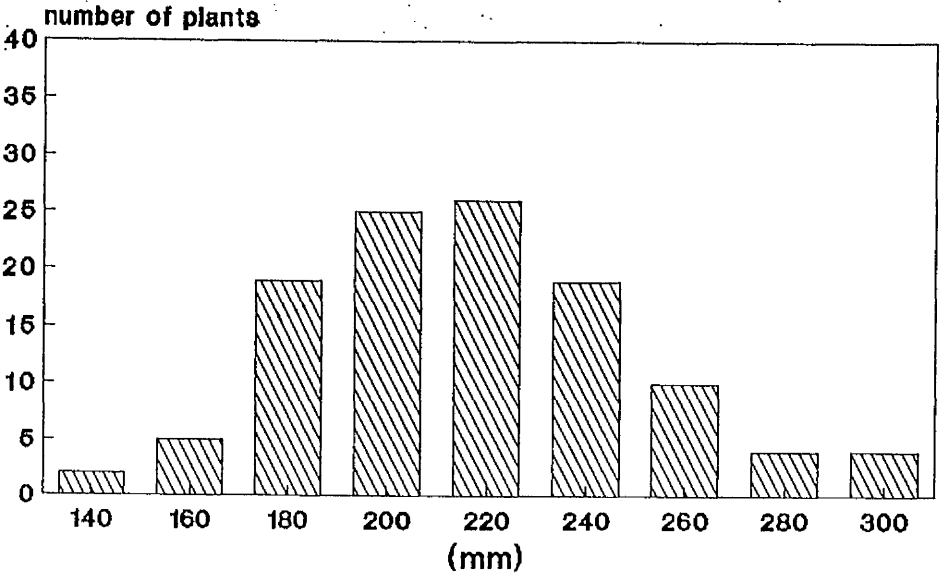
Length (y dimension), 1988
plot E



mean = 206.1; SD = 36.6

Fig. 29

plot G

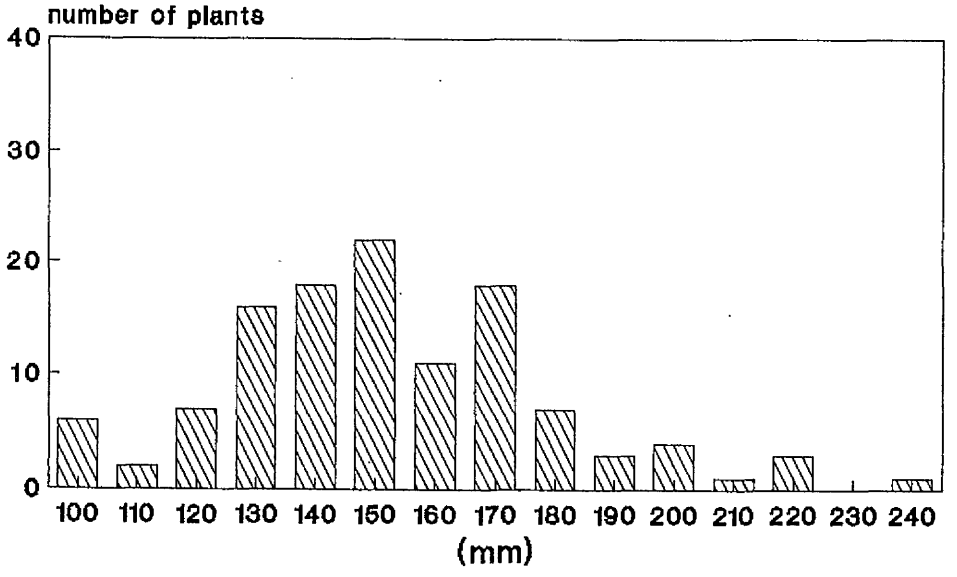


mean = 211.8; SD = 33.6

Fig. 30

Breadth (x dimension), 1988

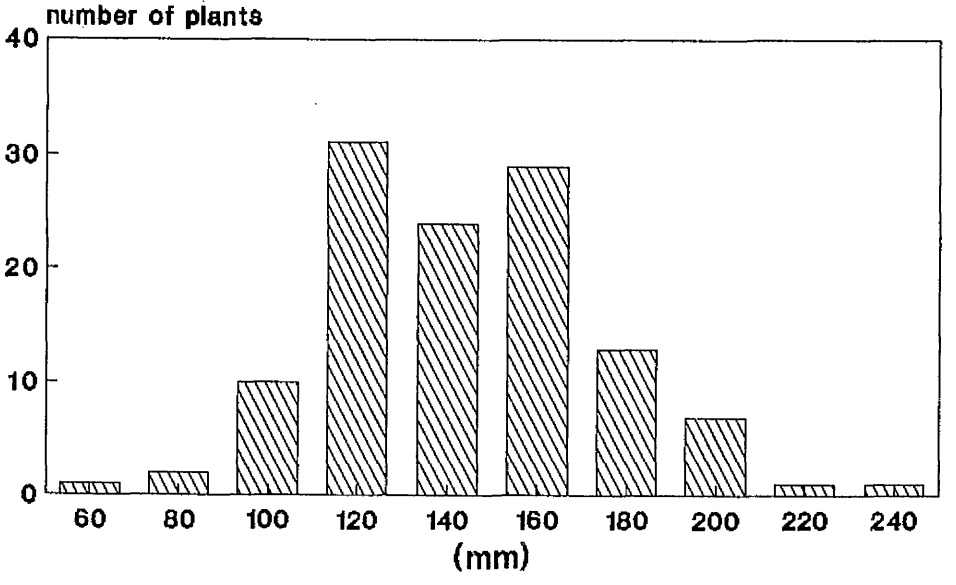
plot A



mean = 150.5; SD = 27.2

Fig. 31

plot B



mean = 140.6; SD = 29.9

Fig. 32

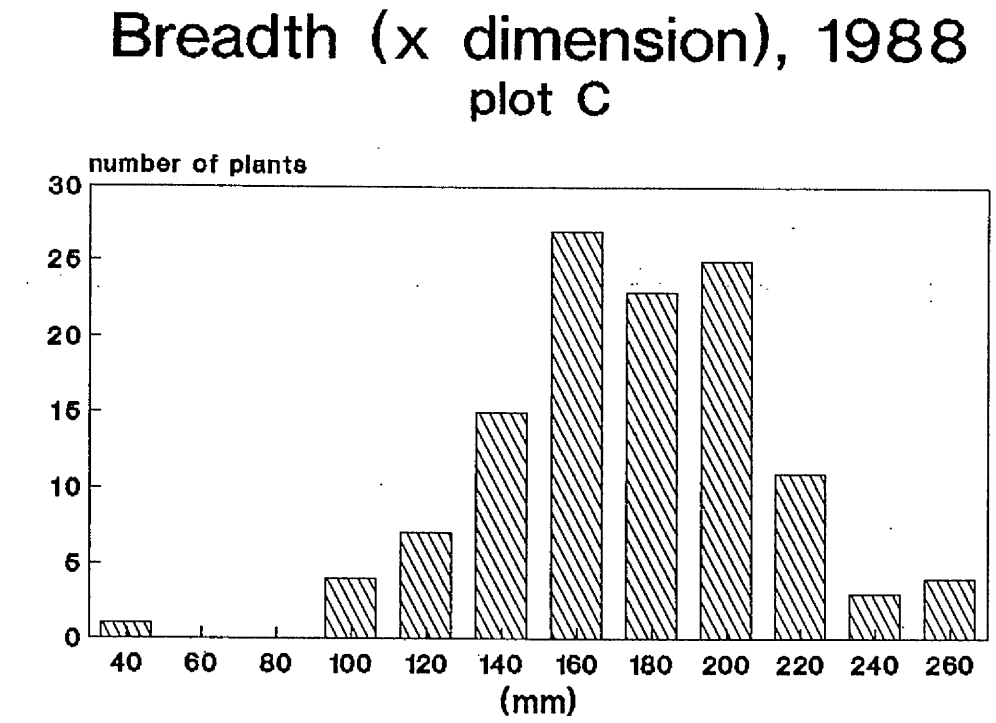

Breadth (x dimension), 1988
plot C
number of plants
30
25
20
15
10
5
0
40 60 80 100 120 140 160 180 200 220 240 260
(mm)
mean = 170.83; SD = 36.44

Fig. 33

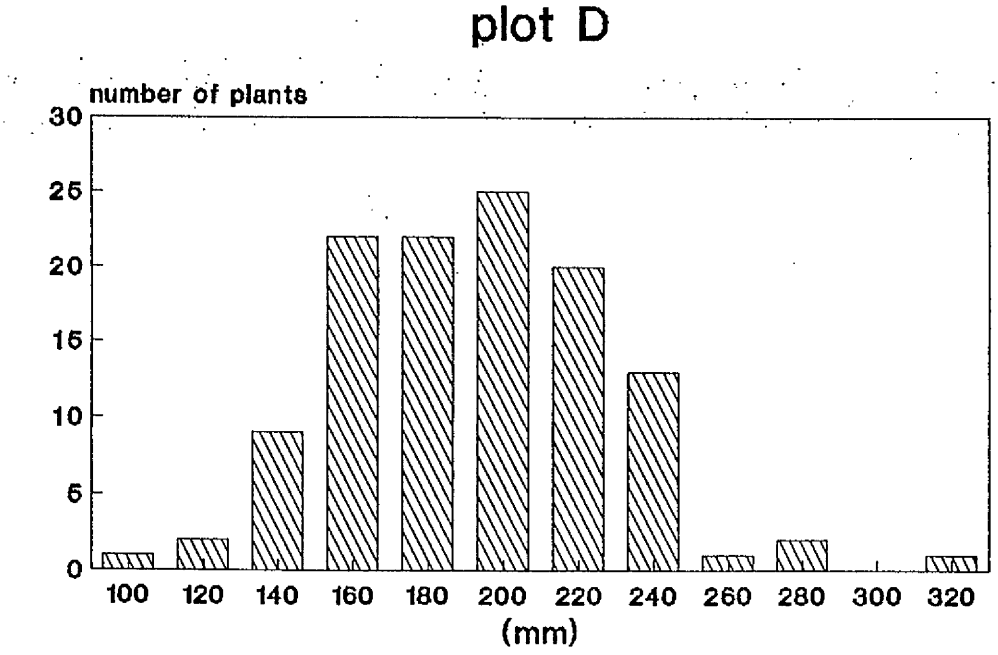
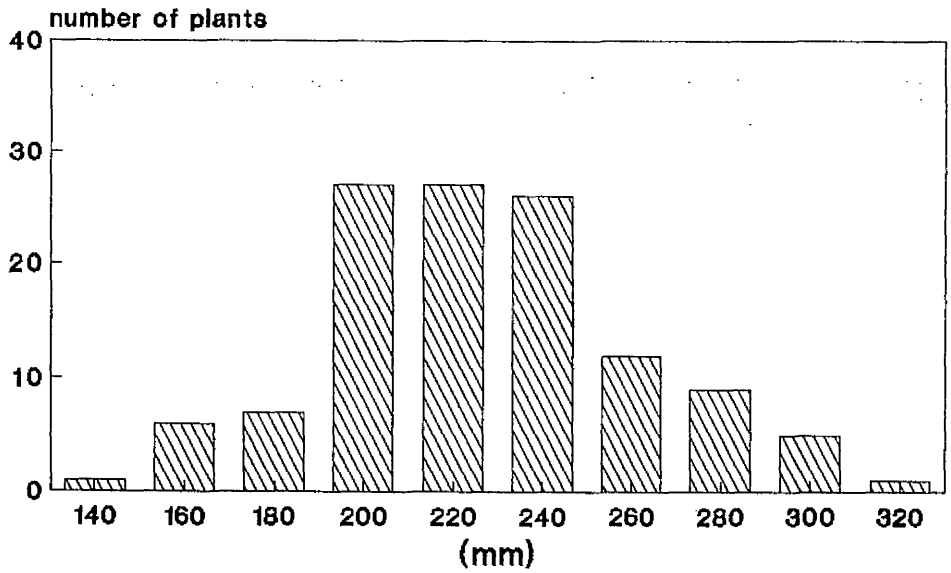

plot D
number of plants
30
25
20
15
10
5
0
100 120 140 160 180 200 220 240 260 280 300 320
(mm)
mean = 189.62; SD = 35.23

Fig. 34

Breadth (x dimension), 1988

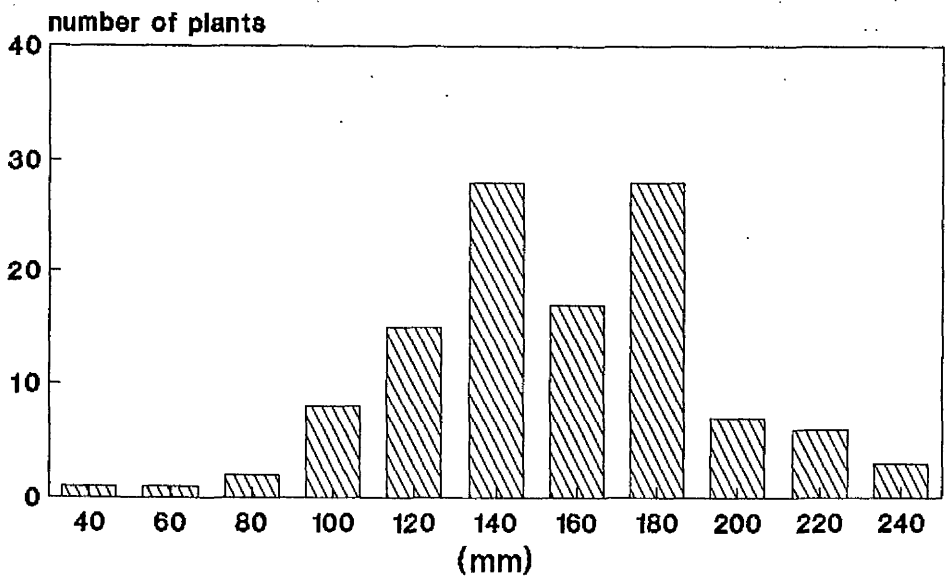
plot F



mean = 221.5; SD = 34.1

Fig. 35

plot H

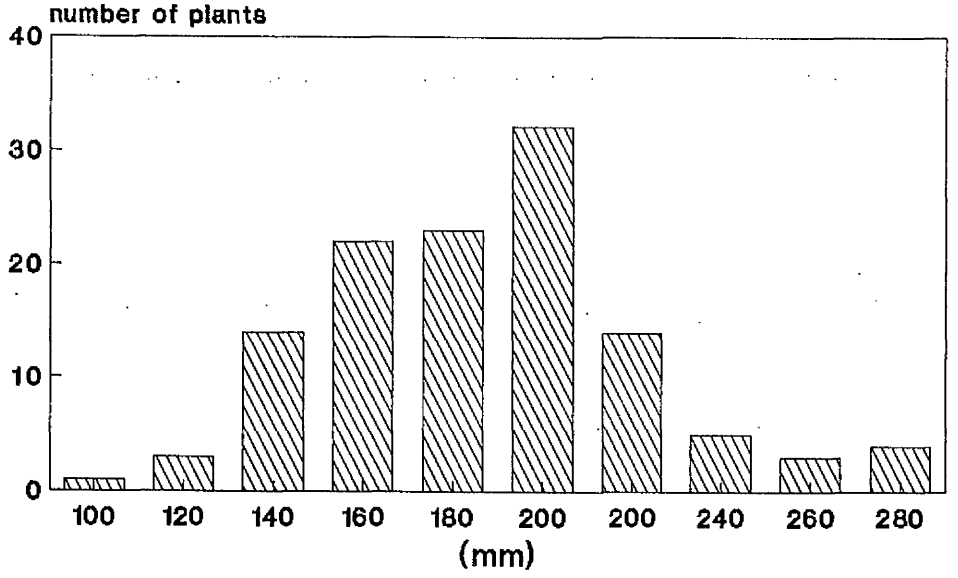


mean = 152.4; SD = 35.6

Fig. 36

Breadth (x dimension), 1988

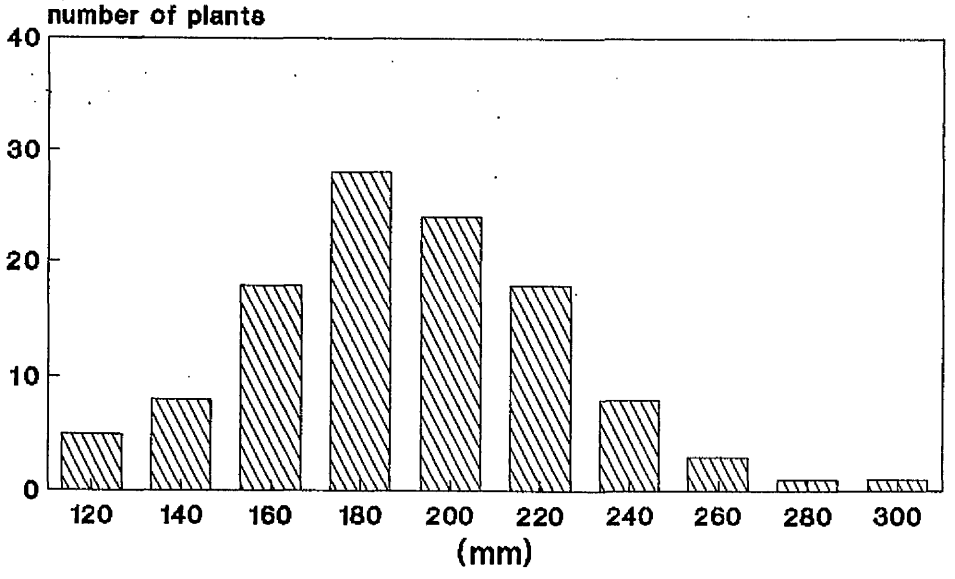
plot E



mean = 183.8; SD = 35.0

Fig. 37

plot G

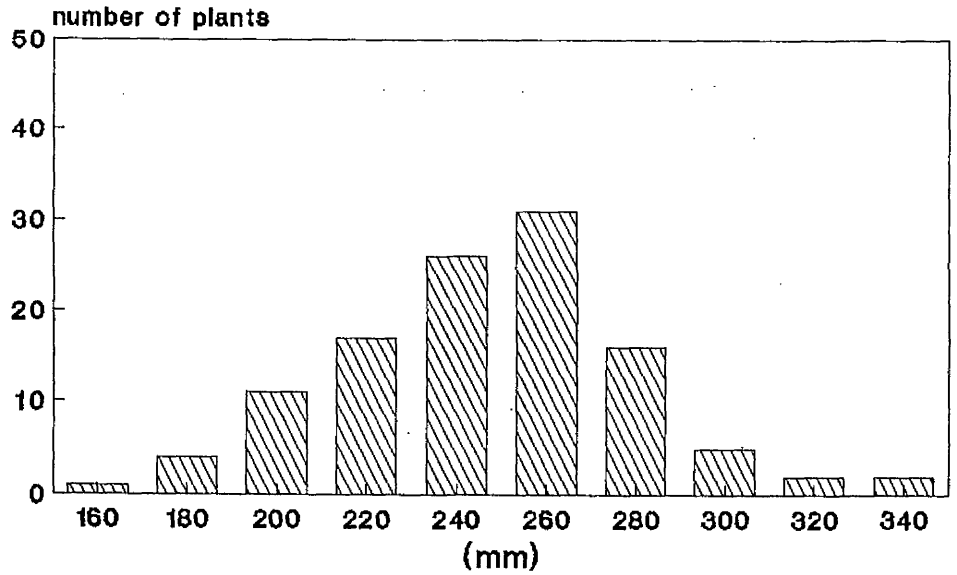


mean = 185.1; SD = 33.9

Fig. 38

Height of thyme plants, 1989

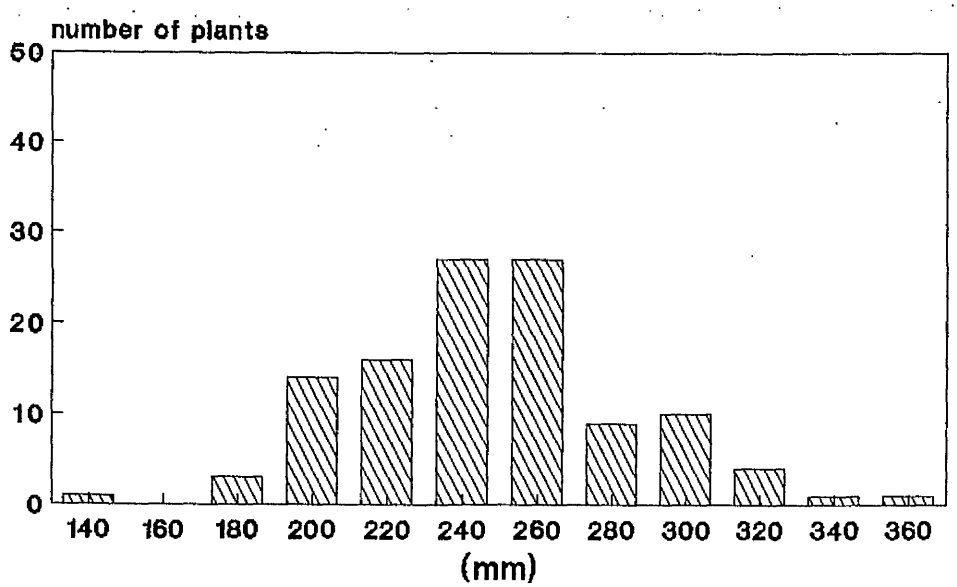
plot A



mean = 243.1; SD = 33.3

Fig. 39

plot B

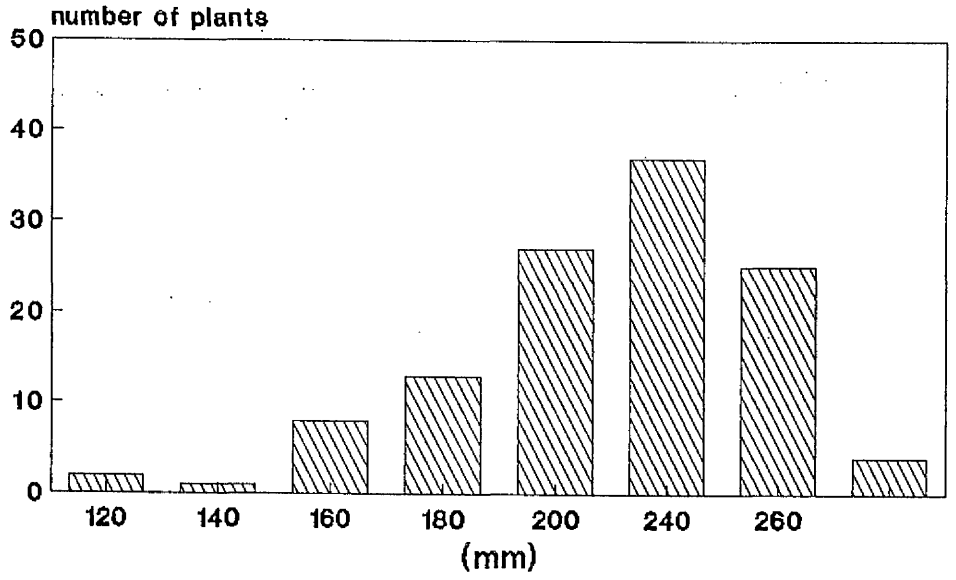


mean = 244.4; SD = 37.6

Fig. 40

Height of thyme plants, 1989

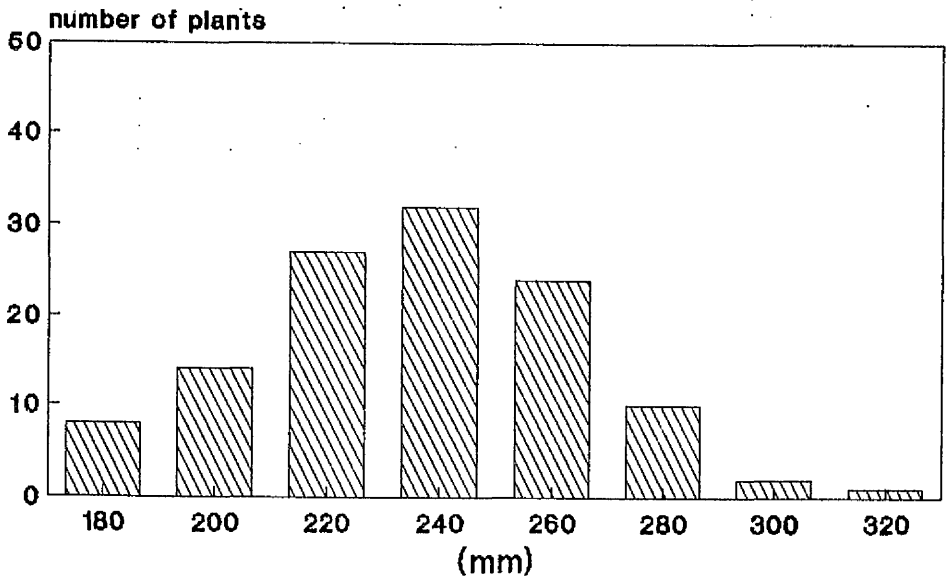
plot C



mean = 206.8; SD = 28.1

Fig. 41

plot D

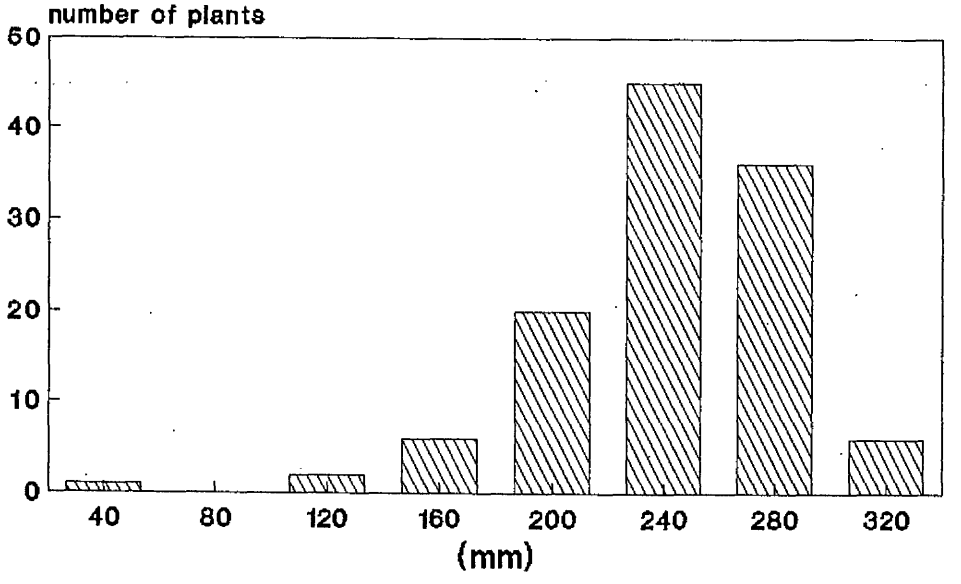


mean = 232.8; SD = 29.2

Fig. 42

Height of thyme plants, 1989

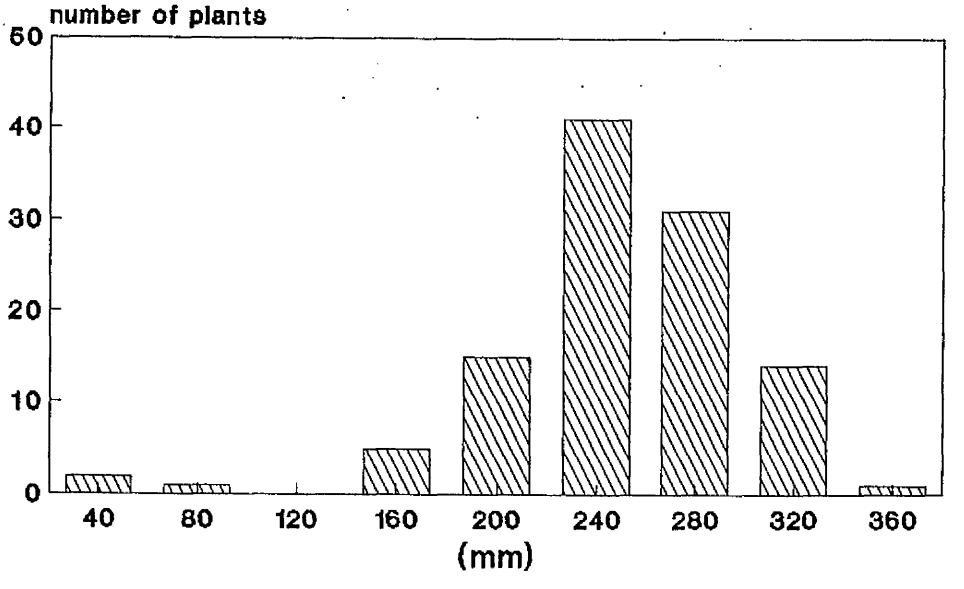
plot F



mean = 236.8; SD = 44.6

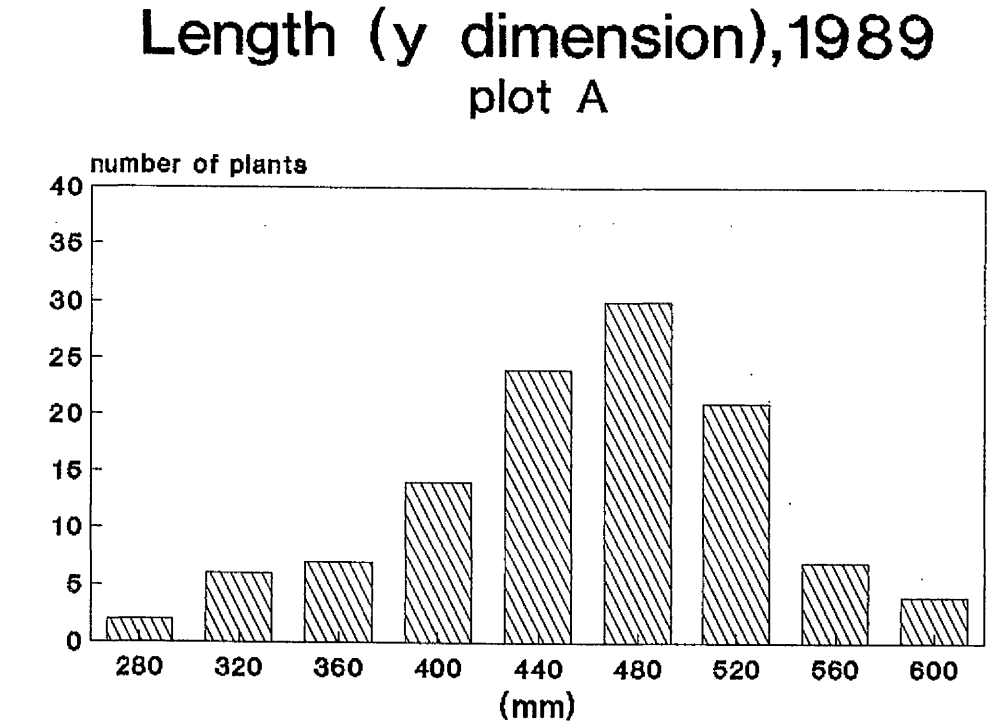
Fig. 43

plot H



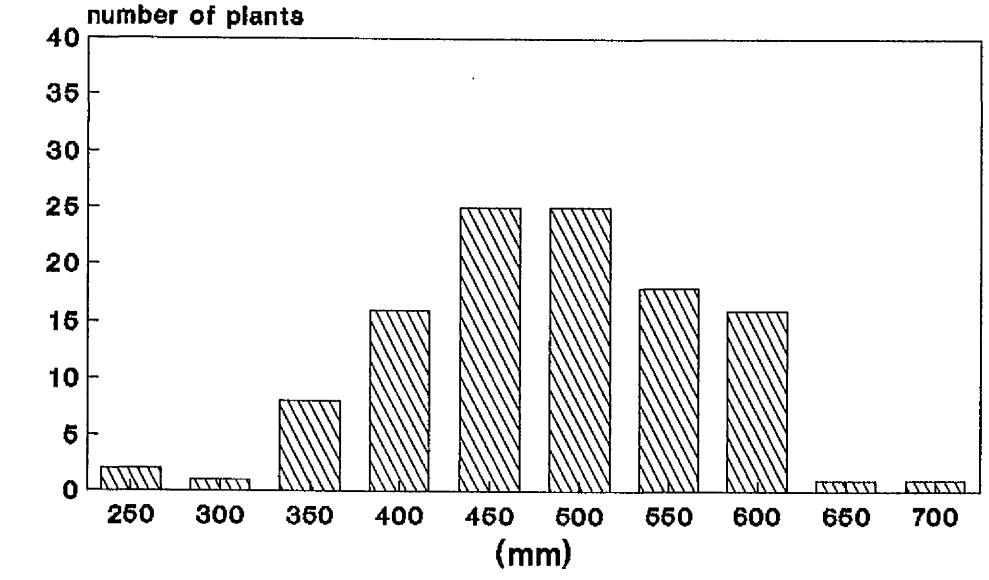
mean = 244.5; SD = 51.4

Fig. 44



mean = 454.1; SD = 67.6

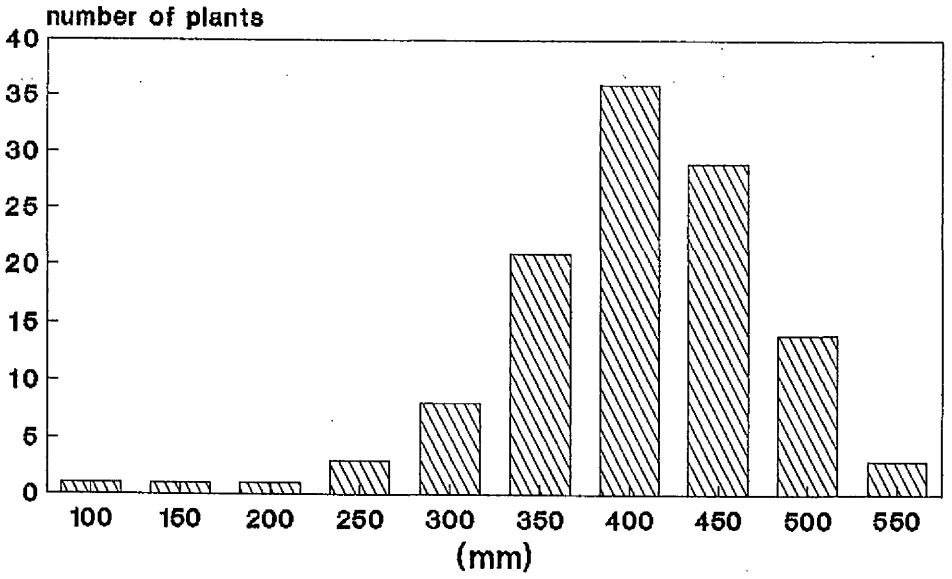
Fig. 45



mean = 482.8; SD = 80.3

Fig. 46

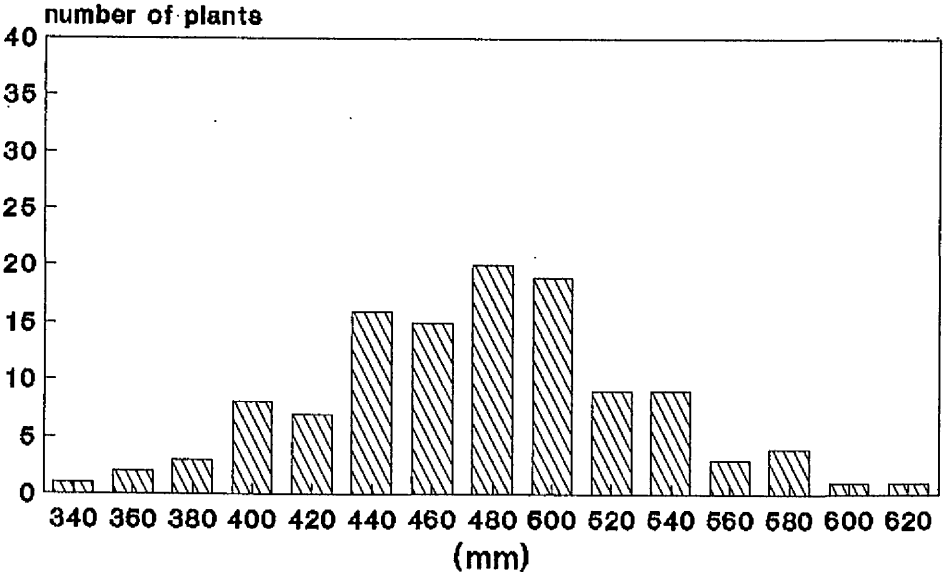
Length (y dimension), 1989
plot C



mean = 403.3; SD = 76.0

Fig. 47

plot D

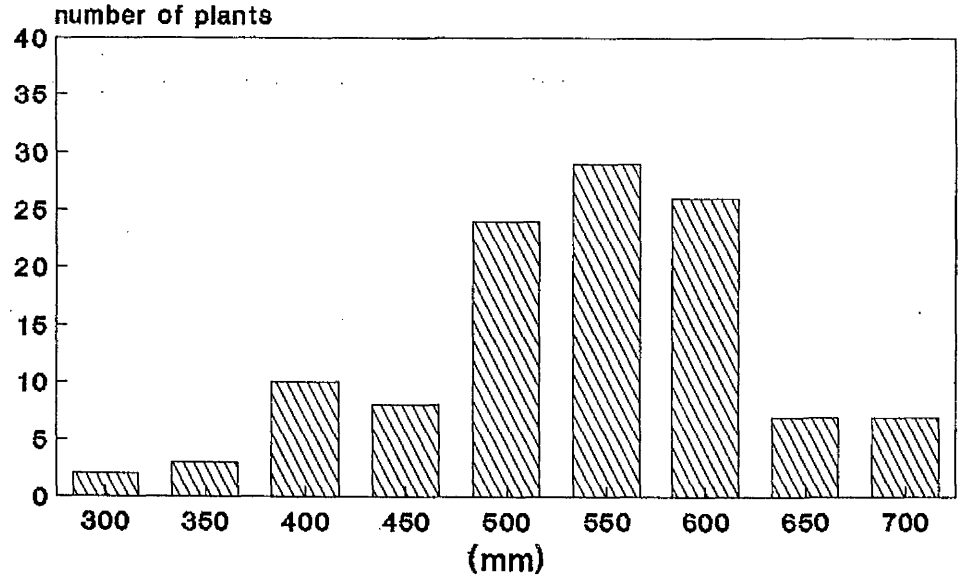


mean = 470.9; SD = 53.5

Fig. 48

Length (y dimension), 1989

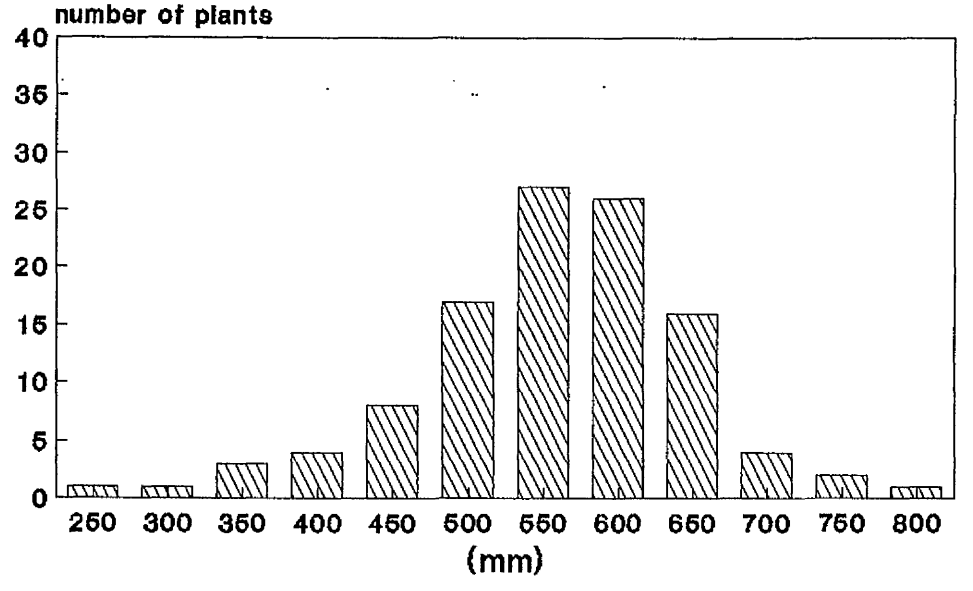
plot F



mean = 537.4; SD = 86.1

Fig. 49

plot H

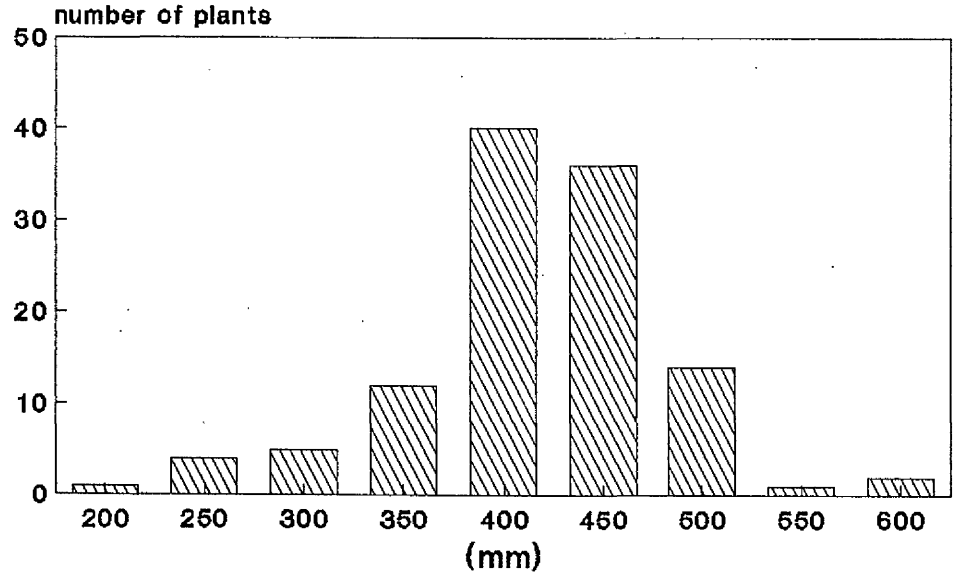


mean = 557.0; SD = 94.0

Fig. 50

Breadth (x dimension), 1989

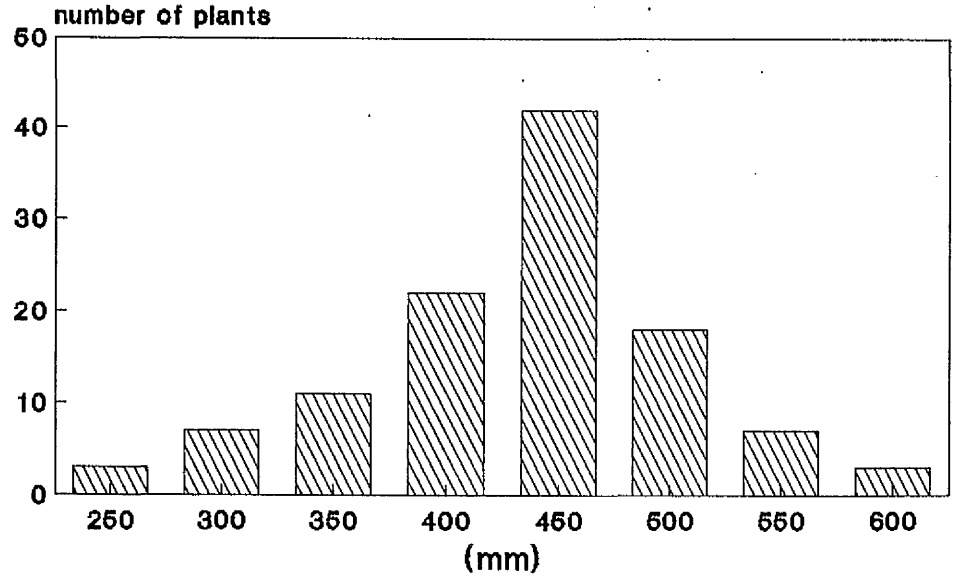
plot A



mean = 415.7; SD = 65.3

Fig. 51

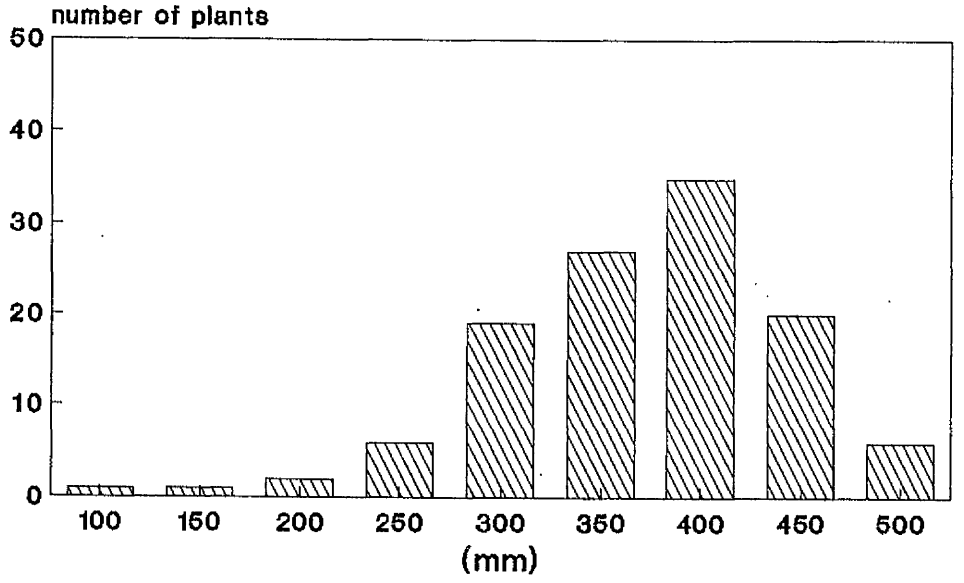
plot B



mean = 433.1; SD = 72.9

Fig. 52

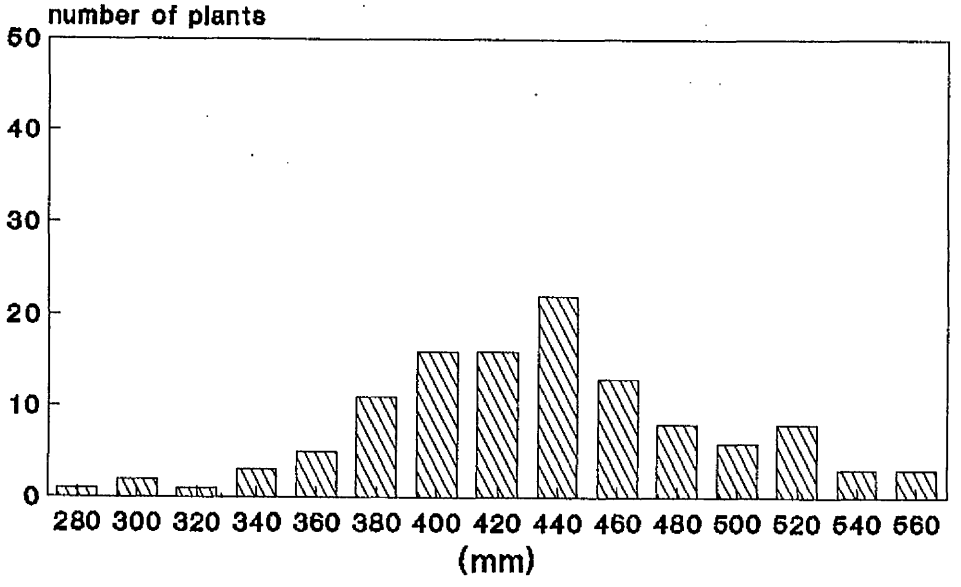
Breadth (x dimension), 1989
plot C



mean = 368.3; SD = 74.6

Fig. 53

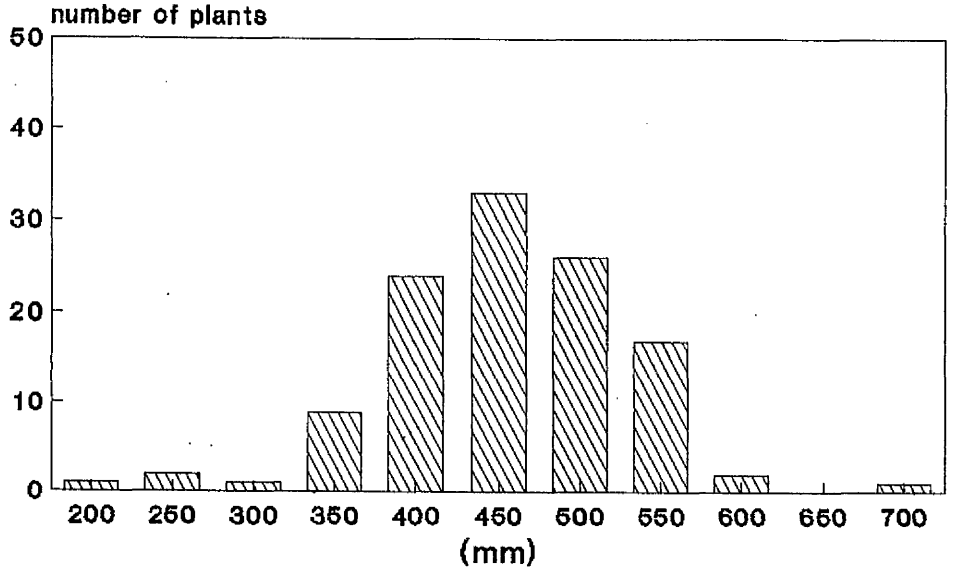
plot D



mean = 429.7; SD = 56.5

Fig. 54

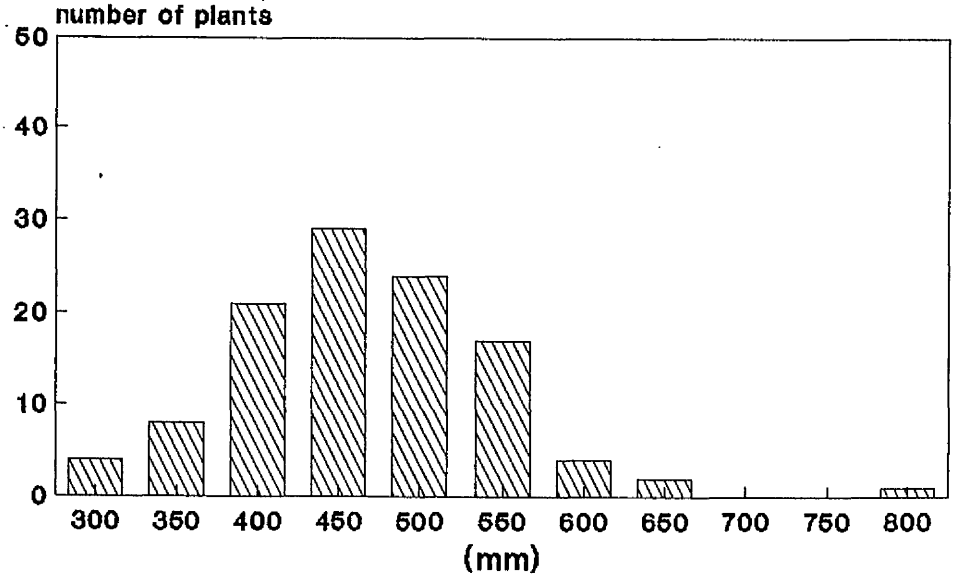
Breadth (x dimension), 1989
plot F



mean = 458.2; SD = 75.7

Fig. 55

plot H



mean = 465.2; SD = 80.3

Fig. 56

5.2 THYME FLOWER MEASUREMENTS

Tables 15 a-c show the difference in mean stamen length between hermaphrodite and intermediate flowers and the difference in flower length (longest petal) between hermaphrodite and intermediate or male sterile flowers. Flowering stems were selected at random and 5 - 7 flowers from each stem were measured.

The mean values of flower length were greatest in the hermaphrodite plants. There was little difference in this characteristic between the intermediate and male sterile flowers. Student's t-tests were carried out, on the first 12 values of flower length for each reproductive type and confirmed that, although the difference in mean petal length was significant ($p < 0.05$) between both hermaphrodite and intermediate and hermaphrodite and male sterile flowers, there was no significant difference between the intermediate and the male sterile flowers.

It was also confirmed, using the same test, that stamen length in hermaphrodite and intermediate flowers was significantly different ($p < 0.05$).

Table 15a: Measurements, in mm, of some of the dimensions of hermaphrodite thyme flowers.

Plant identity	Mean value (with S.D.)		
	Longest petal	Style length	Stamen length
A37	7.8 (0.6)	7.8 (1.1)	6.6 (0.6)
A45	7.4 (0.4)	6.0 (1.0)	6.3 (0.6)
A72	7.8 (0.2)	7.0 (1.1)	6.5 (0.6)
A87	7.9 (0.6)	6.3 (1.8)	6.6 (0.7)
B29	9.3 (0.5)	7.1 (2.4)	7.5 (0.5)
B40	8.3 (0.4)	5.5 (1.3)	6.6 (0.6)
B56	9.3 (0.4)	6.6 (1.8)	8.0 (0.5)
B63	7.7 (0.2)	7.5 (1.1)	6.6 (0.5)
B116	8.5 (1.2)	7.9 (1.1)	5.9 (1.8)
C58	7.3 (0.7)	7.3 (1.2)	6.6 (0.7)
C89	8.4 (0.4)	7.2 (2.0)	7.4 (0.8)
C119	8.6 (0.4)	6.5 (1.1)	7.4 (0.6)
D43	7.5 (0)	7.6 (0.9)	6.6 (0.6)
D72	7.1 (0.7)	6.6 (0.8)	6.6 (0.4)
D95	8.3 (0.5)	8.4 (1.6)	6.9 (0.7)
D10	7.1 (0.5)	5.7 (1.3)	6.1 (0.7)
$\bar{x}$	8.0	6.9	6.8
SD	0.7	0.8	0.6

Some of the variability in style length seen in the individual standard deviations of hermaphrodite flowers may be due to protandry, where the style is immature in newly opened flowers.

Table 15b: Measurements, in mm, of some of the dimensions of intermediate thyme flowers.

Plant identity	Mean value (with S.D.)		
	Longest petal	Style length	Stamen length
A10	4.8 (0.2)	5.6 (0.5)	2.8 (0.3)
A61	6.1 (0.5)	5.8 (0.6)	3.4 (0.4)
B67	5.0 (0.2)	6.8 (0.3)	2.7 (0.3)
B87	6.9 (1.1)	6.8 (0.8)	3.6 (0.5)
C27	7.3 (0.5)	7.1 (0.5)	3.9 (0.3)
C70	5.9 (0.3)	6.2 (0.6)	2.9 (0.3)
C87	6.6 (0.4)	5.8 (0.6)	4.1 (0.6)
C99	6.6 (0.2)	7.3 (0.4)	3.8 (0.4)
D5	5.2 (0.3)	5.1 (0.3)	3.2 (0.3)
D48	5.5 (0.3)	6.8 (1.2)	2.8 (0.4)
D49	6.2 (0.2)	5.8 (0.5)	3.0 (0.1)
D98	6.5 (0.4)	6.6 (0.8)	3.4 (0.3)
x	6.1	6.3	3.3
SD	0.8	0.7	0.5

Table 15c: Measurements, in mm, of some of the dimensions of male sterile thyme flowers.

Plant identity	Mean value (with S.D.)		
	Longest petal	Style length	Stamen length
A27	5.8 (0.5)	5.7 (0.5)	-
A29	5.8 (0.3)	6.2 (0.8)	-
A47	5.9 (0.3)	6.4 (0.4)	-
A107	4.1 (0.2)	4.7 (0.2)	-
A116	5.3 (0.2)	5.6 (0.8)	-
B10	6.8 (0.6)	7.0 (0.6)	-
B16	6.0 (0.5)	6.3 (0.3)	-
B27	6.2 (1.1)	6.4 (0.7)	-
B37	5.6 (0.3)	5.8 (0.6)	-
B45	6.5 (0.7)	6.9 (0.9)	-
B61	6.7 (0.3)	6.9 (0.7)	-
B101	5.5 (0.5)	6.2 (0.4)	-
C9	5.9 (0.1)	5.8 (0.5)	-
C32	5.9 (0.3)	5.9 (1.0)	-
D39	5.0 (0)	5.0 (0)	-
D91	5.9 (0.2)	5.4 (0.3)	-
D111	6.6 (0.3)	6.2 (0.3)	-
x	5.9	6.0	-
SD	0.7	0.6	-

5.3 INTERNODE DISTANCES IN THYME STEMS

Measurements in Tables 16, 17 and Figure 57 were designed to help explain variation in the overall size of thyme plants. They showed that there was considerable variation between the plants of a single lot (plot H) and between lots. These differences are clearly illustrated in Fig. 57, showing a considerable variation in the habit of thyme stems.

Stems were selected, randomly, from throughout each plant but, in each case, the relative similarity of stems within a plant was noted.

Table 16: Length (mm) of internodes of randomly selected stems of plants in plot H (plant codes H1 ... H39) 1989. Single stems from each plant were used.

	Plant number							
Inter-node	1	3	6	12	25	30	37	39
3-4	1.5	1.5	2.0	1.0	2.0	1.0	2.0	1.5
4-5	2.5	3.5	4.0	2.0	6.0	2.0	5.0	5.0
5-6	5.75	7.0	9.0	3.5	14.0	3.5	12.0	9.5
6-7	13.0	14.0	15.0	11.0	19.0	7.0	25.0	21.0
7-8	14.5	17.0	21.0	21.0	27.0	12.0	29.0	27.5
8-9	10.5	19.5	21.0	22.5	34.0	17.0	31.0	27.0
Total*	47.8	62.5	72.0	61.0	102.0	42.5	104.0	91.5

* = the total distance from node 3 to node 9 (nodes counted from the tip of the stem).

The measurements were made during Sept/Oct, 1989.

Table 17: Lengths (mm) of internodes of randomly-selected stems from 5 plots (B, C, D, F, H). Each result is the mean of more than one stem, with standard deviations given in brackets

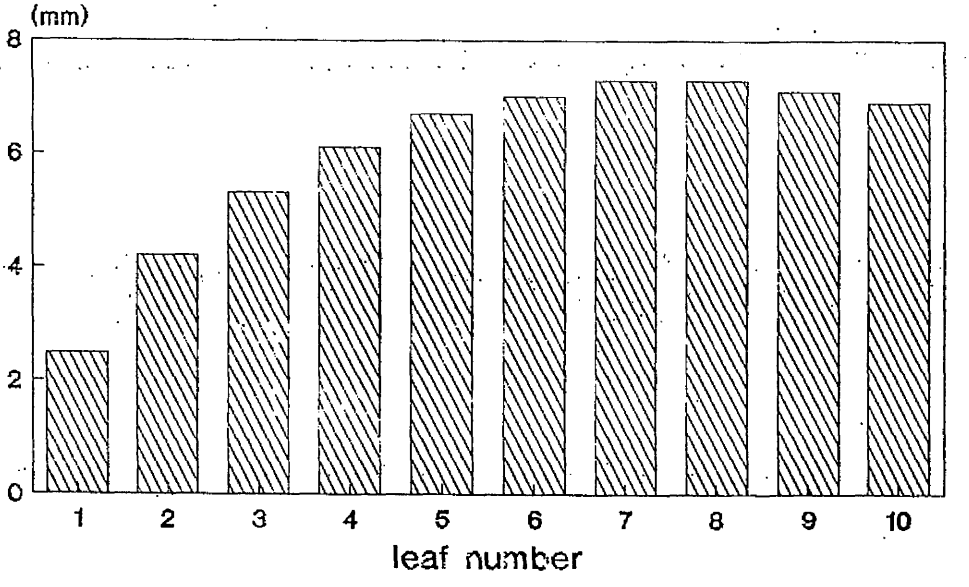
PLANT NO	STEMS	MEAN INTERNODE DISTANCE (mm FROM TIP) 3-4	4-5	5-6	6-7	7-8	8-9
B39	2	1.0 (0)	3.0 (0)	7.0 (1.4)	11.3 (1.1)	13.5 (2.1)	16.0 (2.8)
B40	3	0.7 (0.3)	2.7 (0.6)	5.7 (0.8)	10.5 (1.3)	12.0 (0.9)	9.5 (4.4)
B51	2	1.0 (0.7)	2.5 (0.7)	6.0 (2.8)	11.5 (3.5)	14.3 (2.5)	17.3 (6.0)
B56	2	1.8 (0.4)	5.0 ((0)	14.0 (1.4)	18.3 (0.4)	19.5 (0.7)	18.0 (2.8)
B61	4	3.3 (1.3)	6.6 (2.2)	9.8 (1.9)	10.8 (1.8)	13.0 (1.8)	11.7 (0.6)
B63	2	0.8 (0.4)	2.8 (1.1)	6.5 (3.5)	16.0 (2.8)	21.3 (4.6)	25.3 (3.2)
C31	2	2.8 (2.5)	5.3 (2.5)	9.0 (5.7)	19.0 (5.7)	26.8 (1.8)	26.3 (2.5)
C32	3	1.7 (0.3)	3.3 (0.6)	7.3 (1.5)	15.0 (4.1)	17.7 (5.0)	20.0 (1.8)
C35	2	2.3 (1.1)	4.0 (1.4)	9.8 (4.6)	22.5 (10.6)	26.0 (9.9)	23.5 (1.4)
C39	3	1.5 (0.5)	4.3 (1.8)	8.7 (2.3)	14.5 (2.3)	20.5 (3.1)	22.3 (4.0)
C62	3	4.2 (1.5)	6.8 (1.8)	10.3 (1.5)	13.0 (2.7)	14.2 (4.8)	14.2 (3.6)
C65	2	1.5 (0.7)	3.5 (0.7)	8.0 (2.8)	13.5 (1.4)	19.5 (0.7)	25.8 (1.1)
C89	2	1.3 (0.4)	2.8 (0.4)	6.0 (0)	12.0 (0)	16.3 (0.4)	16.5 (2.1)
D51	4	1.5 (0.4)	2.6 (0.5)	6.0 (1.5)	12.9 (1.6)	17.5 (0.9)	20.8 (1.5)
D102	3	1.1 (0.2)	2.2 (0.3)	4.3 (0.3)	9.5 (0.9)	13.5 (0.5)	15.3 (1.8)
F27	2	1.5 (0.7)	3.3 (1.1)	9.5 (2.1)	13.0 (1.4)	17.5 (0)	17.5 (0.7)
H1	2	1.0 (0.7)	2.3 (0.4)	5.7 (0.2)	12.3 (1.1)	10.0 (6.4)	7.3 (4.6)
H3	3	1.7 (0.3)	4.2 (0.8)	8.3 (2.3)	15.7 (2.1)	18.5 (3.0)	19.5 (0.5)
H6	2	1.5 (0.7)	3.5 (0.7)	8.5 (0.7)	15.5 (0.7)	20.0 (1.4)	19.8 (1.8)
H12	4	2.0 (0.8)	3.5 (1.3)	9.1 (3.9)	14.6 (2.5)	22.6 (3.7)	25.3 (4.8)
H17	3	1.7 (0.6)	4.8 (0.8)	12.5 (2.7)	17.3 (1.2)	21.5 (1.7)	21.0 (1.8)
H25	3	2.0 (0)	5.3 (0.6)	12.0 (2.0)	18.0 (1.0)	22.0 (4.4)	28.0 (5.3)
H30	2	1.0 (0)	2.5 (0.7)	5.3 (2.5)	11.0 (5.7)	16.5 (6.4)	19.8 (3.9)
H39	3	1.5 (0.5)	4.0 (1.0)	10.2 (2.6)	18.3 (3.1)	25.8 (2.1)	27.7 (0.6)
H44	2	2.0 (0)	4.0 (0)	12.0 (0)	20.8 (0.4)	30.3 (1.1)	28.0 (0)
H59	2	1.0 (0)	2.8 (0.4)	5.8 (0.4)	12.8 (1.1)	22.8 (1.8)	26.5 (5.0)
H96	2	1.5 (0)	4.3 (0.4)	11.0 (0)	15.8 (1.8)	19.5 (2.1)	16.5 (0.7)
H115	5	1.4 (0.4)	3.6 (1.1)	7.5 (2.2)	12.5 (2.4)	15.6 (2.2)	17.8 (2.0)
		1.7 (0.8)	3.8 (1.2)	8.4 (2.5)	14.6 (3.3)	18.9 (4.9)	19.9 (5.6)

Fig. 57: A comparison of internode distances between thyme plants (actual size).

5.4 LEAF LENGTHS AND WIDTHS

Figures 58 and 59 show the mean leaf lengths and widths of all leaves measured. Figures 60 - 71 show the mean leaf lengths and widths per plot, of leaves 1 to 10 (numbered from the stem tip). One leaf from one to four stems (from each of 79 plants in total) were measured. The difference in length and width between fully mature and young leaves is seen. In general, leaf length and leaf width between consecutive pairs of leaves, in younger leaves (leaf numbers 1 to 3) show statistical differences ($p < 0.05$), whereas the differences in length and width in older leaves were not significant. There was no significant difference in mean length and width of leaves 1 to 10 between plots.

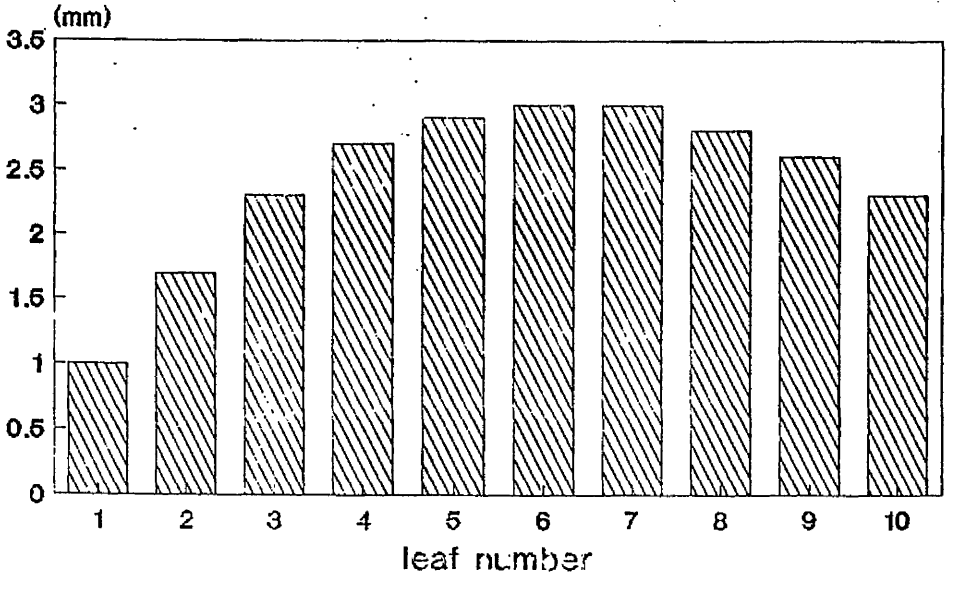
Leaf length in thyme
(plots A - D, F & H)



mean = 5.9; SD = 1.9

Fig. 58

Leaf width in thyme
(plots A - D, F & H)



mean = 2.4; SD = 1.0

Fig. 59

Leaf length in thyme, Oct. 1989

Plot A. Leaves 1-8

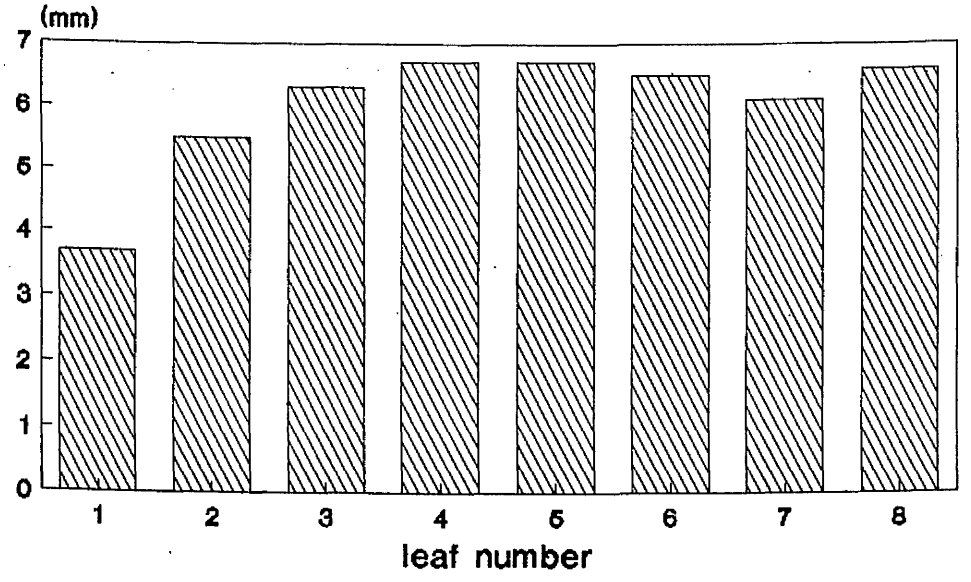


Fig. 60

Plot B. Leaves 1 - 10

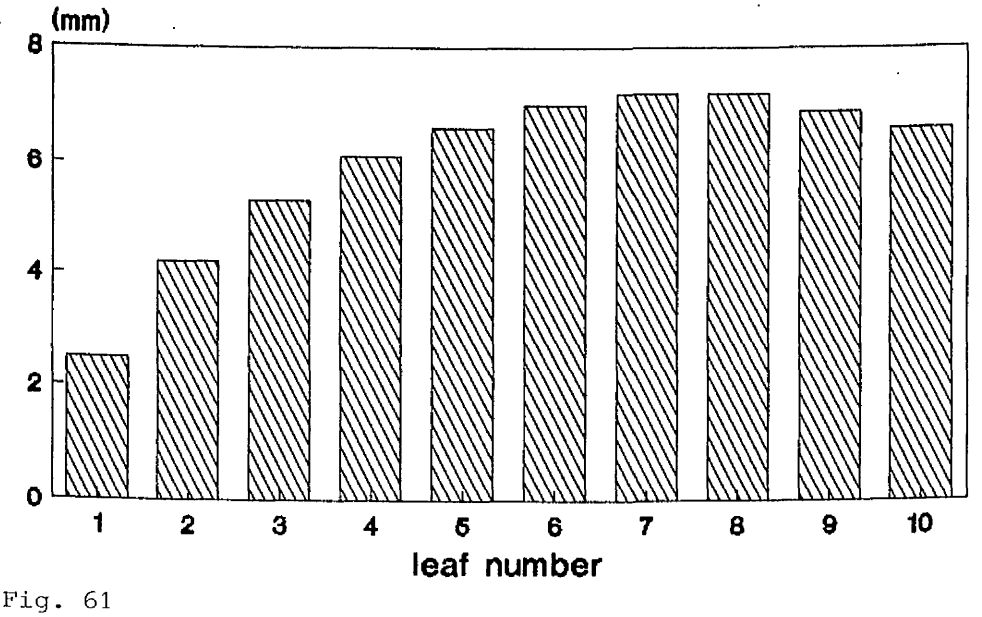


Fig. 61

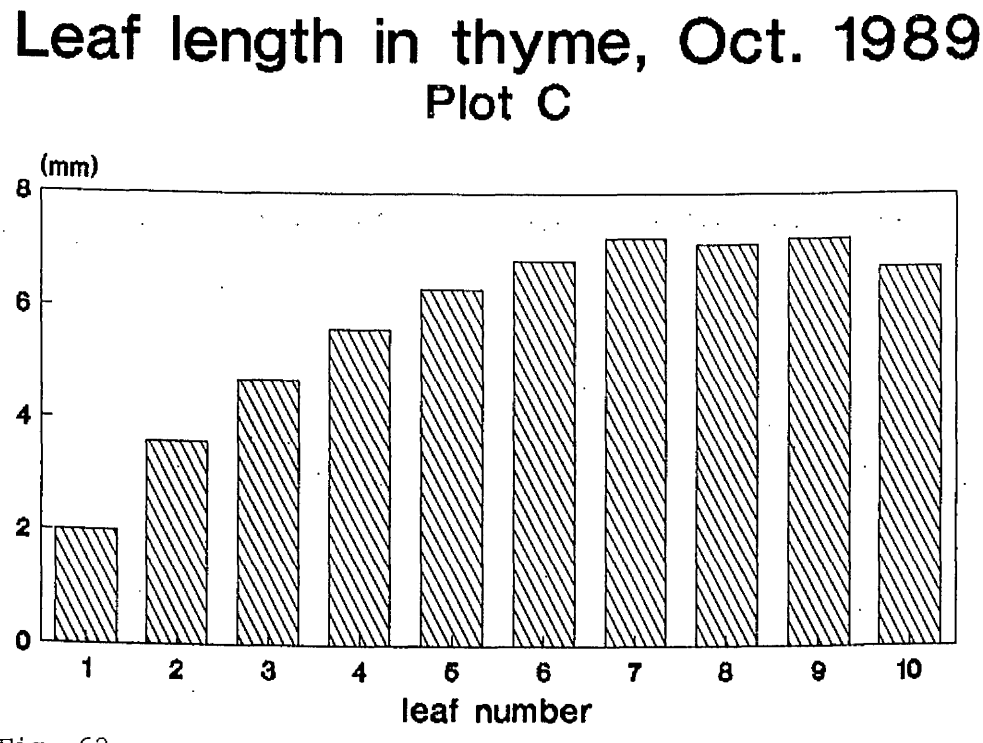

Leaf length in thyme, Oct. 1989
Plot C
(mm)
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
leaf number

Fig. 62

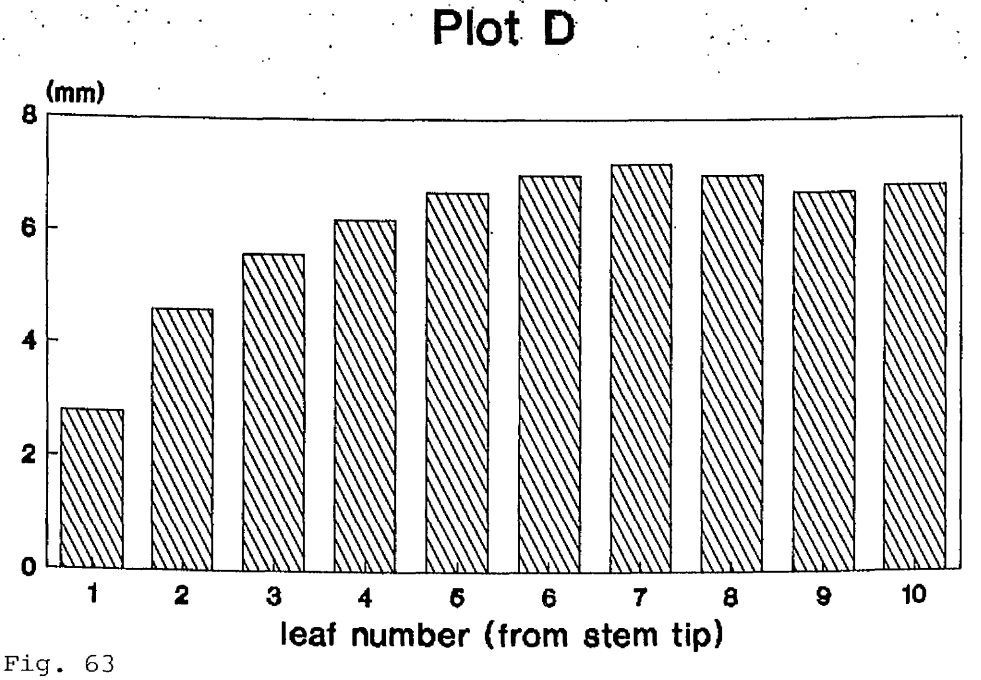

Plot D
(mm)
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
leaf number (from stem tip)

Fig. 63

Leaf length in thyme, Oct. 1989

Plot F. Leaves 1 - 10.

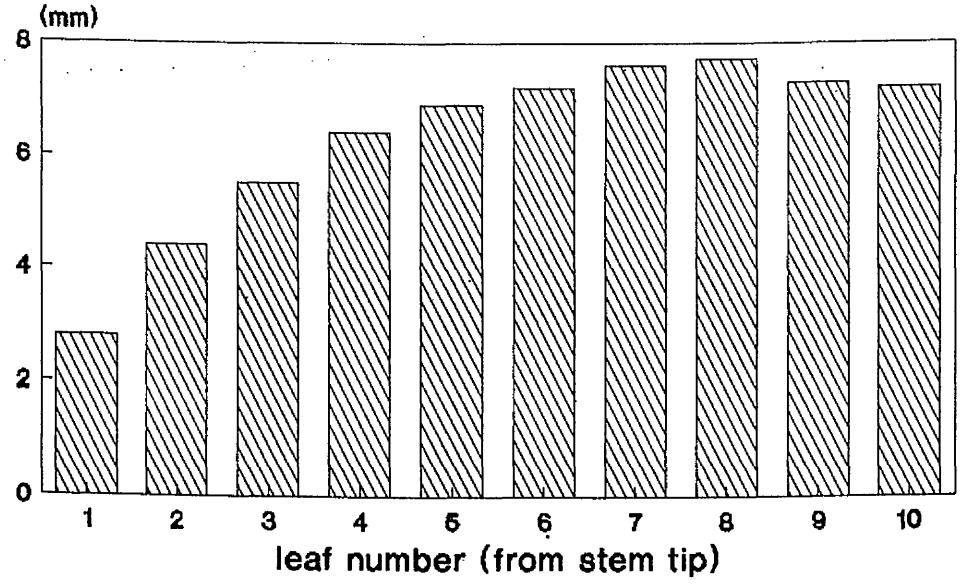


Fig. 64

Plot H. Leaves 1 - 10.

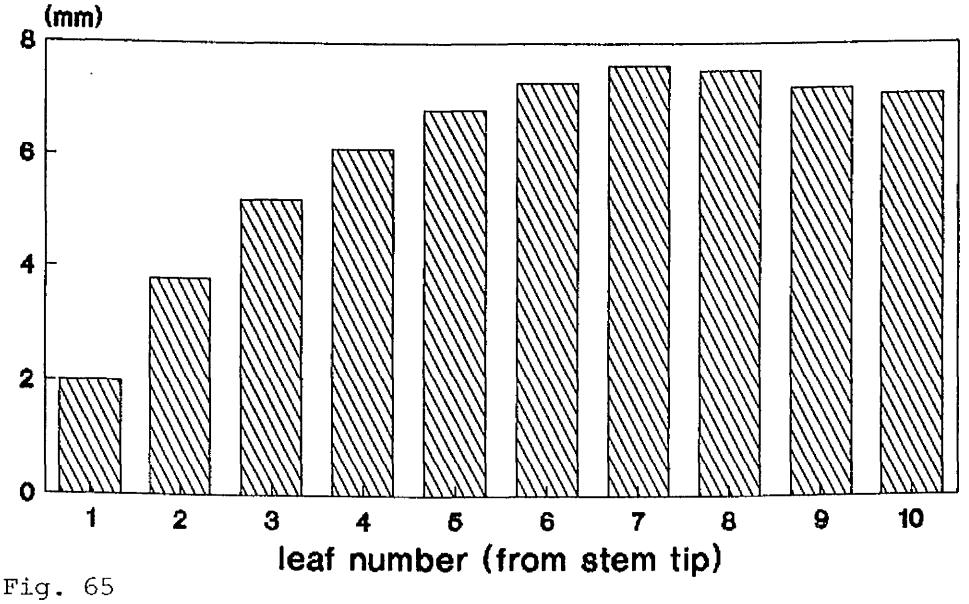


Fig. 65

Leaf width in thyme, Oct. 1989

Plot A

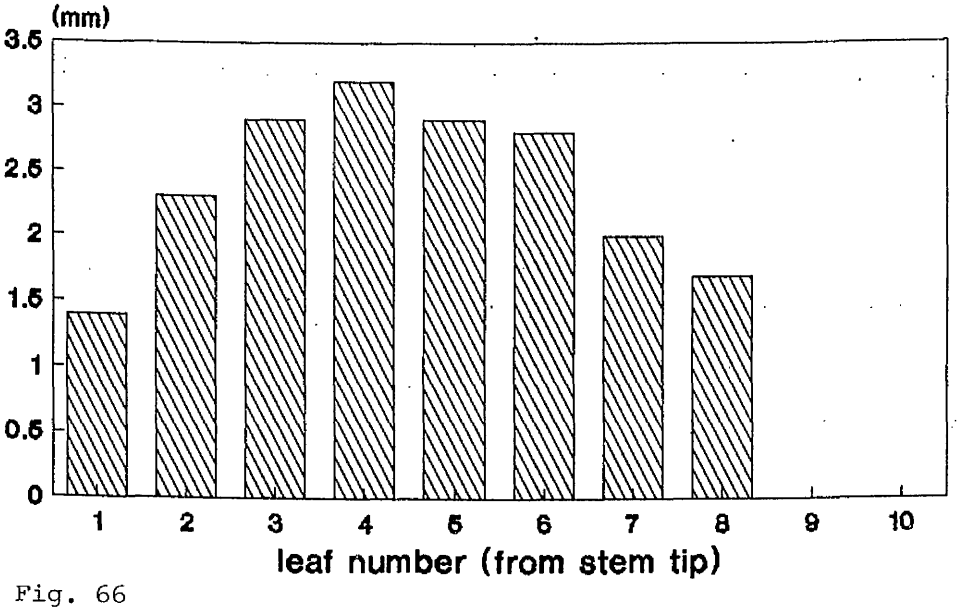


Fig. 66

Plot B

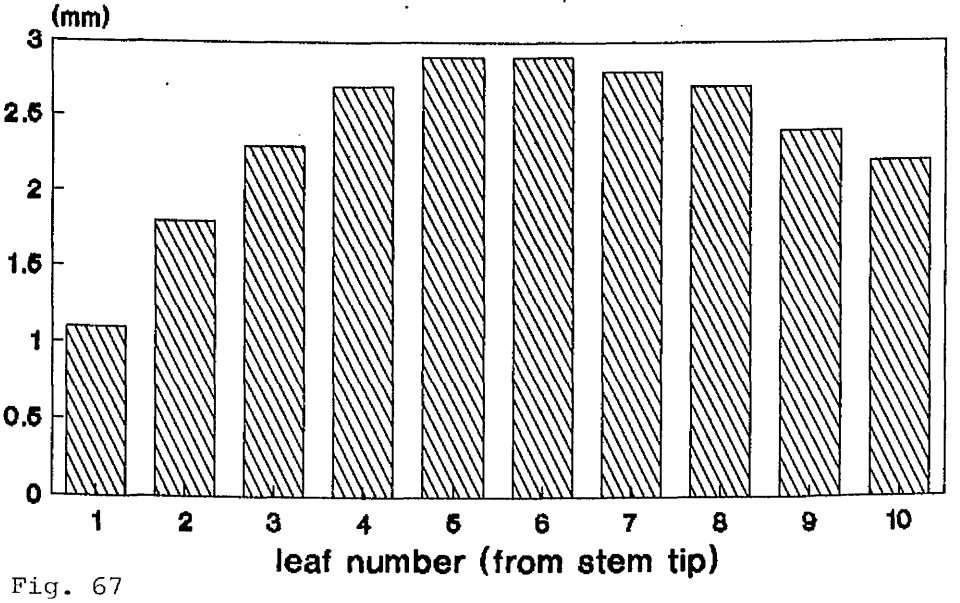


Fig. 67

Leaf width in thyme, Oct. 1989

Plot C

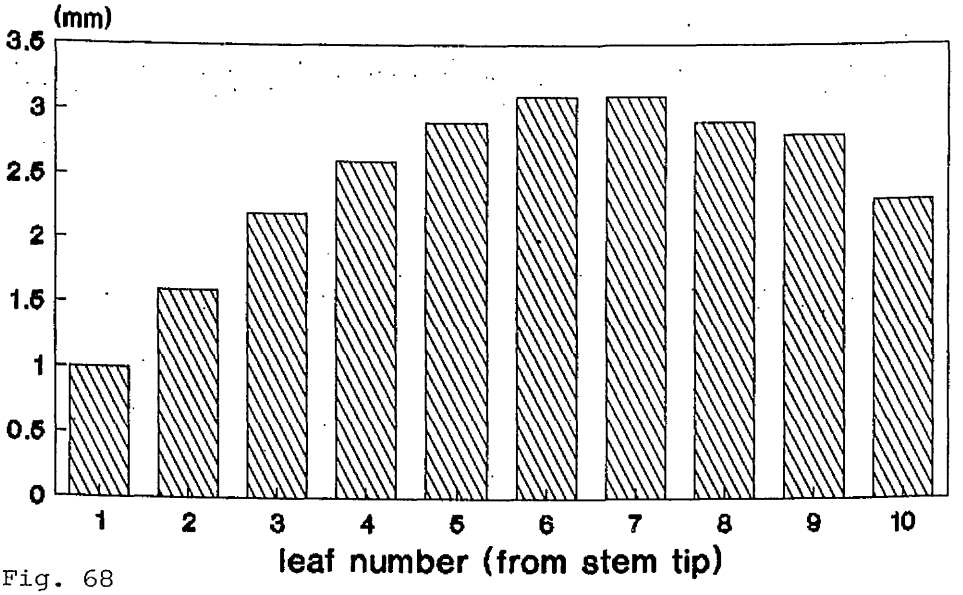


Fig. 68

Plot D

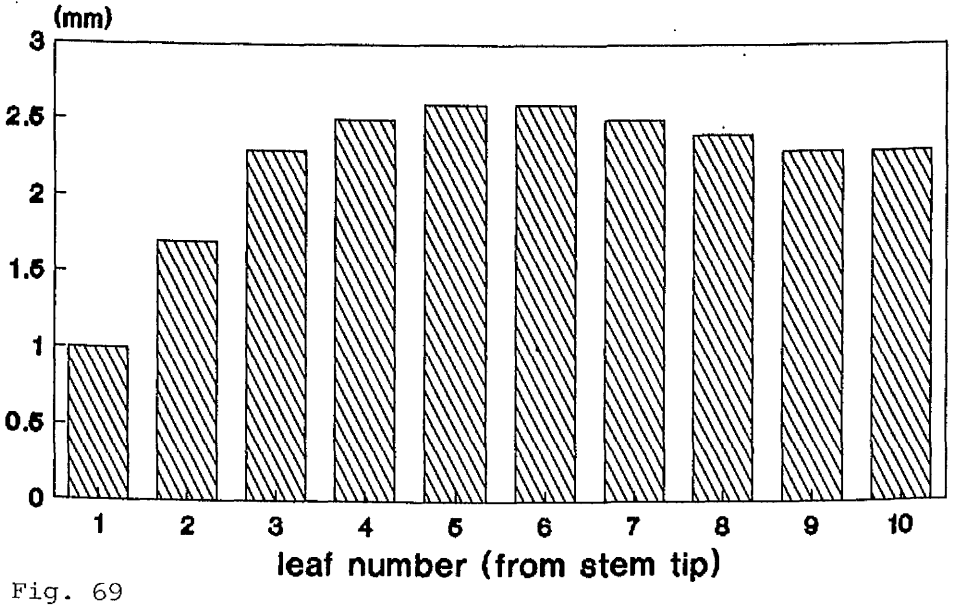


Fig. 69

Leaf width in thyme, Oct. 1989

Plot F. Leaves 1 - 10.

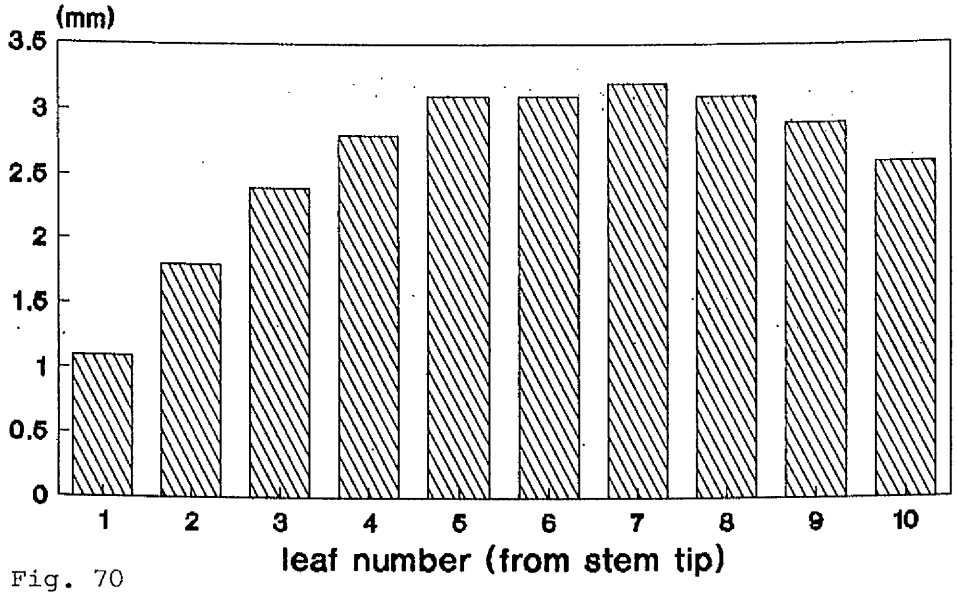


Fig. 70

Plot H. Leaves 1 - 10.

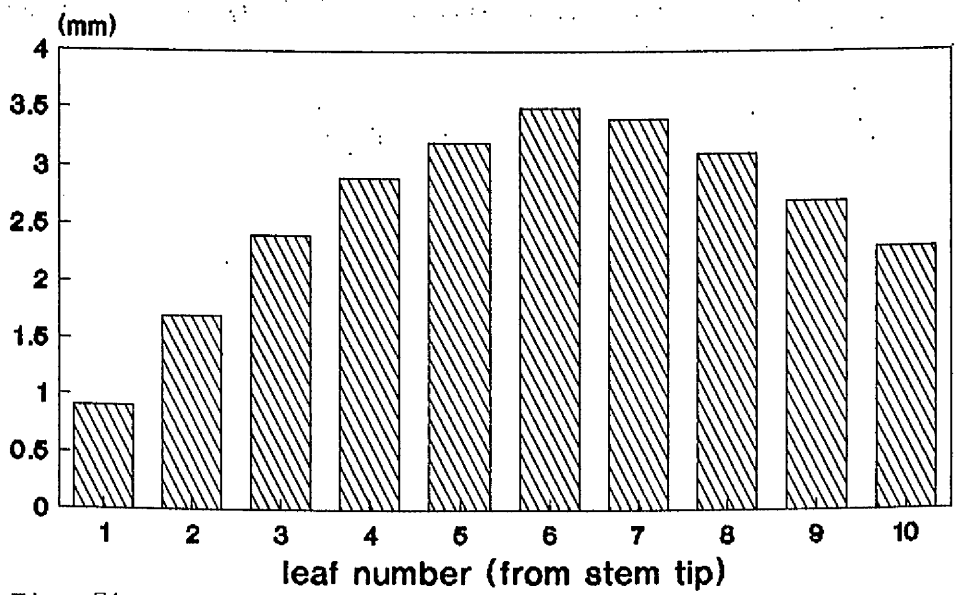


Fig. 71

5.5 FLOWER COLOUR

Table 18 shows the total number of different flower colours (shades) identified in each plot. This varied from 14 in plot F to 22 in plot D. The number of colours shared by more than 5% of the total plants per plot is also given, and this again varied between plots.

Table 18: Number of colours (shades) identified in thyme flowers (plots A - D, F & H).

Plot	Total colours identified (per plot)	Number of colours >5% of plants per plot
A	16	9
B	18	8
C	21	6
D	22	5
F	14	5
H	16	7

In each plot, more than 60% of the plants had flowers which were one of the 5 main shades (Fig. 72). In plot F, this figure was 80.0% and no other shade made up more than 5% of the plot. The main shade was number 1548 (Appendix IX) in all plots, except plot C, where this was number 1540.

Plates 10 and 11 show some flowering plants from plot A (early June, 1990).

Fig. 72

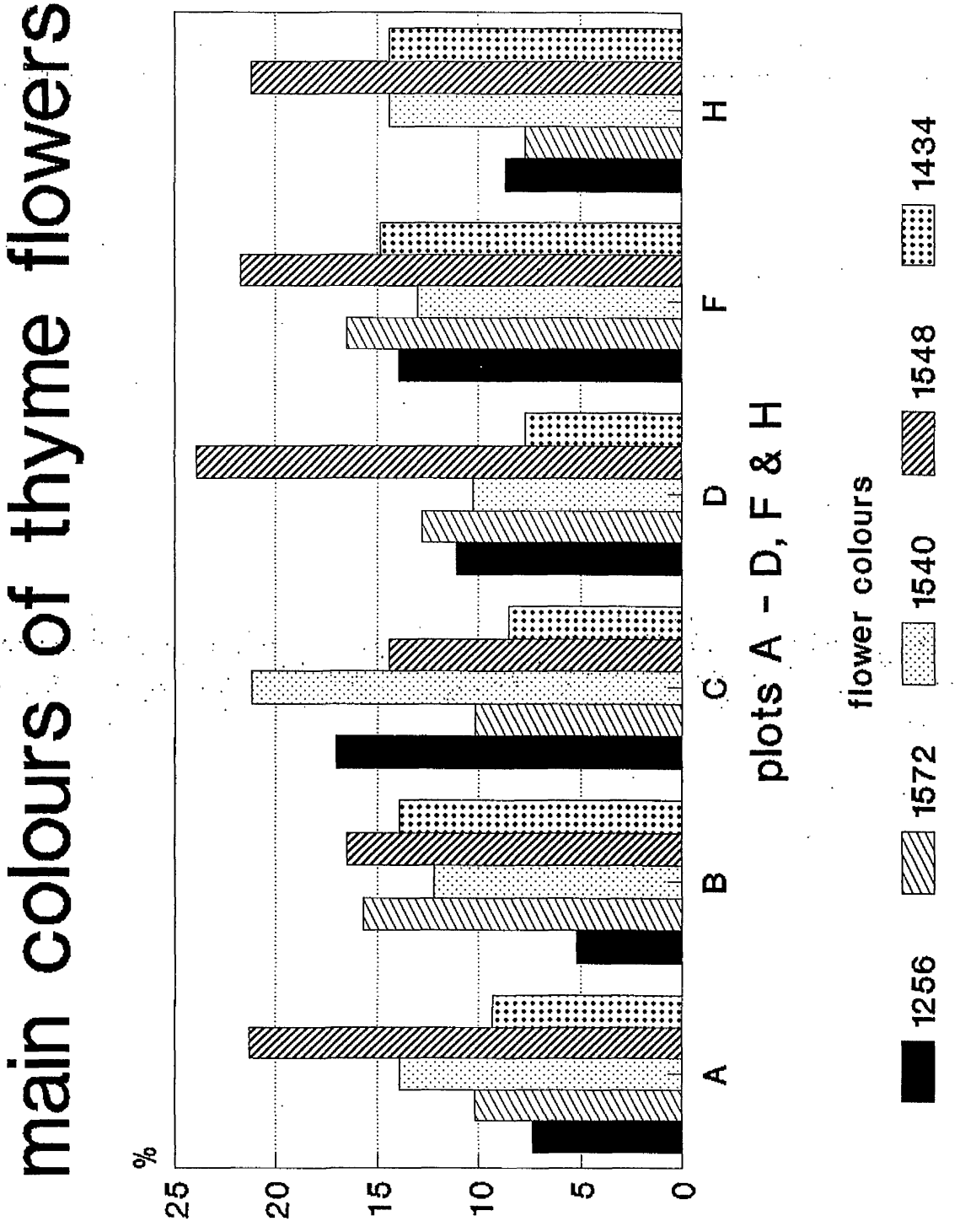

main colours of thyme flowers
%
25
20
15
10
5
0
A
B
C
D
F
H
plots A - D, F & H
flower colours
1256
1572
1540
1548
1434

Plate 10: Plot A, 1990, showing differences in flower shade.

Plate 11: Plot A, 1990, showing differences in flower shade.

5.6 REPRODUCTIVE TYPE (Hermaphrodite, Intermediate, Male Sterile).

There were three reproductive types (Section 4.4) found in the plots examined. Their distributions by plot are given in Table 19. Only those plants which had a single flower type were used here (see pp. 126 - 127).

Table 19: Distribution of reproductive types, in thyme plants, by plot.

	Percentage of total			
Plot	Hermaphrodite	Intermediate	Male Sterile	I+MS
A	21.8	28.2	50.0	78.2
B	14.9	23.7	61.4	85.1
C	23.0	27.4	49.6	77.0
D	12.0	11.1	76.9	88.0
F	17.5	35.1	47.4	82.5
H	17.8	33.7	48.6	82.3
x	17.8	26.5	55.7	82.2
SD	4.1	8.7	11.6	4.1

A chisquared test on the groups, hermaphrodite, intermediate and male sterile (Table 19) showed significant differences at the 1% level but these disappeared when plot D was excluded. When the test was carried out on the groups, hermaphrodite and others (intermediate + male sterile) there was no significant difference.

5.7 THYME FLOWER PHOTOGRAPHS

All of the photographs are of newly opened flowers. Plate 12 shows a protandrous hermaphrodite flower (approx. 2.5x actual size): the anthers were shedding pollen but the style was immature and the stigma was closed.

In Plate 13, an intermediate flower is shown (approx. 2.5x actual size). The style was also immature and the stigma was closed. There were large white anthers present.

In the male sterile flower (approx. 2.5x actual size), Plate 14, the small brown anthers appeared to be on very short pistils which were only slightly detached from the corolla wall. The style was similar to those mentioned above.

The flowering stem (approx. 2x actual size) (Plate 15), from which the above male sterile flower was removed, shows flowers at different stages of development; in the newly opened flower, approximately at the centre of the photograph, the style cannot be seen against the corolla. In older flowers, which were paler in colour, the style was clear of the corolla and different stages of development of the stigma can be seen in several of the flowers. Some were beginning to open whilst others were more advanced, fully opened or senescing. Buds (near the tip) and a spent flower (lower down the

stem) were also present on this flowering stem.

Plate 12: Protandrous hermaphrodite flower of *T. vulgaris*.

Plate 13: Intermediate flower of *T. vulgaris*.

Plate 14: Male sterile flower of *T. vulgaris*.

Plate 15: Flowering stem from a male sterile *T. vulgaris* plant.

5.8 EVIDENCE OF PROTOGYNY IN *T. vulgaris* FLOWERS

Plant number E102 appeared to be male sterile (with brown anthers) when first examined on 5/6/89. By 21/6/89 many hermaphrodite flowers were seen. Five stems, from a rooted cutting of this plant, were examined under the microscope (x10) on 12/2/90, to determine the reproductive type. It was seen that one stem was normal hermaphrodite and one normal male sterile. Two others were nearly normal hermaphrodite with a few male sterile flowers and the fifth was nearly normal male sterile, with two of the flowers examined having one pollen sac almost normal (Table 20

Plants from the same seed lot (Plot E - Vikima) were examined in June, 1989 and it was found that, out of 90 plants, 6 were similar to E102 (6.7%). This phenomenon has since been noted in other plots, but to a lesser degree (Table 20).

Table 20: Apparent changes in the reproductive type of some thyme plants.

Plant identity	3/6/89	11/5/90	17/5/90	13/6/90
A63	H	MS	MS	H
C6	H	I	I*	H
C28	H	I	I*	H
C120	I	H	I*	I*
F42	H	I	MS	H
A60	MS	-	I*	H
D60	I	-	-	H
F42	H	I	MS	H

H = hermaphrodite; I = intermediate; MS = male sterile
* = large white anthers

No hermaphrodite flowers were seen on C120 at the last date.

5.9 FLOWER MUTATION IN PLANT NUMBER 105 FROM PLOT C.

In this mutation there were, usually, 2 - 5 calyces or partial calyces (one within the other), no corolla and from 1 - 4 styles (Table 21). The ovaries were intact and at least one style was gynobasic. There were no stamens or anthers (the pistils usually arise from the walls of the corolla). In some flowers, in the axes of the calyces, there were buds. This was the only plant of this type found amongst more than 1700 plants.

Table 21: Mean number of styles and calyces present in the florets of thyme plant number C105.

Node	Teeth		Calyces	Styles
	Broad	Narrow		
2	3.0 (0)	1.8 (0.5)	2.8 (1.0)	2.0 (0.8)
3	3.0 (0)	2.0 (0)	3.3 (0.5)	2.3 (1.0)
4	3.0 (0)	1.8 (0.5)	4.0 (0.8)	2.8 (1.7)
5	3.0 (0)	2.3 (0.5)	4.3 (1.0)	3.8 (0.5)
6	3.0 (0)	2.8 (1.0)	3.8 (0.5)	2.8 (1.0)
7	3.0 (0)	3.0 (0)	4.5 (0.7)	3.0 (0)
8	3.0	1.0	3.0	-

62 florets from 4 other plants were checked and in each case there were 3 broad and 2 narrow teeth on the calyces. Table 21 shows that although C105 also had 3 broad teeth on each calyx there was some variation in the number of narrow teeth present. The other

plants used were: B5, B8, C3 and C6 (all hermaphrodite, except B8, which was intermediate). It can be seen from Table 22 that there was little difference in calyx length between C105 and the other plants. Figure 13 is a photocopy of flowering stems from C105.

Table 22 : Measurement of calyx length in C105 and 4 other thyme plants.

Node number	C105 (outer calyx)	Other plants
2	3.8 (0.3)	4.1 (0.5)
3	4.0 (0)	4.3 (0.6)
4	4.4 (0.3)	4.4 (0.7)
5	4.6 (0.3)	4.6 (0.6)
6	4.4 (0.3)	4.8 (0.2)
7	4.3 (0.4)	4.4 (0.1)
8	4.5	4.3 (0.4)
x	4.3	4.4
SD	0.3	0.2

Leaves were sampled from C105 and two others from the same plot to examine the oil components present. It can be seen that the essential oil produced by this mutant plant was quite characteristic (Table 23) although the quantity of oil (% v/w leaf dry weight) was lower than in the other two plants and the quantity of thymol (% v/w leaf dry weight) was approximately half that found in the other two plants.

Table 23: Major oil components of C105 and 2 controls.

Plant identity	% Of the oil			Oil (% v/w)	Thymol (% v/w)
	A	B	C		
C105	45.3	9.2	23.4	0.76	0.34
C58	35.8	5.6	36.9	2.02	0.72
C116	59.5	-	16.0	1.06	0.63

A = thymol: B = γ-terpinene: C = ρ-cymene

Figure 73: Plant number C105, showing flower mutation.

5.10 BUDDING AND FLOWERING

In *T. vulgaris*, as in other species, bud initiation is visible only under magnification. However, before the first floret opens, buds are clearly seen on many stems. The term budding, as used here, refers to the phase where the buds were clearly visible to the naked eye but where no florets were open. The term maximum budding was used where the greatest number of plants in a group (hermaphrodite, Intermediate or male sterile) were budding.

In all plots (Figs. 74 - 79), in 1989, hermaphrodite plants were the first to begin to bud. These were followed by the intermediate plants and, lastly, by the male sterile plants. This trend was again evident in 1990, except in plot D. In most plots, during 1990 (Figs. 80 - 85) (except plot D (Fig. 83), where maximum budding in the hermaphrodite plants was delayed, and in plot F (Fig. 84) where maximum budding in the hermaphrodite plants was earlier), maximum budding occurred at the same time in all three flower types.

In plots A (Fig. 74) and D (Fig. 77), in 1989, maximum budding in hermaphrodite plants was earlier than in the remaining plots where this occurred at the same time in all three reproductive types.

In 1989, flowering followed the same trend (Figs. 86 - 91) except in plot H, where a greater number of

intermediate plants were flowering at the second date (Fig. 91) and in plot C where there was very little difference between the three flower types (Fig. 88).

In 1990 (Figs. 92 - 97), flowering progressed as in 1989 except in plot C, where the intermediate plants flowered earliest (Fig. 94) and in plot H, where there was little difference between the flower types up to the fifth measurement, when there were more hermaphrodite than intermediate plants flowering and more intermediate than male sterile (Fig. 97).

Plate 16 shows some plants from plot A on 12/6/90. Three of the four plants in the bottom row were hermaphrodite types, the one to the extreme right of this row had intermediate flowers and the four plants in the second row from the bottom were all male sterile.

In Plate 17, the two hermaphrodite plants (bottom right and top centre) were in full flower (some florets were past in the bottom right plant) whilst the male sterile and intermediate plants (top left) were beginning to flower.

Plate 16: Part of plot A, 1990.

Plate 17: Part of plot A, 1990

Flowering status in thyme, 1989

Budding, plot A

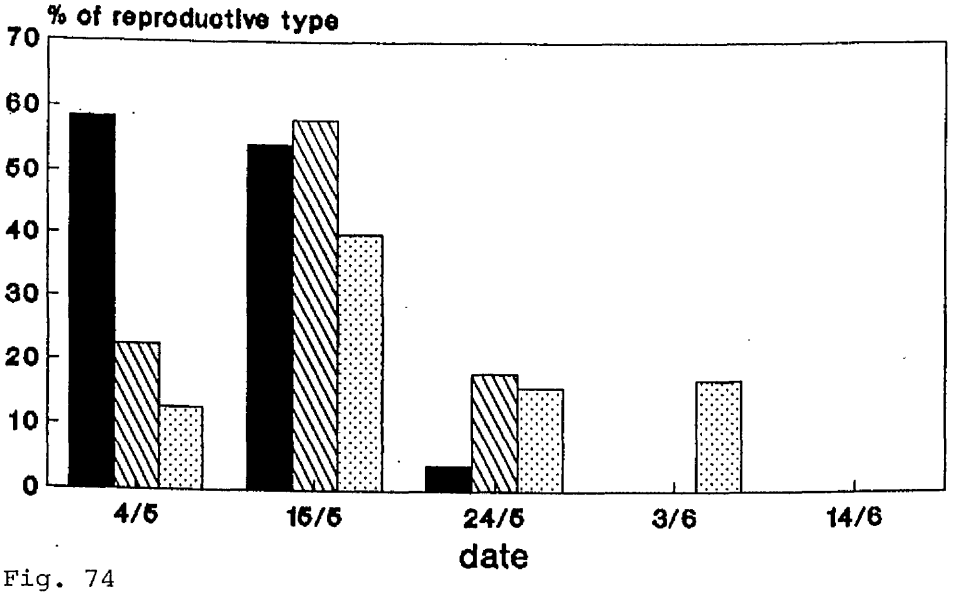


Fig. 74

Flowering, plot A

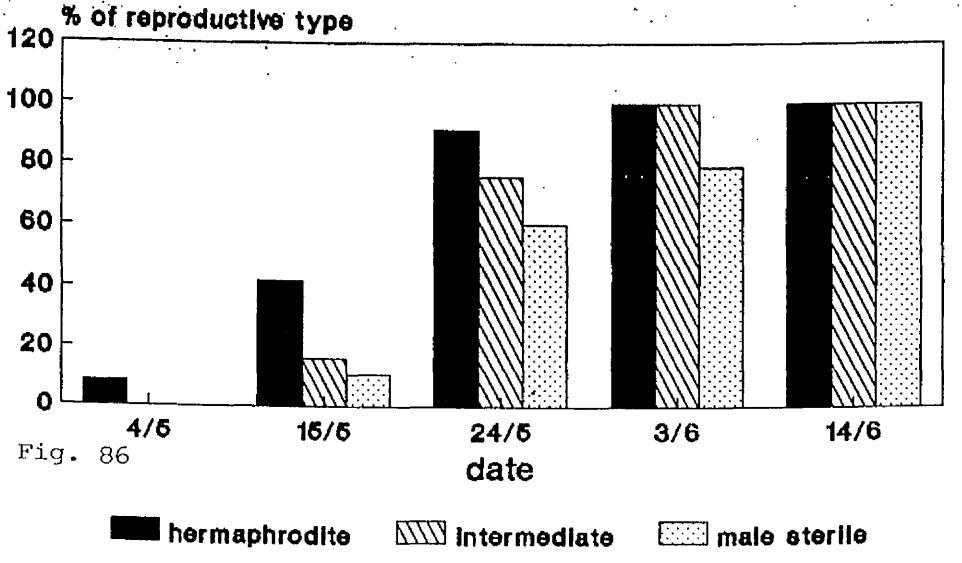


Fig. 86

Flowering status in thyme, 1989

Budding, plot B

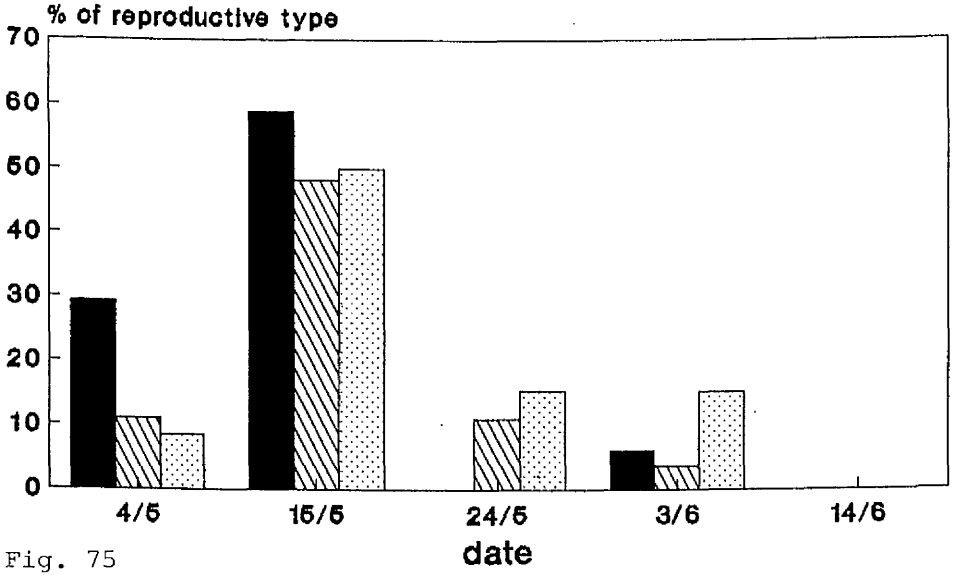


Fig. 75

Flowering, plot B

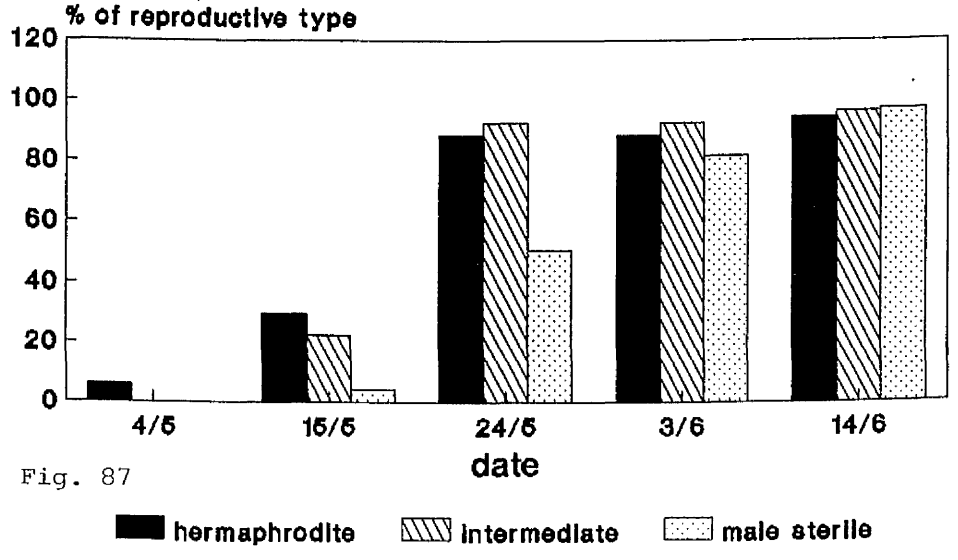


Fig. 87

Flowering status in thyme, 1989

Budding, plot C

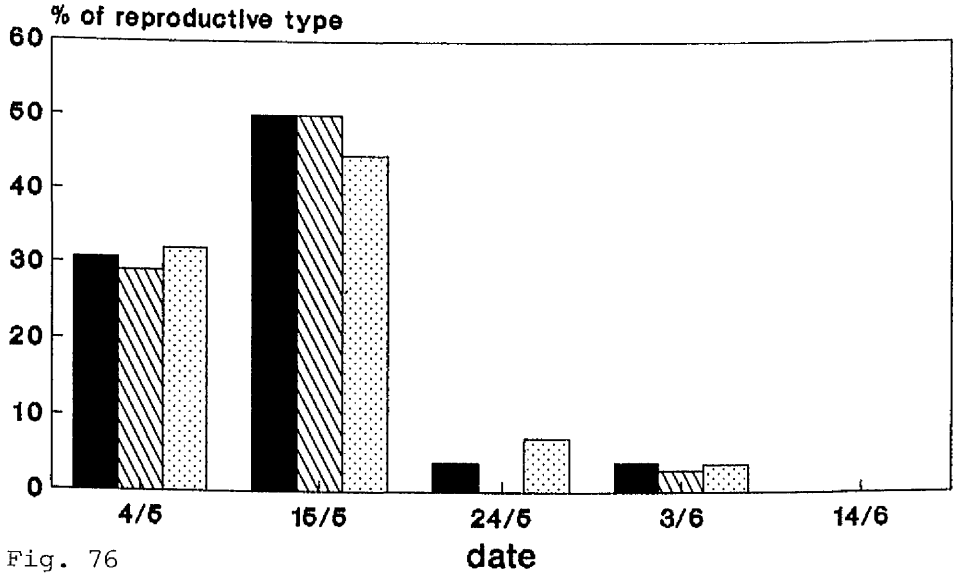


Fig. 76

Flowering, plot C

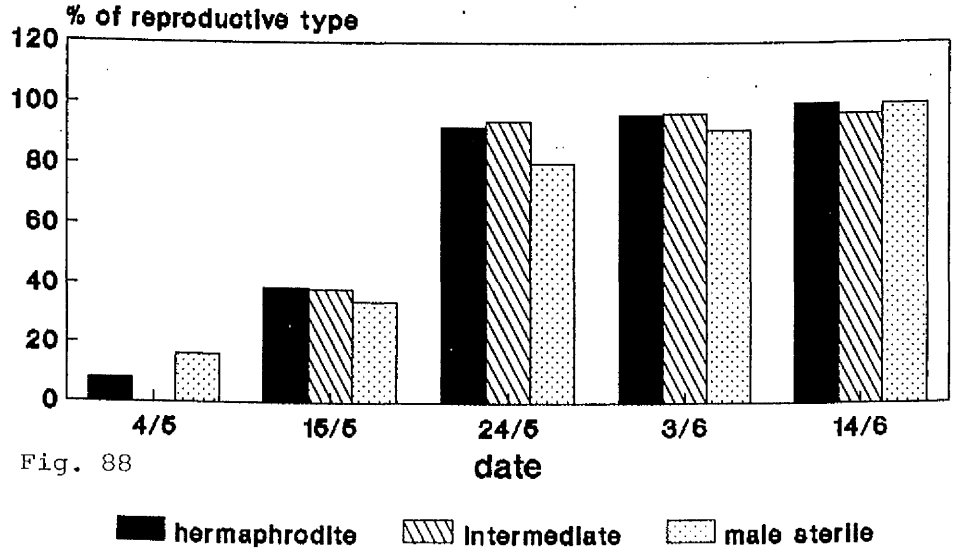


Fig. 88

Flowering status in thyme, 1989

Budding, plot D

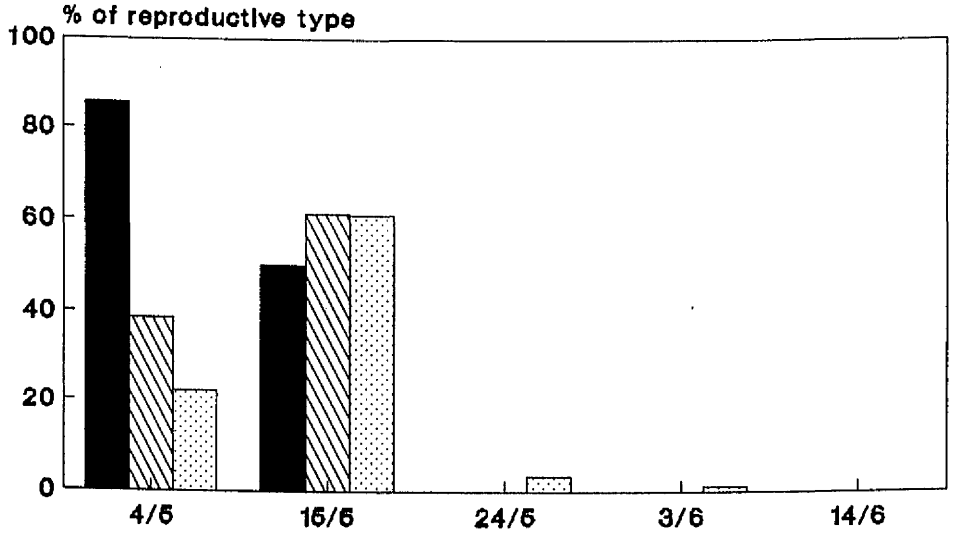


Fig. 77

Flowering, plot D

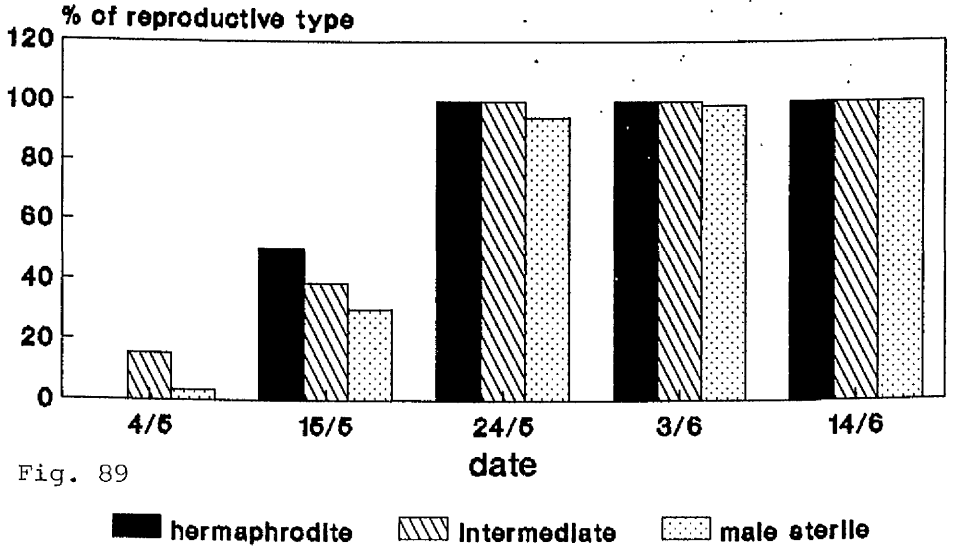


Fig. 89

Flowering status in thyme, 1989

Budding, plot F

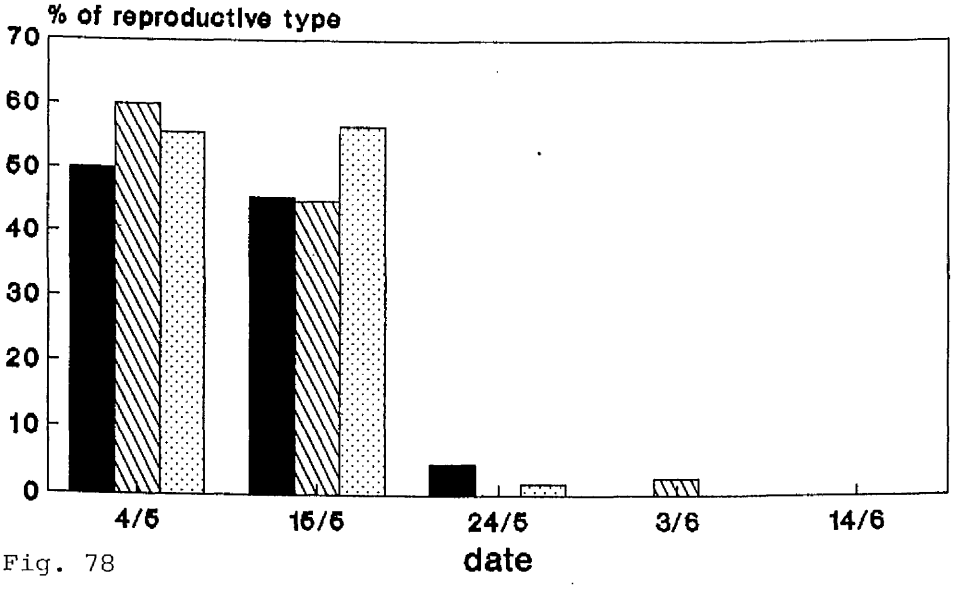


Fig. 78

Flowering, plot F

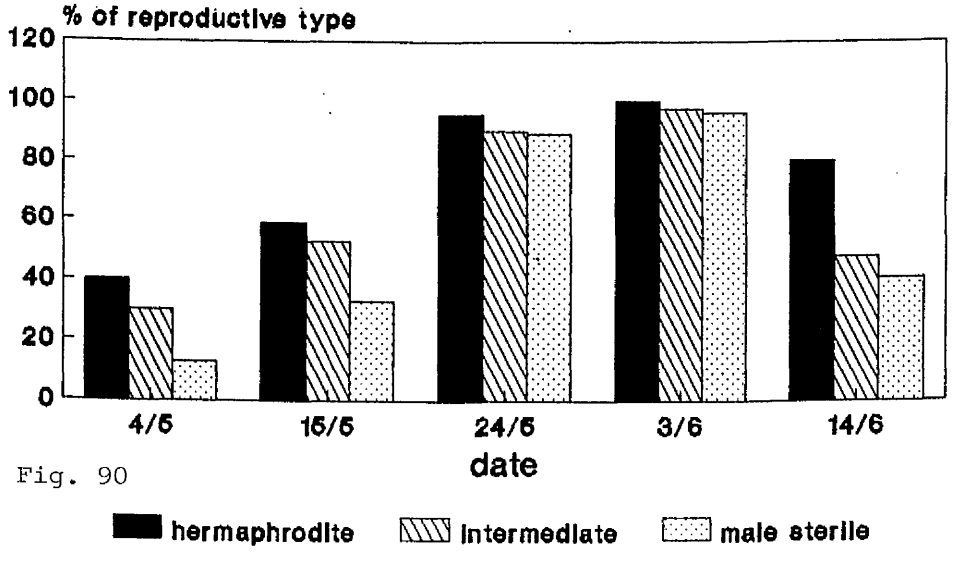


Fig. 90

Flowering status in thyme, 1989

Budding, plot H

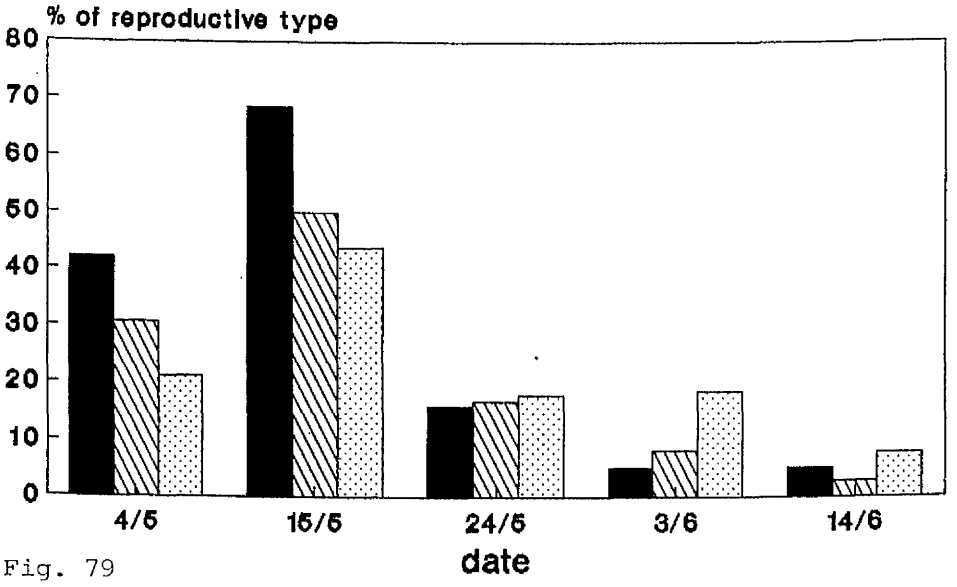


Fig. 79

Flowering, plot H

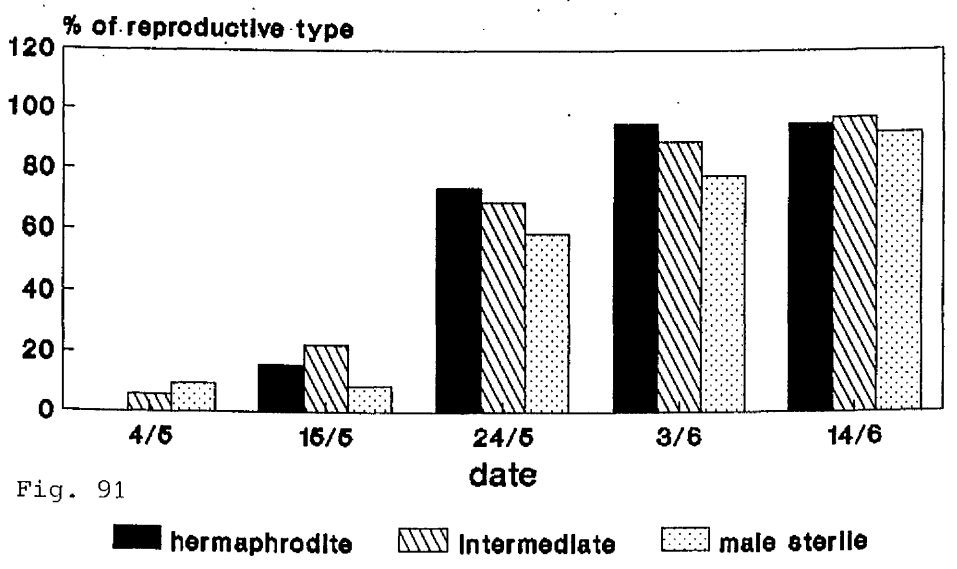


Fig. 91

Flowering status in thyme, 1990

Budding, plot A

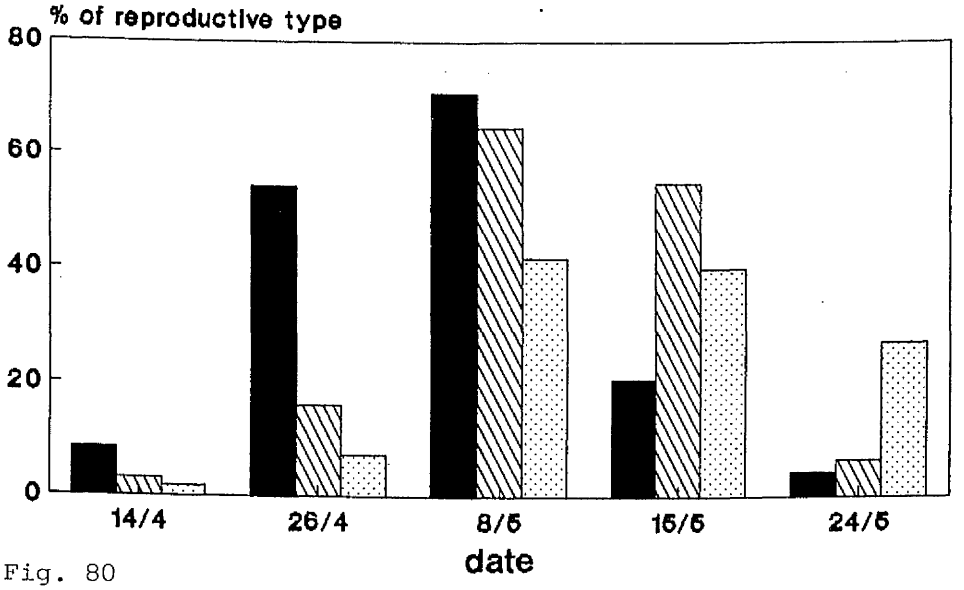


Fig. 80

Flowering, plot A

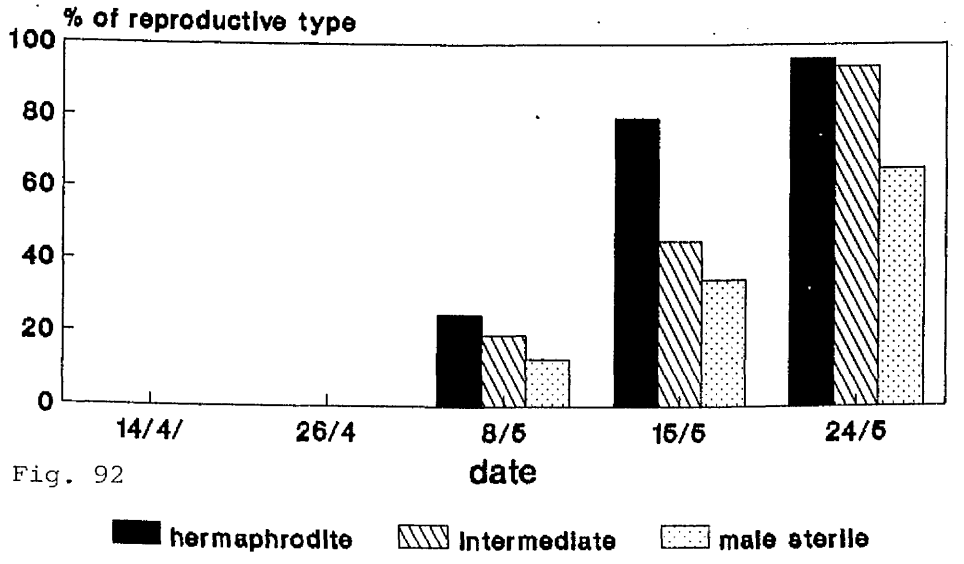


Fig. 92

Flowering status, 1990
Budding, plot B

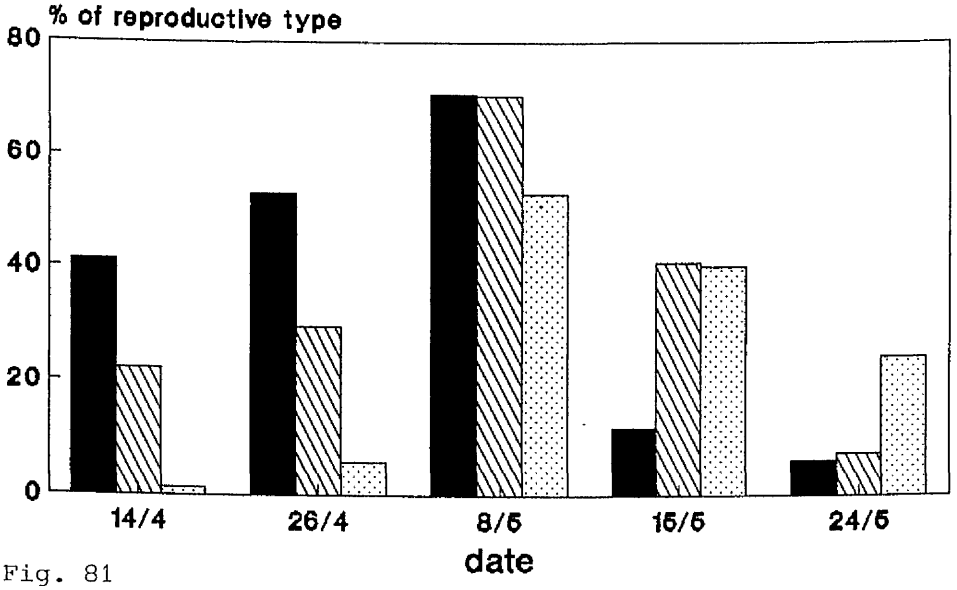


Fig. 81

Flowering, plot B

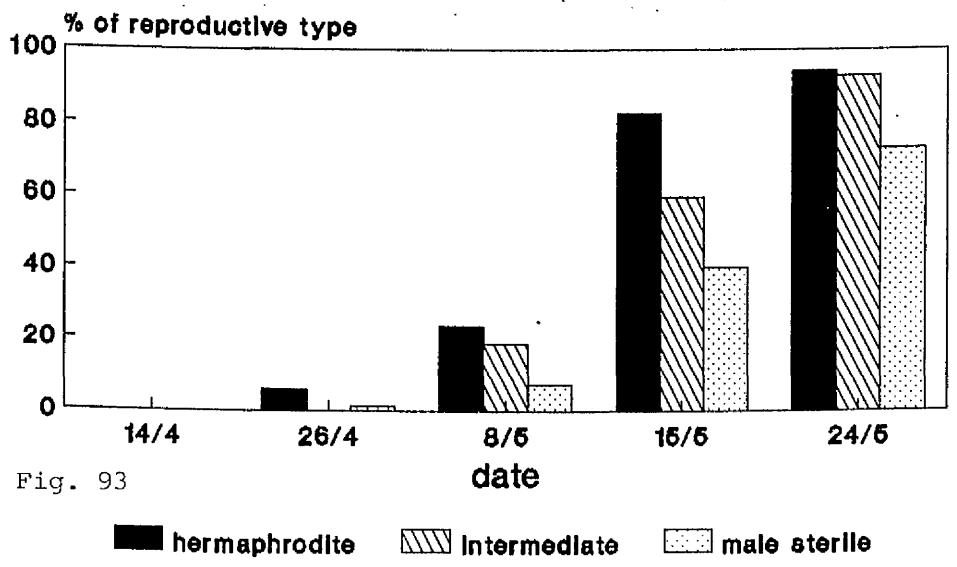


Fig. 93

Flowering status in thyme, 1990

Budding, plot C

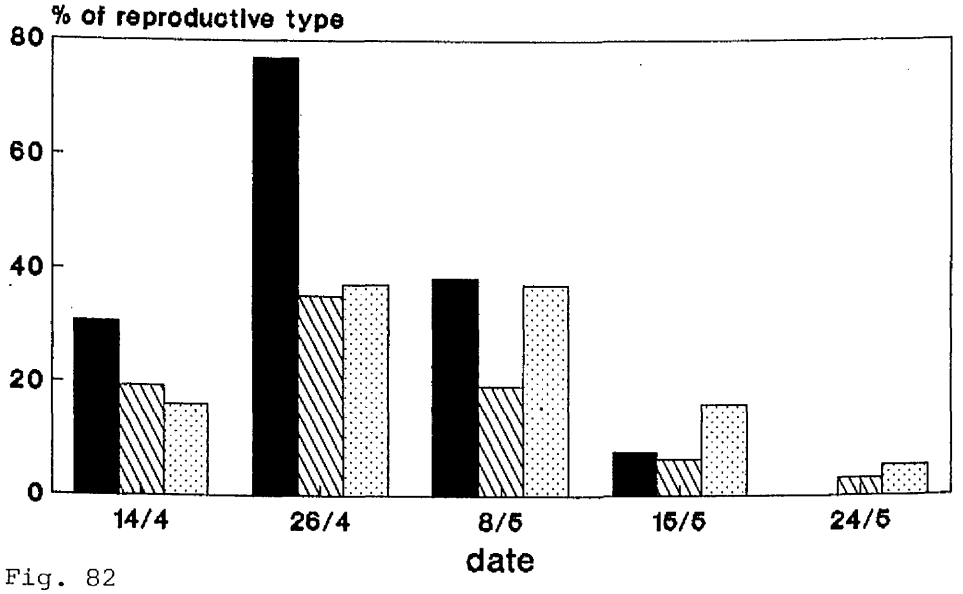


Fig. 82

Flowering, plot C

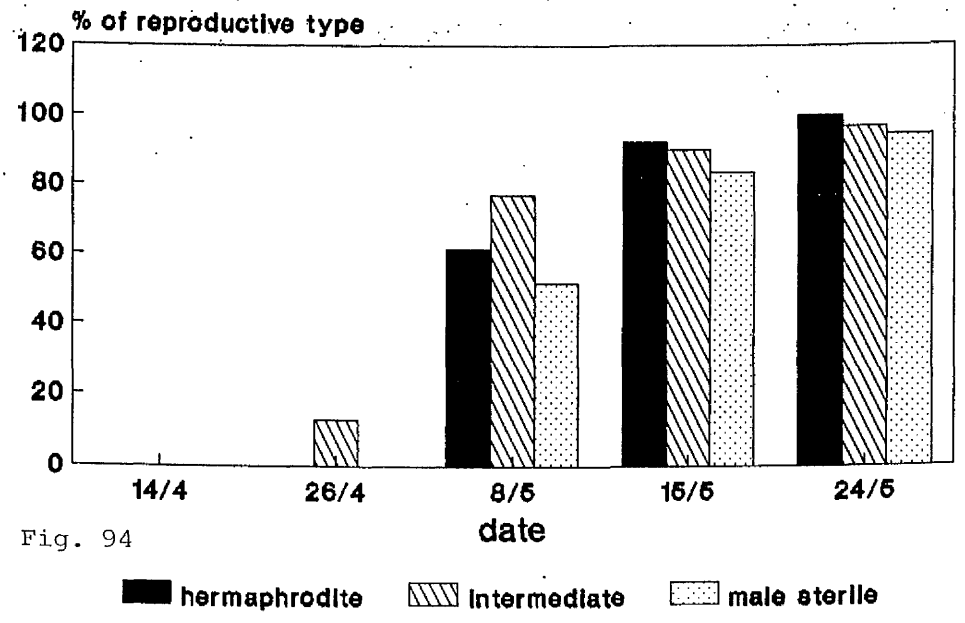


Fig. 94

Flowering status in thyme, 1990

Budding, plot D

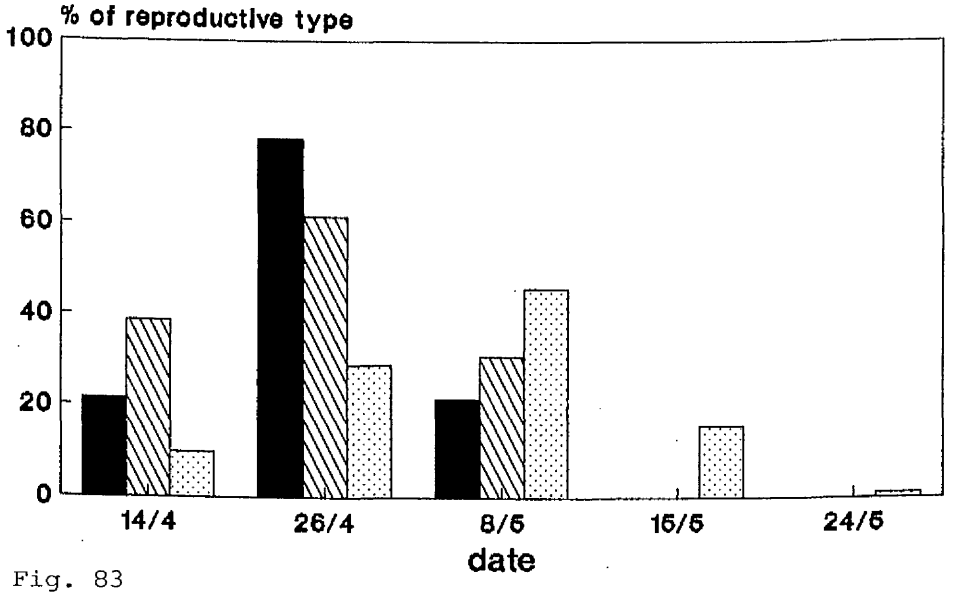


Fig. 83

Flowering, plot D

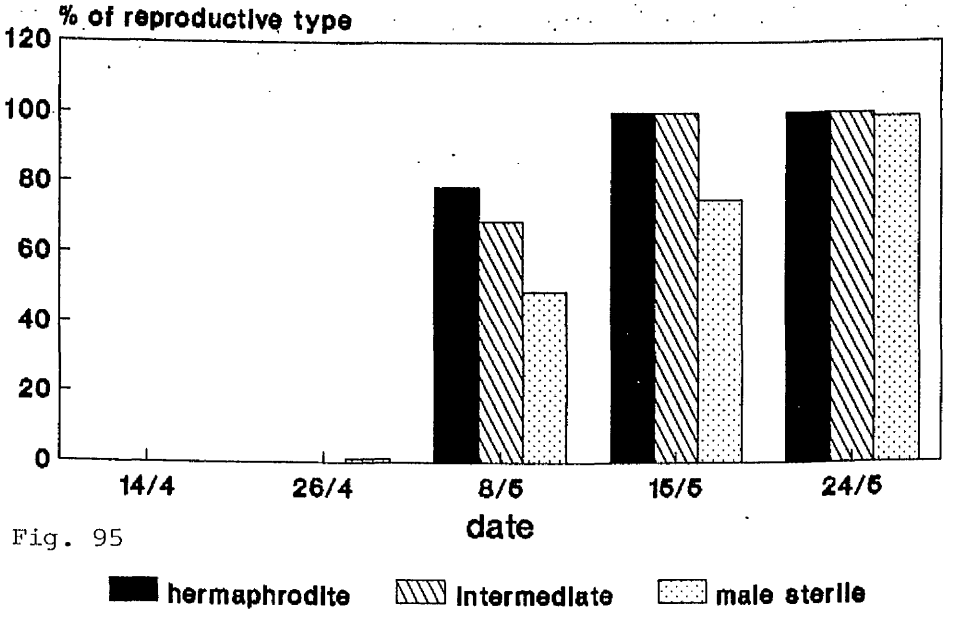


Fig. 95

Flowering status in thyme, 1990

Budding, plot F

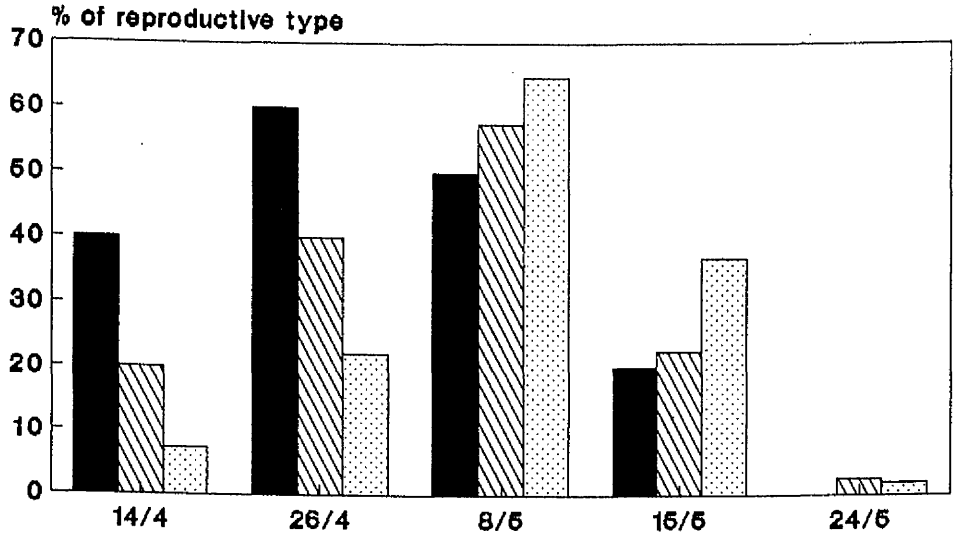


Fig. 84

Flowering, plot F

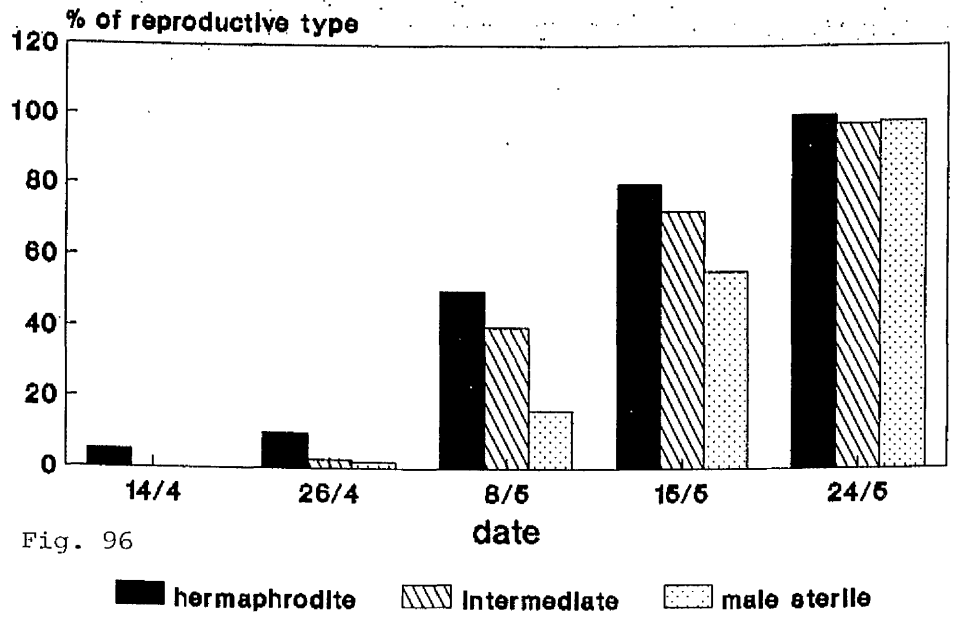


Fig. 96

Flowering status in thyme, 1990

Budding, plot H

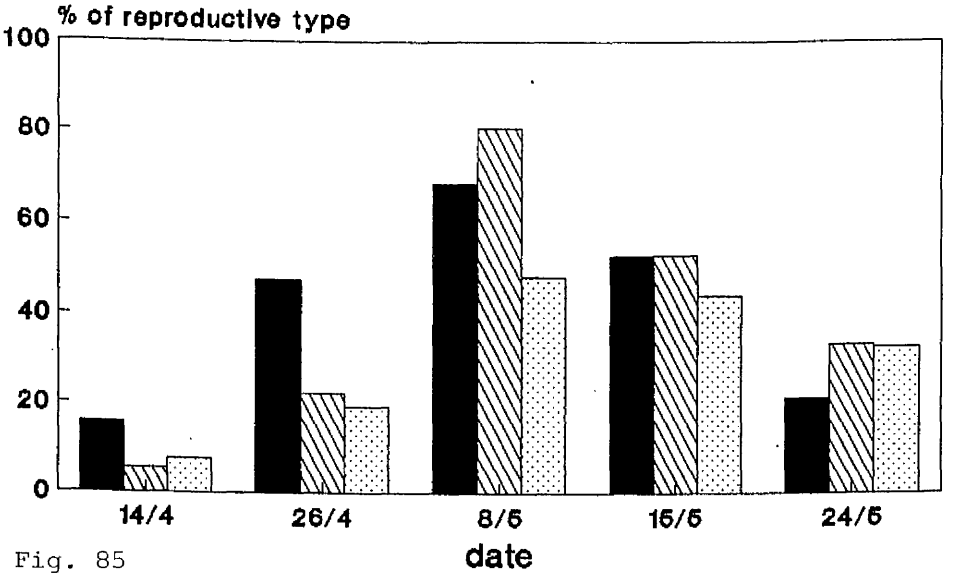


Fig. 85

Flowering, plot H

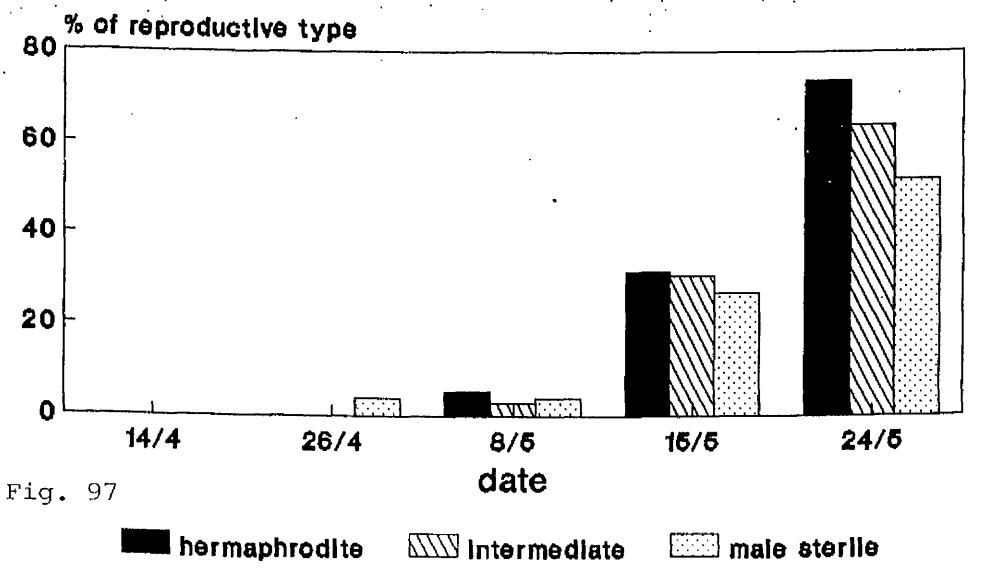


Fig. 97

5.11 OIL COMPONENTS

There were no significant differences, between plots, when comparing the mean total quantities of the 3 main oil components (thymol, ρ-cymene and γ-terpinene or when comparing the mean individual quantities of these compounds (Tables 24 - 29). However, there were differences between plants. In Table 24 the quantity of thymol varied from 45.1% of the oil to 61.7%, ρ-cymene varied from 9.9% to 28.1% and γ-terpinene varied between 7.4% and 13.6%. In general, γ-terpinene varied less than the other 2 components, as can be seen by referring to the relevant standard deviations (Tables 24 - 29). The total amounts of these three components also varied less. In Tables 28 (plot F) and 29 (plot H) a comparison was made between the percentage of the three main oil components in 1988 and 1989.

The plants were harvested at approximately the same ontogenetical stage in each year (Tables 30 & 31). In both plots, there was little difference in the mean values of each component between years although, on examining individual plants, it can be seen that there was some variation. However, in general, there was little difference in the total percentage of these three components between years and in the percentage of thymol. Plants which had a high percentage of thymol in 1988 were again found to have

Table 24: Percentage of the 3 major oil components and flowering status in plot A (1989).

Plant identity	% of major oil components#				Flowering status at harvest *
	A	B	C	total	
A11(R)	52.8	14.1	7.4	74.3	-
A11(L)	45.1	24.4	12.0	81.5	-
A16	45.3	28.1	9.1	82.5	-
A27	51.6	19.3	8.3	79.2	-
A29	50.9	19.3	11.0	81.2	-
A38	45.4	26.9	10.6	82.9	-
A45	51.2	11.2	13.0	75.4	-
A47	47.1	20.3	13.6	81.0	-
A61	47.5	22.9	11.3	81.7	-
A72	61.7	9.9	10.3	81.9	-
x	49.9	19.6	10.7	80.2	
SD	5.1	6.3	2.0	3.0	

* - = no buds or flowers present.

Major oil components:

A = thymol; B = ρ-cymene ; C = γ-terpinene

Table 25: Percentage of the 3 major oil components and flowering status in plot B (1989).

Plant identity	% of major oil components				Flowering status at harvest *
	A	B	C	total	
B10	57.9	15.7	10.0	83.6	B
B16	58.6	12.1	8.2	78.9	F
B27	-	62.4	23.0	85.4	-
B29	62.3	10.2	8.6	81.1	F
B40	57.8	9.0	11.2	78.0	-
B45	60.8	11.1	8.9	80.8	-
B56	64.8	7.6	8.2	80.6	-
B61	54.5	16.3	7.8	78.6	-
B63	54.8	13.4	12.4	80.6	-
B66	52.2	17.9	9.3	79.4	F
B87	51.2	20.1	7.7	79.0	-
B101	48.1	20.6	10.6	79.3	F
B102	51.2	17.6	10.2	79.0	F
B116	52.8	17.2	5.4	75.4	-
x	51.9	17.9	10.1	80.0	
SD	15.7	13.4	4.1	2.4	

- = no buds or flowers present
B = budding
F = flowering

Table 26: Percentage of the 3 major oil components and flowering status in plot C (1989).

Plant identity	% of major oil components				Flowering status at harvest *
	A	B	C	total	
C9	55.1	15.6	10.6	81.3	F
C31	64.2	12.3	7.4	83.9	F
C32	35.7	31.7	10.5	77.9	B
C39	53.3	15.4	5.7	74.4	-
C56	53.5	14.5	10.5	78.5	-
C58	47.2	20.3	10.8	78.3	-
C70	46.6	26.0	9.6	82.2	-
C79	36.7	18.3	5.5	60.5	F
C87	68.1	5.8	7.0	80.9	-
C89	54.7	10.8	8.1	73.6	-
x	51.5	17.1	8.6	77.2	
SD	10.4	7.5	2.1	6.7	

Table 27: Percentage of the 3 major oil components and flowering status in plot D (1989).

Plant identity	% of major oil components				Flowering status at harvest *
	A	B	C	total	
D5	59.1	13.5	8.2	80.8	-
D10	44.6	26.5	9.3	80.4	-
D39	50.0	17.4	10.6	78.0	-
D43	58.4	9.0	11.9	79.3	-
D48	37.3	25.0	11.6	73.9	-
D51	48.8	21.1	10.2	80.1	-
D72	50.6	19.4	9.0	79.0	-
D92	59.1	13.5	8.2	80.8	-
D111	58.6	13.7	9.3	81.6	-
D118	56.0	16.2	7.2	79.4	B
x	52.3	17.5	9.6	79.3	
SD	7.4	5.5	1.5	2.2	

- = no buds or flowers present
B = budding

Table 28: Percentage of the 3 major oil components in thyme plants (plot F).

Plant identity	% of 3 major oil components							
	1988				1989			
	A	B	C	Total	A	B	C	Total
2	57.1	3.1	15.9	76.1	52.9	19.9	8.6	81.4
3	44.7	10.2	23.7	78.6	47.3	17.6	15.1	80.0
4	62.6	7.4	9.8	79.8	58.0	14.0	9.0	81.0
6	51.3	11.7	12.3	75.3	54.2	18.3	9.4	81.9
13	64.5	7.8	8.1	80.4	59.9	13.5	7.4	80.8
14	43.6	22.4	10.2	76.2	44.1	22.4	10.7	77.2
16	60.4	11.2	9.2	80.8	53.3	20.2	7.9	81.4
17	53.4	20.1	7.9	81.4	55.8	17.3	7.5	80.6
26	51.0	17.6	9.3	77.9	54.0	18.4	8.2	80.6
27	55.1	8.7	7.9	71.7	59.8	8.7	8.0	76.5
29	-	-	-	-	56.5	11.3	11.7	79.5
31	65.1	5.5	9.5	80.1	57.1	12.9	10.5	80.5
34	52.5	18.4	9.3	80.2	-	-	-	-
37	62.9	8.1	8.7	79.7	58.1	13.3	8.9	80.3
38	54.1	13.0	13.5	80.6	50.6	19.9	12.2	82.7
40	31.1	30.4	16.0	77.5	39.3	28.6	12.4	80.3
48	57.5	9.5	12.6	79.6	54.4	17.9	8.1	80.4
52	50.0	23.3	10.4	83.7	-	-	-	-
57	56.8	12.2	11.8	80.8	-	-	-	-
58	56.0	14.2	7.7	77.9	58.2	15.7	8.5	82.4
59	58.9	11.1	8.4	78.4	61.1	12.0	8.4	81.5
60	51.2	16.1	15.4	82.7	45.5	24.5	11.6	81.6
63	53.8	20.6	8.2	82.6	53.5	19.6	9.1	82.2
68	60.8	13.9	6.7	81.4	53.3	16.3	9.8	79.4
74	-	-	-	-	51.1	18.3	9.1	78.5
79	58.8	13.4	9.7	81.9	56.6	15.9	9.0	81.5
81	-	-	-	-	52.1	20.1	7.8	80.0
87	48.9	23.8	7.4	80.1	50.0	24.2	6.4	80.6
89	45.6	18.9	13.9	78.4	47.8	22.5	9.3	79.6
93	61.5	10.8	9.1	81.4	63.0	13.3	6.4	82.7
94	31.9	15.2	10.4	57.5	39.9	13.7	11.5	65.1
118	57.0	11.5	13.2	81.7	57.2	16.2	8.2	81.6
x	53.7	14.1	10.9	78.8	53.3	17.5	9.3	80.1
SD	8.4	6.2	3.6	4.8	5.9	4.4	2.0	3.2

Table 29: Percentage of the 3 major oil components in thyme plants (plot H).

Plant identity	% of 3 major oil components							
	1988				1989			
	A	B	C	Total	A	B	C	Total
1	-	-	-	-	52.3	19.2	11.8	83.3
3	46.4	13.1	10.1	69.6	61.2	11.9	5.1	78.2
6	43.9	21.3	9.8	75.0	51.3	16.6	9.0	76.9
7	60.3	8.7	11.3	80.3	61.1	14.0	6.2	81.3
12	45.6	10.9	18.5	75.0	50.8	13.2	14.3	78.3
14	42.2	15.3	18.1	75.6	61.1	12.3	8.3	81.7
17	61.3	13.7	6.8	81.8	60.4	11.6	5.2	77.2
19	54.1	5.6	10.9	70.6	-	-	-	-
25	40.9	14.3	15.5	70.7	58.8	10.4	8.2	77.4
30	36.3	23.0	17.3	76.6	47.0	23.4	8.3	78.7
36	47.8	17.1	10.2	75.1	54.4	14.3	9.9	78.6
37	40.7	25.5	13.3	79.5	51.4	22.8	8.4	82.6
39	-	-	-	-	48.2	17.0	11.3	76.5
44	45.0	10.9	12.5	68.4	55.7	11.7	11.7	79.1
59	37.8	16.9	15.1	69.8	45.2	20.2	10.0	75.4
67	-	-	-	-	46.0	5.1	16.9	68.0
69	42.0	17.4	15.2	74.6	52.6	17.3	4.0	73.9
88	37.0	24.0	16.1	77.1	47.7	13.3	13.6	74.6
95	46.7	16.0	15.0	77.7	52.6	17.0	10.9	80.5
96	55.0	13.8	13.3	82.1	56.9	14.2	10.4	81.5
112	60.4	10.9	8.9	80.2	63.4	12.7	5.8	81.9
115	-	-	-	-	48.9	17.0	13.7	79.6
x	46.9	15.5	13.2	75.5	53.7	15.0	9.7	78.3
SD	8.1	5.4	3.4	4.3	5.6	4.3	3.4	3.5

a high percentage of thymol in 1989 (e.g. F13, F16, F93, H17) and *vice versa* (e.g. F40, F94, H59).

OIL CONTENTS

When the oil contents (% v/w leaf dry weight) of some plants in plots F and H were examined over two years (1988 & 1989) (Tables 30, 31) it was seen that, in most instances, there was a large increase in the values in the second year. This can also be seen when comparing Tables 32 and 33 (1988) with Tables 34 - 39 (1989). Tables 40 - 42 give the height, length and breadth measurements (1988, 1989) of the plants in Tables 30 and 31.

Table 43 shows values for plants with high percent oil content and high total oil yield (ml/m^2) whereas Table 44 gives values for plants with high percentage oil content but low total oil yield. Tables 45 - 46 give the heights, lengths and breadths of these plants, shortly before they were harvested. Similarly, plants in Tables 47 - 48 gave the lowest oil yield and lowest percentage oil respectively. Tables 49 - 50 again give height, length and breadth measurements for the plants in the previous two tables.

Although the mean oil contents (percent) are the same for plants in Tables 43 - 44, the leaf dry weight yields (g/m^2) are more than double in those plants in

the former table. There was no significant difference in the heights, length or breadths of these plants.

Table 30: Comparison of oil contents, in thyme, over two seasons (plot F).

	1988		1989	
Plant identity	Fl. status at harvest	Oil yield (% v/w)*	Fl. status at harvest	Oil yield (% v/w)*
F2	F	1.2	F	4.4
F3	F	1.0	-	2.9
F4	-	2.9	-	4.0
F6	B	3.5	-	4.6
F13	F	2.3	-	3.9
F14	-	2.6	-	3.9
F16	F	2.3	F	3.8
F17	B	3.3	F	4.0
F26	F	2.4	B	2.8
F27	F	1.0	F	2.8
F31	F	2.8	-	4.6
F37	F	0.8	F	2.5
F38	B	1.5	F	3.2
F40	-	2.1	-	3.8
F48	B	1.5	-	3.1
F58	-	1.7	-	4.0
F59	-	1.5	-	3.7
F60	F	1.9	F	3.6
F63	-	2.9	-	3.1
F68	F	3.0	-	2.1
F79	F	2.8	F	2.6
F87	-	3.3	-	4.9
F89	F	2.1	B	2.9
F93	F	3.2	-	3.9
F94	-	0.8	-	1.6
F118	F	1.6	-	3.5
x		2.2		3.5
SD		0.8		0.8

1988: PLANTED - 6/6/88
HARVESTED - 30/9/88

1989: CUT BACK - 26/7/89
HARVESTED - 2/10/89

* = v/w leaf dry weight

Table 31: Comparison of oil contents, in thyme, over two seasons (plot H).

Plant identity	1988 Fl. status at harvest	1988 Oil yield (% v/w)*	1989 Fl. status at harvest	1989 Oil yield (% v/w)*
H3	F	1.1	F	2.7
H6	-	2.4	F	3.6
H7	F	1.6	B	3.3
H12	F	1.3	F	2.5
H14	-	1.4	-	3.1
H17	-	2.7	-	3.8
H25	-	2.0	B	1.0
H30	F	1.3	B	2.2
H36	-	3.2	-	3.2
H37	-	2.1	-	3.2
H44	-	1.8	-	3.3
H59	F	2.9	F	3.8
H69	F	1.9	-	3.0
H88	-	1.9	-	1.0
H95	-	1.9	-	3.7
H96	B	3.9	-	3.7
H112	B	2.0	-	3.7
x		2.1		3.0
SD		0.7		0.9

1988: PLANTED - 15/6/88
HARVESTED - 30/9/88

1989: CUT BACK - 26/7/89
HARVESTED - 2/10/89

Table 32: Flowering status, percent oil contents and percent of 3 main oil components in thyme oil (plot E, 1988).

Plant identity	Flowering status at harvest	Oil* yield (% v/w)	% of major oil components		
			A	B	C
E1	B	2.8	56.8	13.3	11.0
E47	-	2.6	54.0	19.0	2.5
E60	F	1.0	61.6	4.1	9.8
E76	F	2.8	56.9	11.7	12.3
E87	F	2.5	56.9	13.7	11.1
E91	F	2.5	59.4	8.8	9.4
E102	-	2.6	55.0	10.6	11.6
E108	F	1.3	56.7	7.5	11.6
E120	-	1.6	41.9	17.5	12.7
x		2.2	55.5	11.8	10.2
SD		0.7	5.6	4.7	3.1

* = v/w leaf dry weight.

Table 33: Flowering status, percent oil contents and percent of 3 main oil components in thyme oil in leaves of some plants from plot G (1988).

Plant identity	Flowering status at harvest	Oil* yield (% v/w)	% of major oil components		
			A	B	C
G3	F	2.3	61.0	10.0	6.7
G14	-	1.9	55.9	15.0	9.6
G19	-	2.4	18.7	16.4	12.6
G34	-	1.5	54.6	9.5	15.7
G36	-	3.2	44.0	15.7	10.4
G38	-	1.9	46.5	5.0	22.6
G41	B	1.9	61.7	11.1	8.2
G52	F	2.0	45.0	21.3	13.0
G53	F	2.0	50.8	18.1	11.5
G54	-	2.2	47.3	15.3	17.4
G60	F	2.5	48.5	15.3	10.0
G63	F	1.9	50.8	18.1	11.5
G69	-	1.7	46.3	14.6	17.6
G79	F	1.1	53.7	11.6	16.4
G80	F	1.8	39.4	16.4	22.0
G81	pink F	1.8	43.3	14.2	13.0
G100	F	0.8	62.2	5.7	9.9
G101	-	2.2	51.3	11.8	11.9
G103	B	3.4	46.9	14.8	14.2
G117	-	2.0	47.5	16.6	15.8
x		2.0	48.8	13.8	13.5
SD		0.6	9.5	4.1	4.2

F = flowering
B = budding
- = no buds or flowers

* = v/w leaf dry weight.

Table 34: Leaf dry wt., drying ratios and oil contents of thyme plants, 1989 (plot A).

Plant identity	Leaf Dry Wt (g/m^2)	Oil		Drying ratio *		Oil Colour
		(ml/m^2)	(ml/100g)	Leaves	Stems	
A11(R)	48.3	1.4	2.9	22.0	7.6	yellow
A45	36.0	0.7	2.1	23.1	9.7	"
A72	71.1	2.9	4.1	21.2	9.1	"
A16	51.4	2.1	4.0	19.4	6.0	"
A61	44.1	1.5	3.4	21.5	9.2	"
A11(L)	34.9	1.1	3.2	15.0	8.6	"
A27	44.7	2.0	4.5	18.2	5.3	"
A29	44.8	1.3	3.0	19.0	7.2	p yellow
A38	46.7	1.3	2.9	19.6	6.5	yellow
A47	65.5	2.2	3.3	16.9	0.2	p yellow
x	48.8	1.7	3.3	19.6	7.9	
SD	11.6	0.6	0.7	2.5	1.7	

* Drying ratio = (D. Wt./total F. Wt.) x 100

Table 35: Leaf dry wt., drying ratios and oil contents of thyme plants, 1989 (plot B).

Plant identity	Leaf Dry Wt (g/m^2)	Oil		Drying ratio		Oil Colour
		(ml/m^2)	(ml/100g)	Leaves	Stems	
B29	51.4	1.9	3.6	19.7	7.1	p yellow
B40	42.2	1.1	2.6	21.2	10.9	yellow
B56	58.2	1.9	3.2	20.2	8.2	"
B63	52.2	1.6	3.1	20.7	7.8	"
B116	32.6	1.1	3.4	20.0	5.2	"
B27	43.1	1.0	2.4	20.5	7.0	vpy/cl
B87	33.4	0.9	2.7	18.6	6.0	yellow
B10	84.2	1.6	1.9	16.5	8.4	p yellow
B16	64.2	1.5	2.3	16.1	8.0	yellow
B45	52.0	1.3	2.5	20.5	10.3	"
B61	58.8	2.0	3.4	17.0	9.0	p yellow
B66	71.8	1.9	2.6	16.7	9.0	"
B101	53.6	1.3	2.5	19.8	6.1	yellow
B102	89.9	2.6	2.9	15.8	6.3	"
x	56.3	1.6	2.8	18.8	7.8	
SD	17.0	0.5	0.5	2.0	1.7	

Table 36: Leaf dry wt., drying ratios and oil contents of thyme plants, 1989 (plot C).

Plant identity	Leaf Dry Wt (g/m^2)	Oil		Drying ratio		Oil Colour
		(ml/m^2)	(ml/100g)	Leaves	Stems	
C58	58.8	1.8	3.0	15.1	6.9	yellow
C89	28.3	1.0	3.4	22.4	7.6	"
C79	62.8	3.4	5.4	16.0	6.8	"
C87	21.1	0.7	3.2	22.1	6.6	"
C9	62.1	1.5	2.4	13.1	7.0	"
C31	91.4	5.0	5.5	13.2	6.7	"
C32	49.4	0.9	1.8	14.4	5.6	"
C39	30.1	1.5	4.9	15.4	6.7	"
C56	36.2	0.4	1.2	15.0	7.6	d yellow
C70	28.1	1.6	5.8	15.1	6.9	"
x	46.8	1.8	3.7	16.2	6.8	
SD	22.0	1.4	1.7	3.3	0.6	

Table 37: Leaf dry wt., drying ratios and oil contents of thyme plants, 1989 (plot D).

Plant identity	Leaf Dry Wt (g/m^2)	Oil		Drying ratio		Oil Colour
		(ml/m^2)	(ml/100g)	Leaves	Stems	
D10	46.1	2.6	5.6	17.6	4.1	p yellow
D43	27.2	0.8	3.0	17.7	7.1	yellow
D72	34.7	1.5	4.3	17.1	6.3	"
D5	30.3	1.1	3.7	17.5	7.5	"
D48	34.3	0.9	2.6	18.0	6.5	"
D39	22.6	0.7	3.3	18.2	6.2	"
D51	30.9	1.1	3.6	18.1	5.9	"
D92	41.7	1.5	3.6	17.5	5.4	"
D111	31.0	1.3	4.1	16.9	4.5	d yellow
D118	36.1	1.1	3.1	14.0	5.8	"
x	33.5	1.3	3.7	17.3	5.9	
SD	6.8	0.5	0.8	1.2	1.1	

Table 38: Leaf dry wt., drying ratios and oil contents of thyme plants, 1989 (plot F).

Plant identity	Leaf Dry Wt (g/m^2)	Oil		Drying ratio		Oil Colour
		(ml/m^2)	(ml/100g)	Leaves	Stems	
F13	54.9	2.2	3.9	18.2	6.4	yellow
F16	50.5	1.9	3.8	17.4	7.5	"
F29	46.0	1.3	2.7	20.6	7.3	"
F74	23.4	0.7	3.2	17.7	9.4	"
F81	97.8	3.0	3.0	14.6	6.9	p yellow
F2	91.8	4.1	4.4	17.7	4.8	"
F3	50.9	1.5	2.9	19.6	5.8	"
F27	32.3	0.9	2.8	15.8	7.9	"
F38	61.7	2.0	3.2	17.8	9.8	"
F40	65.6	2.5	3.8	17.5	6.7	"
F79	42.6	1.1	2.6	14.0	5.1	"
F89	33.0	1.0	2.9	15.6	6.8	"
F4	53.8	2.2	4.0	17.8	8.4	"
F6	77.1	3.6	4.6	19.5	7.3	p yellow
F14	57.6	2.2	3.9	17.2	7.6	"
F17	86.8	3.5	4.0	13.5	7.5	"
F26	57.7	1.6	2.8	14.7	9.8	"
F31	40.3	1.9	4.6	19.1	8.9	"
F37	60.0	1.5	2.5	16.3	6.8	"
F48	47.4	1.5	3.1	18.3	6.5	"
F58	172.0	6.9	4.0	14.2	10.1	"
F59	44.2	1.6	3.7	17.2	7.0	"
F60	28.5	1.0	3.6	17.4	5.2	d yellow
F63	62.9	1.9	3.1	21.2	7.5	p yellow
F68	55.4	1.2	2.1	20.2	6.3	yellow
F87	67.0	3.3	4.9	16.7	6.2	p yellow
F93	57.7	2.2	3.9	16.2	6.7	"
F94	57.3	0.9	1.6	15.3	5.9	"
F118	85.3	3.0	3.5	14.9	6.2	yellow
x	60.7	2.1	3.4	17.1	7.2	
SD	28.1	1.3	0.8	2.0	1.4	

Table 39: Leaf dry wt., drying ratios and oil contents of thyme plants, 1989 (plot H).

Plant identity	Leaf Dry Wt (g/m^2)	Oil		Drying ratio		Oil Colour
		(ml/m^2)	(ml/100g)	Leaves	Stems	
H6	49.5	1.8	3.6	20.1	9.3	yellow
H12	42.0	1.0	2.5	20.7	8.4	"
H25	109.7	1.1	1.0	14.5	4.8	"
H88	68.5	0.7	1.0	16.9	5.5 d	yellow
H96	32.4	1.2	3.7	19.8	7.0	yellow
H115	65.7	-	-	14.4	6.3	-
H1	109.9	2.4	2.2	-	-	yellow
H3	65.5	1.8	2.7	19.5	6.1 p	yellow
H14	48.5	1.5	3.1	14.9	7.3	yellow
H30	67.5	1.5	2.2	15.3	6.8	"
H44	34.0	1.1	3.3	17.6	9.0	"
H67	29.9	0.3	1.0	20.1	1.8 d	yellow
H7	54.5	1.8	3.3	14.6	5.3	yellow
H17	76.4	2.9	3.8	17.0	6.9 p	y/y
H36	80.5	2.6	3.2	15.6	9.8	yellow
H37	81.3	2.6	3.2	13.2	8.3	"
H59	60.6	2.3	3.8	16.5	9.2	"
H69	17.2	0.5	3.0	19.9	6.1	"
H95	48.3	1.8	3.7	17.5	7.9 d	yellow
H112	79.0	2.9	3.7	18.6	5.4 p	y/y
H39	54.7	2.9	3.1	15.4	6.9	yellow
x	60.7	1.7	2.9	17.3	7.5	
SD	24.1	0.8	0.9	2.3	1.8	

Table 40: Measurements (mm) made over 2 seasons of plants harvested for oil determination (plot F).

Plant identity	1988			1989		
	Height	Width (x)	Width (y)	Height	Width (x)	Width (y)
F2	180	300	230	200	460	420
F3	190	260	210	210	420	390
F4	175	170	160	270	450	450
F6	195	240	180	260	510	460
F13	165	270	240	210	510	450
F14	170	270	200	220	580	510
F16	165	260	210	220	550	470
F17	200	250	230	220	500	490
F26	165	270	230	255	660	550
F27	145	190	160	290	560	410
F31	170	250	230	310	610	480
F37	165	270	240	250	570	440
F38	160	310	270	230	680	560
F40	135	250	210	260	690	470
F48	165	310	240	260	580	530
F58	165	270	200	230	620	470
F59	160	230	230	320	680	570
F60	155	240	230	290	570	440
F63	170	260	200	230	520	410
F68	140	220	200	200	490	440
F79	190	270	220	250	560	560
F87	165	250	180	235	540	540
F89	165	230	210	230	570	540
F93	145	240	240	280	590	500
F94	160	280	250	275	560	490
F118	150	250	240	230	510	490
x	165.8	254.2	216.9	247.5	559.2	481.9
SD	16.1	31.5	27.0	32.8	70.3	51.3

x and y = measurements made along axes at right angles to each other.

MEASURED: 11/8/88, 27/6/89

Table 41: Measurements (mm) made over 2 seasons of plants harvested for oil determination (plot H).

Plant identity	1988			1989		
	Height	Width (x)	Width (y)	Height	Width (x)	Width (y)
H3	170	220	160	240	590	580
H6	170	145	145	280	600	460
H7	160	190	170	300	580	400
H12	155	300	230	230	660	470
H14	205	180	170	320	620	460
H17	155	235	200	270	645	545
H25	155	160	140	230	500	380
H30	175	185	175	250	520	430
H36	110	185	180	230	550	430
H37	205	230	185	325	685	480
H44	265	270	170	250	620	540
H59	250	220	175	350	690	490
H69	145	185	170	230	470	420
H88	165	220	220	240	600	510
H95	180	240	235	280	650	510
H96	120	180	180	230	520	430
H112	175	200	200	310	740	400
x	174.1	208.5	282.7	268.5	602.4	466.8
SD	39.7	39.4	26.8	39.7	73.5	56.8

Some of the above plants were remeasured on 3/10/89, two months after all of the plots were cut back to the woody stems (26/7/89).

Table 42: Measurements of some plants in plot H, 2 months after they were cut back to old wood.

Plant identity	Height (mm)	Width (mm)	
		(x)	(y)
H3	230	520	470
H6	225	470	400
H7	260	460	420
H12	220	410	390
H14	240	420	400
H17	245	500	470
H25	220	420	340
H30	235	410	400
H37	285	580	430
H39	290	490	460
H44	255	450	450
H59	275	510	440
H96	220	440	400
x	246.2	467.7	420.8
SD	24.9	50.9	37.5

Table 43: Plants with high % oil contents and high total oil yield (ml/m^2) (>4.0% oil) (1989).

Plant identity	Total leaf dry weight (g/m^2)	Oil (ml/m^2)	Oil (%)	Oil colour
A72	71.1	2.9	4.1	yellow
C31	91.4	5.0	5.4	"
C79	62.8	3.4	5.5	"
F2	91.8	4.1	4.4	pale yellow
F6	77.1	3.6	4.6	"
F17	86.8	3.5	4.0	"
F58	172.0	6.9	4.0	"
F87	67.0	3.3	4.9	"
x	90.0	4.1	4.6	
SD	34.9	1.3	0.6	

Table 44: Plants with high % oil contents but low total oil yield (ml/m^2) (>4.0% oil) (1989).

Plant identity	Total leaf dry weight (g/m^2)	Oil (ml/m^2)	Oil (%)	Oil colour
A16	51.4	2.1	4.0	yellow
A27	44.7	2.0	4.5	"
C39	30.1	1.5	4.9	"
C70	28.1	1.6	5.8	"
D10	46.1	2.6	5.6	pale yellow
D72	34.7	1.5	4.3	yellow
D111	31.0	1.3	4.1	dark yellow
F4	53.8	2.2	4.0	pale yellow
F31	40.3	1.9	4.6	"
x	40.0	1.9	4.6	
SD	9.5	0.4	0.7	

Table 45: Height and width (x and y dimensions) of plants in Table 43 before they were cut back (1989).

Plant identity	Height (mm)	Width (x)	Width (y)
A72	245	540	470
C79	210	470	440
C31	235	430	460
F2	200	460	420
F6	260	510	460
F17	220	500	490
F58	230	470	620
F87	235	540	540
x	229.4	490.0	487.5
SD	19.2	39.3	64.3

Table 46: Height width (x and y dimensions) of plants in Table 44 before they were cut back (1989).

Plant identity	Height (mm)	Width (mm) (x)	Width (mm) (y)
A16	235	450	480
A27	280	430	450
C39	180	390	370
D10	210	460	430
D72	180	400	480
D111	210	420	480
F4	270	450	450
F31	310	610	480
x	234.4	451.3	452.2
SD	48.0	68.8	38.5

x and y measurements were made across the plants at right angles to each other.

Table 47: Lowest total oil yield (<1.0 ml/m^2) (1989).

Plant identity	Leaf dry weight (g)	Oil (ml/m^2)	Oil (%)	Oil colour
A45	36.0	0.7	2.1	yellow
B87	33.4	0.9	2.7	"
C87	21.1	0.7	3.2	"
C32	49.4	0.9	1.8	"
C56	36.2	0.4	1.2	dark yellow
D43	27.2	0.8	3.0	yellow
D48	34.3	0.9	2.6	"
D39	22.6	0.7	3.3	"
F74	23.4	0.7	3.2	"
F27	32.2	0.9	2.8	pale yellow
F94	57.3	0.9	1.6	"
H88	68.5	0.7	1.0	dark yellow
H67	29.9	0.3	1.0	"
H69	17.2	0.5	3.0	yellow
x	34.9	0.7	2.3	
SD	14.5	0.2	0.9	

Table 48: Lowest % oil (1989).

Plant identity	Leaf dry weight (g)	Oil (ml/m^2)	Oil (%)	Oil colour
B10	84.2	1.6	1.9	pale yellow
C32	49.4	0.9	1.8	yellow
C56	36.2	0.4	1.2	dark yellow
F94	57.3	0.9	1.6	pale yellow
H25	109.7	1.1	1.0	yellow
H88	68.5	0.7	1.0	dark yellow
H67	29.9	0.3	1.0	"
X	62.2	0.8	1.4	
SD	28.0	0.4	0.4	

Table 49: Height and width (x and y dimensions) of plants in Table 47 (1989).

Plant identity	Height (mm)	Width (mm) (x)	Width (mm) (y)
A45	230	440	410
B87	320	460	530
C87	190	350	390
C32	175	340	330
C56	160	340	310
D43	205	480	450
D48	255	490	480
D39	250	460	500
F74	230	620	470
F27	290	560	410
F94	275	560	490
H88	240	510	600
H67	270	610	430
H69	230	420	470
x	237.1	474.3	447.9
SD	44.6	92.8	76.4

Table 50: Height and width (x and y dimensions) of plants in Table 48 (1989).

Plant identity	Height (mm)	Width (mm) (x)	Width (mm) (y)
B10	210	470	530
C32	175	340	330
C56	160	340	310
F94	275	560	490
H25	235	500	380
H88	240	510	600
H67	270	610	430
x	223.6	475.7	438.6
SD	44.3	103.0	107.2

5.12 THYME PLANT PRODUCING NO THYMOL

Plant number B27 was sampled in October, 1989, and the oil was found to contain no thymol. When a further sample was collected and analysed in April, 1990, there was again no thymol. Values for B (ρ-cymene) and C (γ-terpinene) were very similar in the two samples and these were both much higher than in other, thymol-producing plants.

Table 51: Major oil components (> 5%) in B27 at 2 dates

Sample	Major oil components as % of oil			
date	A	B	C	total
2/10/89	-	62.4	23.0	85.4
18/4/90	-	64.5	21.3	85.8

The mean values of the main oil components for plants harvested from plot B on 2/10/89 (1) and mean values excluding B27 (2) are given below:

Table 52: Mean values of the major oil components from (1) plot B, (2) plot B excluding B27.

	Major components as % of oil			
	A	B	C	TOTAL
1	51.9 (15.7)	17.9 (13.4)	10.1 (4.1)	80.0 (2.4)
2	55.9 (4.9)	14.5 (4.3)	9.1 (1.8)	79.4 (2.0)

5.13 ANALYSIS OF OIL FROM OLD AND NEW GROWTH IN THYME PLANTS

Two plants from which half of the growth was removed on 2/10/89 were again sampled on 20/4/90. A sample was taken from the half which was previously sampled (new), in October, and from the half not sampled then (old). (See Table 51).

Table 53: Fresh and dry weights and oil quantity and colour from thyme leaves.

Plant Identity/ age	Total F. Wt. (g)	D. Wt.		Oil (ml)	Oil Colour
		Leaves	Stems		
H115					
(new)	59.71	9.26	3.85	0.10	p y/yellow
(old)	59.71	10.54	4.56	0.10	"
C31					
(new)	13.77	3.00	0.30	0.06	yellow
(old)	40.83	7.22	4.41	0.12	"

Table 54: Oil % and components in thyme leaves.

Plant identity/ age	Oil (%)	Major components as % of oil*			
	leaf D. Wt.	A	B	C	TOTAL
H115 (new)	1.1	48.0	20.9	10.7	79.6
(old)	1.0	48.9	17.0	13.7	79.6
C31 (new)	2.0	38.7	22.4	12.6	73.7
(old)	1.7	34.7	21.9	22.1	78.7

* A = thymol; B = ρ-cymene ; C = γ-terpinene

5.14 CHANGES IN THYME OIL (% V/W) AND MAIN COMPONENTS AT TWO GROWTH STAGES.

Plant material harvested from 6 plants at two different growth stages showed differences in the proportions of the main oil components (Figs. 98, 99). There were also differences in the amount of oil (& v/w leaf dry weight) (Fig. 100) at the two harvests (July, 1989 [end of flowering]; October, 1989 [young growth]). The July plant material was harvested at the same time that the plants were cut back to old wood, after flowering. The October harvest consisted of the new growth which had taken place since July. The young growth showed little difference in the quantity of thymol in each of the six plants and the total of the three main components was almost constant for all of the plants. However, there were slight differences in the amounts of ρ-cymene and γ-terpinene, which had mean values of 16.4 (4.5) and 9.8 (2.6) respectively. From the July sample, the six plants could be split into two groups: group 1 contained the three plants from plot F and all of these contained ρ-cymene in the oil; in group 2 none of the plants contained ρ-cymene and the amount of γ-terpinene was less than in group 1. However, these latter plants contained more thymol in the oil (mean = 59.9% [2.6]) than the former (mean = 36.9 [4.7]). This may be due to the fact that many plants in plot F

were noted to have new growth present at around the time that these plants were harvested. It has been shown that -terpinene is produced in greater amounts in young leaves and is gradually converted to thymol, via ρ-cymene, as the leaves (glands) age (Fig. 11). The plant material harvested in October contained more thymol (v/w leaf dry weight) than that harvested in July (1.8 [0.48]) compared with 0.6 [0.05] (Fig. 101).

In all except one of the plants the percentage of oil (v/w leaf dry weight) was greater in the young growth than at the end of flowering (seeds set). The mean value for the former was 3.9 (0.6) and the latter 1.5 (0.7).

Thyme oil at 2 growth stages

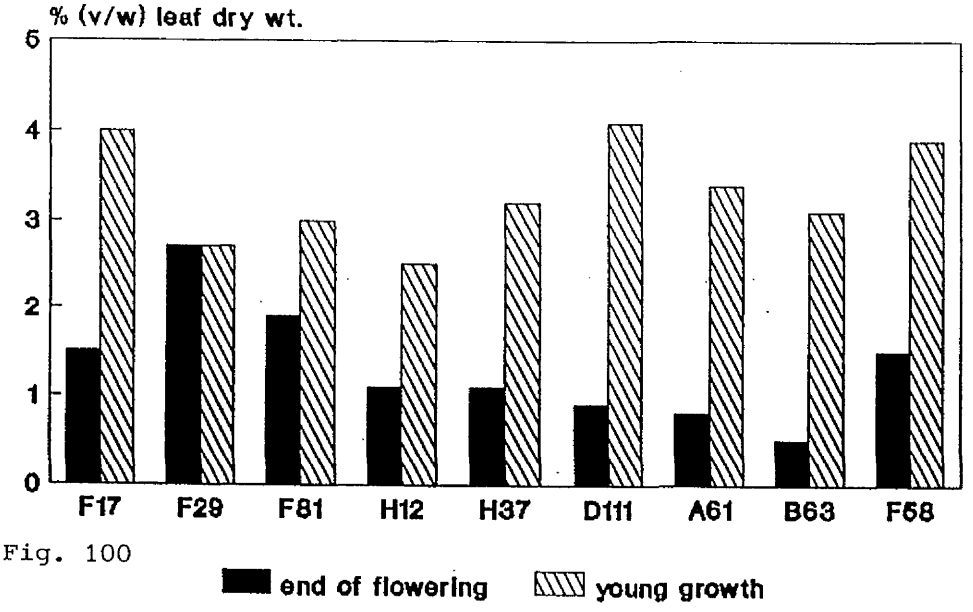


Fig. 100

1989

Thymol in thyme leaves

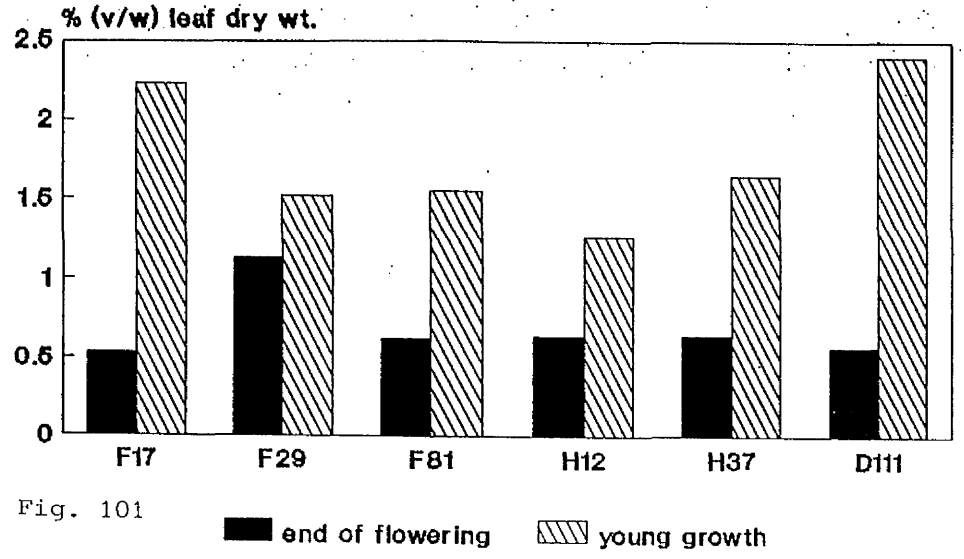


Fig. 101

1989

THYME OIL GLC results
July 1989

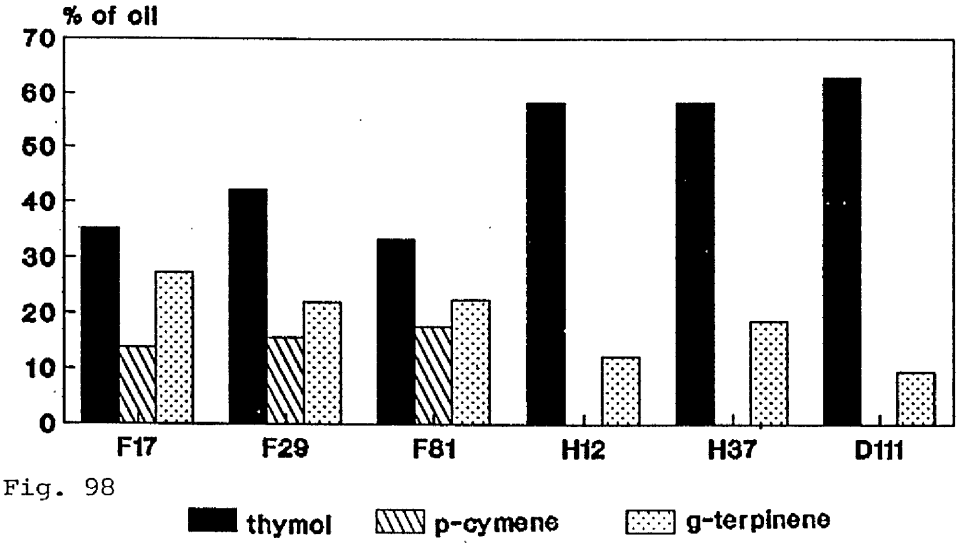


Fig. 98

thymol p-cymene g-terpinene

end of flowering

October, 1989

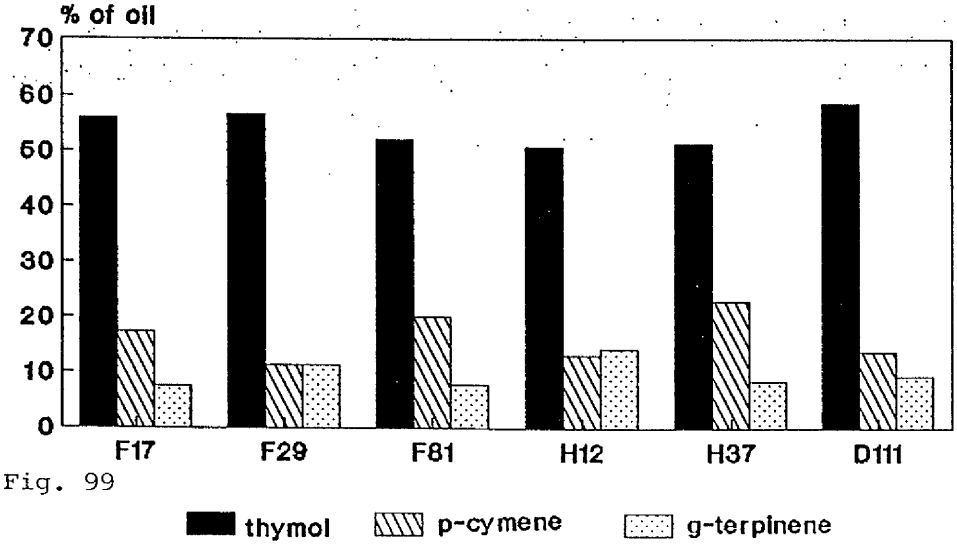


Fig. 99

thymol p-cymene g-terpinene

young growth

5.15 DIFFERENCES IN THYME OIL IN YOUNG AND OLD LEAVES

On examining the oil glands seen on leaves which were being measured (Figs. 58 - 71) it was found that there was a change in the colour of the oil on passing along the stem, from the tip to the base. A representative sample of the plants was sampled, between 18/4/90 and 26/4/90, to find if the difference in oil colour coincided with differences in the oil components.

With F75 and B97 the change in colour occurred at around 4.5 cm from the tip of the stem and with B14 at around 2.5 cm from the tip. The plant material was, therefore, separated at these points and the tops and bases of the stems dried, distilled and run on the GC separately. The results were as follows:

Table 55: Fresh and dry weights of tops and bases of thyme stems run separately to determine differences in oil in young and old thyme leaves.

Plant identity/ age	Total F. Wt. (g)		Total D. Wt.(g)	
	Tops	Bases	Tops	Bases
F75	38.59	21.81	9.38	6.40
B14	31.32	46.76	6.44	10.65
B97	49.30	33.85	11.05	8.07

Table 56: Leaf and stem dry weights and oil quantity and quality in dry leaves from tops and bases of thyme stems.

Plant identity/ age	Dry weights		Oil		Oil components *		
	Leaves	stems	(%)	(ml)	A	B	C
F75							
(tops)	8.14	1.21	1.11	0.09	37.1	19.7	22.3
(bases)	3.69	2.85	0.54	0.02	19.2	-	52.4
B14							
(tops)	6.47	0.32	1.55	0.10	39.6	21.9	12.5
(bases)	5.26	5.65	0.76	0.04	39.9	-	29.2
B97							
(tops)	8.82	2.23	1.6	0.14	51.6	13.5	11.7
(bases)	3.25	4.82	1.2	0.04	45.6	-	27.4

* Major oil components as % of oil:

A = thymol; B = ρ-cymene; C = γ-terpinene

There was half the quantity of oil (% v/w leaf dry weight) in the leaves on the lower part of the stems (bases) of F75 and B14 as in the tops (Table 56). There was only slightly less oil in the lower leaves of B97. No ρ-cymene was found in the lower leaves from any plant and there was considerable variation in the amount of γ-terpinene between upper and lower leaves in all of the plants. Only F75 showed a marked difference in the quantity of thymol (as % of oil) in the top and base leaves.

5.16 ANALYSIS OF THE OIL FROM FRESH & DRY THYME

In order to be satisfied that the drying process did not alter the quality or quantity of oil produced on distillation, samples from three plants were divided so that half could be distilled fresh and the other half dry. There was virtually no difference in the quality of oil from plant number F102 but the other two showed a slight variation in the values of some of the main components. The quantities of oil obtained in the fresh and dry samples were almost identical.

Table 57: Fresh and dry weights and quantity of oil obtained from fresh and dry thyme leaves.

Plant identity/ age	Total weight (g)		Dry weight (g)		Oil
	Fresh	Dry	Leaves	Stems	(ml)
F102 (fresh)	42.74	-	-	-	0.10
(dry)	42.74	10.91	6.94	4.00	0.09
H114 (fresh)	47.00	-	-	-	0.05
(dry)	47.00	10.73	6.89	3.84	0.05
B97 (fresh)	43.00	-	-	-	0.09
(dry)	43.00	9.29	5.78	3.51	0.08

Table 58: Major oil components in fresh and dry thyme.

Plant identity/ age	Major oil components as % of oil *				
	A	B	C	D	(A+B+C)
F102(fresh)	31.5	19.7	26.3	-	77.5
(dry)	32.1	18.6	26.9	-	77.6
H114(fresh)	26.6	35.1	13.3	9.1	75.0
(dry)	27.2	29.5	14.5	15.3	71.2
B97 (fresh)	50.7	13.0	13.8	-	77.5
(dry)	48.7	12.3	15.4	5.5	76.4

* OIL COMPONENTS:

A = thymol; B = ρ-cymene; C = δ-terpinene;
D = unknown

6 DISCUSSION

Of the many edible species of thyme, the most commonly used, commercially, are *T. vulgaris* and, in Spain, *T. zygis* (Greenhalgh, 1979a). The main commercial thyme producers are in the Mediterranean region, although Britain and the U.S.A. produce a small proportion of their own annual requirements from cultivated plants. Most of the thyme collected for oil production comes from the wild, since it is uneconomical to cultivate the plant for this purpose (Greenhalgh, 1979b).

Although improved varities of *T. vulgaris* are grown, commercially, in Central and Eastern Europe (Technologii, 1982), the seed lots available in this country show great morphological variability. The eight seed lots supplied by Nutting and Son Limited, at the start of this work, were from different 'varieties' of *T. vulgaris* and each of these was grown separately in order to compare inter-plot and inter-plant variability in growth, time of flowering and oil production.

Differences in height, length and breadth between plots were not statistically significant; however, there were differences in these characteristics between plants, both within and between plots, and plants were, in general, much larger in 1989 than in 1988. There was around a fifty percent increase in

mean oil content (v/w leaf dry wt.) between 1988 and 1989. However, the relative amounts of the main components (thymol, ρ-cymene, γ-terpinene) were very similar in each year. There were, again, inter-plant differences in these components, but individual plants gave similar proportions of these three compounds in both years and the total amount of thymol, ρ-cymene and γ-terpinene was very similar in most of the plants. Examination of leaves from different parts of the stem showed a disappearance of ρ-cymene in older leaves. This was also seen in leaves from senescing flower stems.

It was noted, in 1988, that plants with hermaphrodite flowers began to flower somewhat earlier than plants with intermediate or male sterile flowers. This also occurred in 1989. It also appeared that a very small proportion of the plants produced flowers which seemed to be male sterile or intermediate at the start of the flowering season but produced hermaphrodite flowers later.

In thyme, seeds are shed close to the parent plant (Encyclopaedia Britannica, 1974; Valdeyron *et al.*, 1977) and they are capable of germinating when shed, under suitable conditions, as found in this investigation. This causes problems for the grower and the seed producer. Since thyme is a perennial species, seedlings may establish between the parent

plants, making harvesting difficult. The young seedlings, as well as weeds, can be prevented from germinating by applying a black polythene mulch around the plants, although this may be difficult to carry out on a large scale.

Seed producers must decide on the best time to harvest for maximum seed production; too soon and few seeds will be ripe, too late and many will have been shed. The problem of seed loss occurs with, for example, oilseed rape (*Brassica napus* L.) and beet (*Beta vulgaris* L.) where ripe seeds are easily shed. Timing of the harvest is crucial in order to maximise seed harvest. In oilseed rape, breeders have developed varieties with more erect seed pods, where the pod bearing branches are compressed into a 'mop head', which are less prone to lose seeds and then only those from the outer pods (Scarisbrick and Daniels, 1986). Although there is little variation in the shattering characteristics of varieties, some resistance to pod shattering has been transferred from brown mustard (*B. juncea* L.) by workers in Australia (Scarisbrick and Daniels, 1986), leading to increased seed yields at harvest.

6.1 PLANT SIZE

There were no statistically significant differences in height between plots (i.e. between seed

between plots, in length and breadth, in 1988 were not maintained in 1989. There were no statistical differences in these characters between sites (Templefield *vs.* Diamondfield) and no significant edge effects within plots. However, there was a great range in height, length and breadth between plants (i.e. within a 'variety') which was a result of differences in internode distance and/or extra growth (these effects could not be distinguished by the present observations).

Although the mechanism for these differences in internode length is not understood, gibberellins are known to increase cell division (Moore, 1979; Phillips, 1971), in sub-apical meristems (Phillips, 1971). They are also involved in cell elongation, in the presence of auxins (indole acetic acid or I.A.A.), in the internodes of young stems, whereas ethylene inhibits internode elongation (Phillips, 1971). I.A.A. promotes cell wall plasticity, and gibberellins may regulate membrane permeability (Moore, 1979). Abscisic Acid (A.B.A.) is thought to reduce activity in sub-apical meristems and to oppose gibberellin-induced cell division. It is suggested that they are antagonists, and act to regulate the rate of cell division in the sub-apical meristem (Phillips, 1971).

In the barley aleurone layer, gibberellins are known to turn on the synthesis of some hydrolytic enzymes selectively, and this may occur in the

regulation of growth and development in higher plants (Moore, 1971).

Phytochrome has been implicated in the synthesis of gibberellins and work on plants illuminated with red light showed an increase in gibberellin-like substances which were biologically active. When the red light was followed by illumination with far red light this effect was no longer seen (Moore, 1979).

It is possible that, in a species where little selection has been done by man, specific hormone levels vary. Within such a variable species, some plants may be more or less adapted to the different levels of radiation. As a result, differences in internode distance may be found in a given radiation environment. If radiation levels were higher, or lower, clonal plants might produce stems with shorter, or longer internodes, or they may remain unchanged.

When selecting for this characteristic, therefore, it would be necessary to grow the plants in a variety of radiation environments in order to determine the variability of the characteristic.

Differences in internode distance, if genotypic rather than phenotypic, would allow selection of plants with a particular growth habit for use in breeding to obtain a compact plant, for example, with short internodes. This type of plant would be ideal for rockeries and exposed sites where more dwarfed

plants would be less exposed to the mechanical and drought stresses than taller plants. A plant with very large distances between internodes is likely to become straggly and unsightly and, unless there is some other redeeming feature, will be rejected for ornamental use. Where internode distances are intermediate, the plant may remain fairly upright and this type would be ideal for mechanical harvesting for herb or essential oil where the previous examples would not. This was the type selected in the present experiment. The permanence of this morphology will be examined in later generations.

6.2 VARIATION IN OIL CONTENT AND MAJOR OIL COMPONENTS

Variation in oil content (v/w leaf dry wt.) between years was noted in many of the plants examined (Tables 30, 31). Overall, oil content increased from around 2% to more than 3%, giving an increase in plant oil yield, which was in addition to the increase due to plant biomass noted from 1988 to 1989. This difference in oil content was not associated with changes in levels of the major oil components from year to year. Although the total percentage of phenols and their precursors (thymol, γ-terpinene and ρ- cymene) in the oil was very similar in all of the plants (Figs. 24 - 29, 32 and 33), and all of the harvested material was of the same age (in days),

there were differences in individual percentages of these three main components. This may have been due to differences in the rate of development of the plants (see pp. 34 - 35).

An ontogenetical study of some of these plants would determine whether lower thymol levels, for example, were a result of intrinsically lower levels or differences in the rate of development.

6.3 YIELD PER UNIT AREA

Oil yield per unit area was examined only in 1989, and differences between plants were found. The marked difference in leaf dry weight (g/m^2), oil yield (ml/m^2) and percentage of oil (ml/100g) which were observed between individual plants (Table 34 - 39) showed that there is a great potential for selection for these characters in a breeding programme, or for direct utilisation of plants with the desirable characters for clonal production of dried leaf material and/or oil production.

Harvest Index is a measure of the economic yield of a crop as a proportion of the biological yield (dry weights). In cereal crops, the economic yield is the weight of grains produced and the Harvest Index may be affected by environmental factors throughout the growing period of the crop (Downes, 1988). It is thought by some that the efficient use of radiation

and photosynthate plays a large part in crops with high Harvest Index (Downes, 1988). In modern wheat cultivars, incorporating dwarfing genes, which were selected for high grain yield, it was found that Harvest Index was also increased (Hay and Walker, 1989; Downes, 1988). Increased grain yields and Harvest Indices (up to 0.5) resulted from partitioning of assimilate away from stem production (reduced stem wt./unit area) and towards the ear to give greater survival of florets (Hay and Walker, 1989). This effect was due to the presence of the dwarfing genes, although the connection is not yet understood.

Since Harvest Index is heritable (Downes, 1988), it should be possible to select plants with high Harvest Index, although high Harvest Index does not automatically give high economic yield. In cereal crops, tillering, pests, diseases, soil fertility, available water and temperature at germination, flowering and seed set may affect Harvest Index (Pearson, 1984; Gutschick, 1987; Downes, 1988). Crops such as kale or forage rape, which are used as animal feeds, where the whole of the above ground crop is utilised, are not expressed in terms of Harvest Index (Harper, 1983). In crops whose economic yield consists of leaves, the Harvest Index tends to be higher than in cereal crops. For example, in thyme this is 0.74 (S.D. = 0.05) (unpublished data). A delay in flowering may increase biological yield

(Downes, 1988) and may affect Harvest Index by prolonging vegetative growth, since flowering and seed production can divert photosynthate away from vegetative growth.

The relative stability of the composition of the oil of a given plant may also be used in selecting types which produce a greater percentage of, for example, thymol which is a valuable component in pharmacy and in some cosmetic preparations (Simon *et al*, 1984).

As already noted (p. 190) and in contrast to the inter-plant variability in other characters (e.g. height, length, breadth) the total percentage of thymol plus its immediate precursors (γ-terpinene and ρ-cymene) in the oil was remarkably similar in all of the plants. This finding is similar to that of Svoboda (1988) for summer savoury (*Satureja hortensis* L.)

6.4 VARIATION IN PHENOLIC PRECURSORS

All of the work previously done on this and other species suggests single steps between γ-terpinene -> ρ-cymene -> thymol (Fig. 11). The oil from plant number B27 was found to contain no thymol. The proportion of ρ-cymene in the oil was very much higher than was seen in any other plant examined and the proportion of γ-terpinene was also higher (Table 51).

It appears, therefore, that the enzyme necessary for the conversion of ρ-cymene to thymol is missing in this plant, leading to a build up of ρ-cymene and, to a lesser extent, γ-terpinene. It can be seen that the total proportion of these two compounds in the oil of B27 is approximately equal to or a little higher than the thymol plus precursors found in the oil from other plants examined (Tables 51, 52).

It appeared, from two different pieces of evidence, that as leaves (glands) aged they ceased producing ρ-cymene. Firstly, when stems from three plants were divided up, so that the older leaves (bases of stems) and the younger leaves (tops of stems) (Table 55) were distilled separately, it was seen that there was no ρ-cymene in the oil samples from the older leaves. There was also less oil (% v/w leaf dry wt.) in the older leaves from each of the plants. The total percentages of the three main components (thymol, ρ-cymene, γ-terpinene) were somewhat lower in the older leaves. It appeared from two of the results in Table 56 that the disappearance of ρ-cymene led to a similar increase in the proportion of γ-terpinene, its precursor. Table 59 shows that although there was a large increase in the proportion of γ-terpinene in the oil of the base leaves (134%) this translated to a smaller increase in the actual quantity of γ-terpinene in these leaves (12

- 74%) (% v/w leaf dry wt.). In each case the amount of thymol (% v/w leaf dry wt.) was much lower in the base leaves (34 - 75% lower).

Table 59: Quantity (% v/w leaf dry wt.) of the 3 major oil components in leaves from tops and bases of thyme plants.

Plant identity	Oil component*			
	A	B	C	A+B+C
F75 (tops)	0.41	0.22	0.25	0.88
(bases)	0.10	0	0.28	0.38
B14 (tops)	0.61	0.34	0.19	1.14
(bases)	0.30	0	0.22	0.52
B97 (tops)	0.83	0.22	0.19	1.24
(bases)	0.55	0	0.33	0.88

* A = thymol; B = ρ-cymene; C = γ-terpinene

Secondly, when leaves were harvested from 6 plants at two different ontogenetical stages (before flowering and after flowering) there was ρ-cymene in the oil from all of these plants at the former stage but none in three of the latter. In the case of the three plants which gave ρ-cymene in the oil at this stage (all from plot F), it may be that there was some new growth collected when this material was harvested. It was noted that there was new growth in this plot at the time of harvest. Here again, there was less oil

(% v/w leaf dry wt.) in the leaves harvested at the end of flowering (Fig. 100) and the quantity of thymol was lower in the older leaves (Fig. 101). At this stage the plants from plot F gave slightly more oil than the others.

Tanaka *et al.* (1988) showed that monoterpenes present in young *T. vulgaris* leaves were closely associated with the number of peltate glands present on the leaves and Werker *et al.* (1984) found that , in the species they examined, cells of peltate glands secreted oil until the leaf was mature. In *Mentha piperita* (Loomis and Croteau, 1980) and *Origanum dictamnus* (Bosabalidis and Tsekos, 1982) secretory cells disintegrated after secretion was complete and the oil remained in the space formed between the head cells and the cuticle (Plates 6 - 9) as there were no pores and the cuticle was covered with a thin layer of wax. Although each of the plants examined was separated into tops and bases at a predetermined point (where the colour of the oil in the glands changed from yellow to dark yellow) it may be assumed that there were differences in leaf maturity on passing along the length of the stem. By separating the stems nearer to the top or the base the proportion of the main components would have been different, in the first example. In the second example, it must be assumed that all of the leaves on the flowering stems were mature at the time of harvest and that secretion

of oil had ceased. It seems possible that, in these mature leaves, the cuticle may be more easily torn, allowing evaporation of the stored monoterpenes. However, Croteau *et al.* (1984, 1987) showed that plants can convert terpenes to primary metabolites and that specific products may change from day to day or over longer periods. This may be controlled by the balance between the rate of photosynthesis and the use of photosynthate or by the balance between growth and differentiation (Croteau and Johnson, 1984). Croteau and Sood (1985) suggest that the metabolic turnover of monoterpenes may be a mechanism for recycling carbon and energy from mature leaves, of *Mentha piperita*, to give metabolites for use by the rhizome. Movement of these metabolites from the leaves to the roots of flowering sage plants has also been demonstrated (Croteau *et al.*, 1987). Here, in the older leaves of the thyme plants, recycling of stored oil to provide primary metabolites may be involved .

Alternatively, the disappearance of ρ-cymene in the older leaves may be due to feedback, from the biosynthetic pathway leading to production of thymol, blocking or switching off the enzymes which produce ρ-cymene from γ-terpinene or to an ontogenetical decrease in the production of the enzyme necessary for the formation of ρ-cymene from its precursors a) by degradation of the RNA molecules responsible for

production of ρ-cymene, b) by switching off the genes which produce these RNA molecules. Although the results obtained here for oil components reflect the situation with the oil glands only at the time of harvest, the fact that the total percentage of phenol and precursors appears to be fairly constant may imply that these three components are in a state of dynamic equilibrium. As the quantity of one increases the quantity of at least one of the others should decrease. It may be that, as the proportion of thymol reaches a predetermined maximum in the oil, there is a feedback mechanism operating on its precursors. This maximum percentage of thymol may be genetically determined in each plant but may possibly be affected by the environment and how well the plant is adapted to that environment.

6.5 REPRODUCTIVE TYPE

The somewhat earlier flowering of the hermaphrodite plants may ensure that bees will visit the plants because pollen, as well as nectar, is required early in the season for rearing the brood (Park, 1949; Vernon, 1986). Pollen, which provides protein, vitamins, fats and minerals in the bee diet is mainly eaten by nurse bees which produce 'royal jelly' for the queen and young larvae to eat (Hodges, 1952). It is also used as an food source for adult

bees when they manufacture comb (Hambleton, 1949). Although bees may visit more than one flower species during a season, and sometimes collect only pollen or nectar on a single trip, they remain constant to a single pollen and nectar source until these are exhausted (Vernon, 1986).

Sight and smell allow bees to find pollen and nectar but they cannot tell if there is nectar in a flower until their proboscis is inserted. If there is, they remain to drain off all of the nectar which is used to produce honey. If there is none, they quickly pass on to another floret or flower. If the floret has recently been visited by a bee the odour puts off other bees from landing. It is thought that the odour disappears after a time. A bee may visit from 50 to 1000 florets to collect a full load of nectar, depending on the species and, given a choice, will visit plants with the richest, most concentrated nectar. The average sugar concentration of nectar varies (e.g. chickweed - 50%; pear trees - 3 to 40%) (Grout, 1949).

The larger hemaphrodite flowers which provide both pollen and nectar for the bees early in the year may fix the interest of the bees until all of the flowers (including later opening intermediate and male sterile flowers) are finished. It is possible that if the bees were to encounter a group of these plants where mostly intermediate and male sterile flowers

were open they might move on to another site where pollen was also available as this is an invaluable food source both for adult bees and for larvae. If all of the reproductive types began to flower at the same time the same problem would arise. Since the ratio of male sterile and intermediate plants to hermaphrodite plants is approximately four to one then, on average, a bee would be four times as likely to encounter a non-pollen producing plant as a pollen producer (assuming that the hermaphrodite plants are not intrinsically more attractive to the bees than the other types are). This may also be an adaptation which ensures successful pollination of hermaphrodite flowers (see p. 62).

6.6 BREEDING

Although self-pollination is possible in this species, there is usually inbreeding depression of vigour in cross-pollinated species (Poehlman, 1959). The small size of the individual florets and the large number present on each branch makes emasculation a very time-consuming and labour-intensive job for the plant-breeder. However, in breeding thyme, it is possible to use the fact that there are both hermaphrodite and male sterile plants. When crossing plants, a hermaphrodite plant is used as the pollen donor and a male sterile plant as the female parent.

It is only necessary then to transfer the ripe pollen to a ripe stigma. This should be quite possible since there is a continual production of ripe florets over some weeks.

In a breeding programme, plants which were shown to give high dry weight yields of leaves and a high percentage of oil (Table 43) would be selected and allowed either to cross-pollinate freely (mass selection) or be crossed, individually, with other selected plants. The progeny from these crosses would be grown on and tested for high yield (progeny selection), and back-crosses to the parental plants used to produce plants homozygous for the required characters.

By using progeny selection it is possible to establish the effect of the environment on the line. If around 50 plants are grown, from a single parent the variability (heterozygosity) of the line can be seen (Poehlman, 1959). Selfing (where possible) gives progeny which are homozygous. This may be continued for more than one generation, to find the desired combination of characters, depending on the loss of vigour with each selfing and the selected homozygote crossed to restore lost vigour. A number of progeny lines showing the required characters may be grouped and allowed to cross-pollinate freely (Poehlman, 1959).

It would also be possible to reproduce selected plants, after testing in different environments or in different years to ensure the stability of the characters, by conventional means (cuttings, layering) or by tissue culture. By using the latter method it may be possible to produce thousands of plants at a reasonably competitive price.

For ornamental use, flower colour and size may determine the plants acceptability. Although most of the plants in the plots examined had flowers of a single shade, there was sufficient variation in flower colour between plants to allow selection of those with darker or lighter flowers (Plates 7 & 8) to suit different requirements. Where the flowers are the most important visual requirement, it may be better to select a hermaphrodite plant with the required flower shade. The larger size of these flowers gives the impression of a more floriferous plant and they also flower slightly earlier.

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APPENDIX I

A LIST OF LABIATE SPECIES FOUND IN THE BRITISH HERBAL PHARMACOPOEIA.

Ballota nigra L. - black horehound (a native)

Betonica officinalis L. - betony; bishopswort; wood betony; *Stachys officinalis* (L) Trev; *Stachys betonica* Benth. (a native)

Collinsonia canadensis L. - stone root; knob root; heal all

Hyssopus officinalis L. - hyssop

Lavandula officinalis Chaix. - lavender; lavender flowers; *L. spica* L.

Leonorus cardiaca L. - common motherwort

Lycopus europaeus L. - gypsywort; water horehound; bugle weed (a native)

Marrubium vulgare L. - hoarhound; white horehound; horehound (a native)

Melissa officinalis L. - balm; lemon balm

Mentha piperita L. - peppermint; *M aquatica x M spicata* (a native)

Mentha pulegium L. - penny royal; pulegium; European pennyroyal (a native)

Nepeta cataria L. - catmint; catnip; catnep (a native)

Nepeta hederacea L. - ground ivy; *Glechoma hederacea* [L] Trev.; *Nepeta glechoma* Benth. (a native)

Rosmarinus officinalis L. - rosemary

Salvia officinalis L. - sage; red sage

Scutellaria laterifolia L. - skullcap; hoodwort; helmet flower; Quaker bonnet

Teucrium chamaedrys L. - germander; wall germander

Teucrium scorodonia L. - wood sage; garlic sage: wood germander (a native)

Thymus serpyllum L. - wild thyme; mother of thyme; quendel serpyllum; herba serpylli; serpolet (a native)

Thymus vulgaris L. - common thyme; garden thyme; thyme; rubbed thyme

APPENDIX II

THYMUS SPECIES USED BY MAN (Uphof, 1959; Clapham *et al.*, 1962; Simon *et al.*, 1984)

Thymus acinos L. - *Satureja acinos*

Thymus capitatus Hoffm. & Link. = *Coridothymus capitatus* (L) Reichb. (syn - see appendix III, conehead thyme) - native to the Mediterranean Portugal and Middle East. The essential oil known as Spanish origanum oil is distilled from this plant and is used as a flavouring. It contains a high percentage of carvacrol. This is the main Thymus species grown in Spain. Used locally in place of oregano and was used to alleviate bronchial asthma by early settlers in America.

Thymus chamissonis Benth. = *Micromeria chamissonis* (Benth.) Greene (yerba buena) - found in the west of North America the Californian Indians use the dried leaves in place of tea.

Thymus hirtus Willd. - (syn - see appendix III) a herbaceous perennial found in North Africa and Spain. The ground leaves are used for seasoning and a decoction of the leaves is used to relieve stomach disorders.

Thymus mastichina L. (mastic thyme; Spanish marjoram)

- native to the Mediterranean and Portugal and a herbaceous perennial this species gives an essential oil on distillation which is used for flavouring (oil of marjoram; oil of wild marjoram).

Thymus serpyllum L. (creeping thyme; wild thyme; mother-of-thyme; 2 sub-species known [appendix III]) - native to Northern Europe, temperate Asia, Africa and North America. Height is 2 - 7 cm and spread is 60 cm or more. The steam distilled essential oil obtained from creeping thyme contains carvacrol, thymol and cymene as its major components. A decoction of the dried leaves and flowers is said to be antispasmodic and has been used to relieve whooping cough. Safe for human consumption for flavouring, seasoning and essential oil and oleoresin. Used as an ornamental.

Thymus pulegioides L. (syn - *T. ovatus* Mill; *T. glaber* Mill; appendix III) - very aromatic creeping undershrub native to parts of England, Scotland and Ireland. Widespread in Europe from Russia to Spain. Leaves slightly folded upwards from the midrib. Used as an ornamental. Height up to 25 cm. Leaves oval to elliptic and 6 - 10 cm by 3 - 6 cm, with short stalks. Leaves non-hairy except at the base. Flowers rose-purple. 2n = 28.

Thymus drucei Ronn. (synonyms - *T. arcticus* (E. Durand) Ronn; *T. britannicus* Ronn; *T. neglectus* Ronn;

T. zetlandicus Ronn; *T. pseudolanuginosus* Ronn; *T. carniolicus auct. brit.*; *T. pycnotricus auct.*; *T. serpyllum auct. brit.*.) - less aromatic than *T. pulegioides*. Lateral veins on the undersides of leaves are prominent when dry (also in *T. serpyllum*). Used as an ornamental. Height always less than 7 cm and leaves are 4 - 8 mm by 1.5 - 4 mm. These are round to elliptical and may be hairy or not. Flowers are rose-purple. 2n = 50 - 56. There is more than one ecotype.

[Distribution of *T. Drucei* and *T. serpyllum* is similar. Differences in taxonomy are relatively minor: *T. drucei* has definitely quadrangular stems with hairs of varying lengths of dense hairs on opposite sides , the other sides being less hairy or hairless. *T. serpyllum* stems are less angular and have short white hairs all around.(S. Ross-Craig) The best way to classify these two species is by examining the chromosome numbers: *T. drucei* - 2n = 54; *T. serpyllum* - 2n = 24 (Clapham *et al.*, 2nd edition, 1962)]

Thymus zygis L. (Spanish thyme; a variety is known as *T. zygis* var *gracilis* Boiss., appendix III) - collected from the wild in Spain and Portugal and used to produce Spanish thyme oil. This is a short but erect shrub with relatively large white hairs at the base of the leaves which distinguish is from *T.*

vulgaris. The essential oil and oleoresin are safe to use.

Thymus x citriodorus (Pers) Schreb ex Schweigg and Korte (*T. serpyllum* L var *vulgaris* Benth; lemon thyme) - a cross between *T. vulgaris* and *T. pulegioides* it is similar in habit to common thyme but the leaves are a richer green and lemon scented. The plant is highly branched and semi-erect, the latter making it easier to harvest. It is the second most popular commercial thyme after *T. vulgaris*.

Thymus herba-barona Loisel (caraway thyme) - native to Corsica & Sardinia, it is a low growing (less than 10cm with a spread of 30 - 37 cm) evergreen with hairy leaves up to 1 cm long. The pale purple flowers in terminal heads are produced in the summer. Used with bacon and beef and can be used in *bouquets garnis*. 2n = 56.

Thymus caespititius Brot. - (syn. appendix III) a native of the Mediterranean, Portugal, North West Spain and the Azores and used in medicines and perfumery it is a compact and bushy plant up to 25cm tall. The narrow leaves have hairy margins and the pink or purple flowers are in dense and rounded heads. This plant is attractive to bees and is also used in rock gardens. The essential oil is safe for use.

Thymus praecox subsp. *arcticus* (E Durand) Jalas - (*T. drucei* Ronniger) a low growing creeping species found with different flower colours it is used horticulturally as a ground cover. A native of west Europe. 2n = c. 50, 51, 54.

Thymbra spicata L. - a native of Israel and other Mediterranean countries it is not a thyme species but has a thyme-like odour and is used locally in its place.

Basil thyme is *Satureja calamintha* and 'oil of thyme' is obtained from *Monarda didyma*. Also called basil thyme in S. Ross-Craigs Book of British Labiates is *Acinos arvensis* (Lam.) Dandy (synonyms - *Calamintha acinos* [L] Clairv; *Satureja acinos* [L] Scheele; *Clinopodium acinos* [L] Kuntze). This has light green toothed leaves.

APPENDIX III

EUROPEAN *Thymus* SPECIES (Compiled by J. Jalas [Clapham *et al.*, 1972])

SUBGENUS Coridothymus (Reichenb. fil.) Borbas has only one species:

Thymus capitatus (L) Hoffmans. & Link otherwise classified as *Satureja capitata* L and *Coridothymus capitatus* (L) Reichenb. fil.

SUBGENUS *Thymus*

SECTION MASTICHINA

T. mastichina L
T. tomentosus Willd.

SECTION MICANTES

T. caespititius Brot.

SECTION PIPERELLA Willk.

T. piperella L

SECTION TEUCRIOIDES Jalas

T. teucrioides Boiss.

SECTION PSEUDOTHYMBRA

T. cephalotos L
T. villosus L
T. longiflorus Boiss. (variable especially size of leaves & flowers and shape of bracts)
T. membranaceus Boiss. (possibly not separate from *T. longiflorus*)
T. antoninae Rouy & Coincy (*T. portae* Freyn)
T. mastigophorus Lacaita (*T. hirtus* auct., non Willd.,
T. hispanicus auct., non Poiret). Possibly a subspecies of *T. munbyanus* Boiss. & Reuter (*T. ciliatus* (Desf.) Bentham, non Lam.)
T. dolopicus Form.
T. Leucotrichus Halascy
T. cherlerioides (*T. boisseri* Halacsy, *T. hirsutus*

auct., non Bieb; incl. T pseudohumillimus Klokov & Schost., *T. tauricus* Klokov & Schost)
T. parnassicus Halacsy (possibly a sub-species of *T. cherlerioides*)

SECTION *Thymus* **(Section** *Vulgares* **Velen., section** *Zygis* **Willk.)**

T capitellatus Hoffmans. & Link
T. camphoratus Hoffmans. & Link (*T. algarbiensis* Lange)
T. carnosus Boiss.
T. vulgaris L (incl. *T. aestivus* Reuter ex Willk., *T. ilerdensis* F. Gonzalez ex Costa, *T. valentinus* Rouy, *T.webbianus* Rouy)
T. hymenalis Lange
T. glandulosus Lag. ex H. del Villar
T. zygis L (incl. *T. sabulicola* Cosson, *T. sylvestris* Hoffmans. & Link)
T. baeticus Boiss. & Lacaita (*T. hirtus* auct., non Willd.)
T. hirtus Willd. (*T. diffusus* Salzm. ex Bentham)
T. loscosii Willk. in Willk. & Lange
T. loscosii subsp. *loscosii*
T. loscosii subsp. *fontqueri* Jalas (*T. augustifolius* auct., non Pers.)
T. serpylloides Bory
T. serpylloides subsp. *serpylloides*
T. serpylloides subsp. *gadorensis* (Pau) Jalas (*T. zygis* var*gadorensis* Pau, *T. sylvestris* auct., non Hoffmans. & Link)

SECTION HYPHODROMI

T. hololsericeus Celak.
T. laconicus Jalas (*T. pastoralis* Turrill, non Iljin)
T. bracteosus Vis. ex Bentham
T. granatensis Boiss. (variable especially corolla length)
T. aranjeuzii Jalas (*T. granatensis* var *micranthus* Willk., *T hirtus* auct., non Willd., *T. hispanicus* auct., non Poiret, *T. mastigophorus* auct., non Lacaita)
T. bracteatus Lange ex Cutanda
T. leptophyllus Lange (*T. augustifolius* auct., non Pers)
T. atticus Celak
T. plasonii Adamovic
T. striatus Vahl (*T. acicularis* Walst. & Kit., *T. comptus* auct., non Friv.; incl. *T. pseudoatticus* Ronniger)
T. spinulosus Ten. (*T. conspersus* Celak.)
T. aznavourii Velen. (*T. sintenisii* auct., non Celak)
T. kirgisorum Dubjanski (incl. *T. calcareus* Klokov &

Schost., *T. cretaceus* Klokov & Schost., *T. graniticus* Klokov & Schost., *T. kaljmijussicus* Klokov & Schost.)

T. dubjanskii Klokov & Schost. (may be identical to *T. rariflorus* C Koch)

T. zygioides Griseb.

T. eupatoriensis Klokov & Schost. (may be the same species as*T. zygioides* Griseb.)

SECTION SERPYLLUM (Miller) Bentham

T. comptus Friv. (*T. glaucus* Friv. ex Podp.)

T. sibthorpii Bentham (incl. *T. tosevii* Velen., *T. korthiaticus* Adamovic, *T. macedonicus* (Degen & Urum.) Ronniger)

T. degenii H. Braun (*T. tosevii* subsp. *degenii* (H. Braun) Ronniger

T. grisebachii Ronniger

T. substriatus Borbas

T. heterotrichus Griseb.

T. pannonicus All. (incl. *T dzevanovskyi* Klokov & Schost., *T. latifolius* (Besser) Andrz., *T. marschallianus* Willd., *T. serpyllum* subsp. *auctus* Lyka, subsp. *brachyphyllus* Lyka & subsp *marschallianus* (Willd.) Nyman, *T. stepposus* Klokov & Schost.)

T. dimnorphus Klokov & Schost. (incl. *T. amictus* Klokov, *T. litoralis* Klokov & Schost.)

T. bulgaricus (Domin & Podp.) Ronniger

T. glabrescens Willd.

T. glabrescens subsp. *glabrescens* (*T. serpyllum* subs. *glabrescens* (Willd.) Lyka, *T. austriacus* Bernh. ex Reichenb., *T. loevyanus* Opiz, *T. tschernjajevii* Klokov & Schost.)

T. glabrescens subsp. *decipiens* (H. Braun) Domin (*T. oenipontanus* H. Braun, *T. serpyllum* subsp. *decipiens* (H. Braun) Lyka

T. glabrescens subsp. *urumovii* (velen.) Jalas (*T. calleri* subsp. *urumovii* Velen.; incl. *T. callieri* Borbas ex Velen., *T. hirsutus* Bieb., nom. ambig., *T jailae* (Klokov & Schost.) Stankov, *T. zelenetzkyi* Klokov & Schost.)

T. longedentatus (Degen & Urum.) Ronniger

T. conspersus var. *lycaonicus* Celak (*T. zygioides* var. *lycaonicus* (Celak.) Ronniger

T. pallasianus H. Braun

T. pallasianus subsp. *pallasianus*

T. pallasianus subsp. *brachyodon* (Borbas) Jalas (*T. brachyodon* Borbas, *T. eltonicus* Klokov & Schost.; incl. *T. lanulosus* Klokov & Schost.)

T. borysthenicus Klokov & Schost.

T. ciliatissimus Klokov & Kotov

T. herba-barona Loisel

T. nitens Lamotte

T. willkommii Ronniger
T. richardii Pers.
T. richardii subsp. *richardii* (*T. aureopunctatus* (G. Beck) K. Maly)
T. richardii subsp. *nitidus* (Guss.) Jalas (*T. nitidus* Guss.)
T. richardii subsp. *ebusitanus* (Font Quer) Jalas
T. guberlinensis Iljin (*T. zheguliensis* Klokov & Schost.)
T. mugodzharicus Klokov & Schost.
T. bashkiriensis Klokov & Schost.
T. binervulatus Klokov & Schost.
T. ocheus Heldr. & Sart. ex Boiss. (*T. chaubardii* (Boiss. & Heldr. ex Reichenb. fil.)
T. thracicus Velen (incl. *T. alsarensis* Ronniger, *T. longidens* (Velen.) Podp.)
T. stojanovii Degen

T. longicaulis C. Presl (incl. *T. illyricus* Ronniger, *T. kosaninii* Ronniger, *T. lykae* Degen & Jav., *T. malyi* Ronniger, *T. moesiacus* Velen., *T. rohlenae* Velen., *T. serpyllum* subsp. *dalmaticus* (Reichenb.) Nyman)
T. dolomiticus Coste
T. sintenisii Celak
T. adamovicii Velen.
T. praecox Opiz
T. praecox subsp. *praecox* (*T. humifusus* Bernh., *T. serpyllum* subsp.*clivorum* Lyka, subsp. *hesperites* Lyka & subsp. *praecox* (Opiz) Vollman)
T. praecox subsp. *skorpilii* (Velen.) Jalas.
T. praecox subsp. *polytrichus* (A. Kerner ex Borbas) Jalas (*T. alpigenus* (A. Kerner ex H. Braun) Ronniger, *T. balcanus* Borbas, *T. kerneri* Borbas, *T. polytrichus* A. Kerner ex Borbas, *T. serpyllum* subsp. *polytrichus* (A. Kerner ex Borbas) Briq. subsp. *trachselianus* (Opiz) Lyka)
T. praecox subsp. *zygiformis* (H. Braun) Jalas (*T. albanus* H. Braun, *T. zygiformis* H. Braun)
T. praecox subsp. *arcticus* (E. Durand) Jalas (*T. drucei* Ronniger)
T. widderi Ronniger ex Machule
T. doerfleri Ronniger (*T. hirsutus* var. *doerfleri* (Ronniger) Ronniger)
T. nervosus Gay ex Willk.
T. pulcherrimus Schur (incl. *T. carpathicus* Celak., *T. circumcinctus* Klokov, *T. serpyllum* subsp. *sudeticus* Lyka, non *T. sudeticus* Opiz ex Borbas)
T. comosus Heuffel ex Grizeb.
T. bihoriensis Jalas (*T. marginatus* A Kerner, Non Sm.)
T. pulegioides L (*T. alpestris* auct., non Tausch ex A. Kerner, *T. chamaedrys* Fries, *T. enervius* Klokov, *T. groelichianus* Opiz, *T. montanus* Waldst.

& Kit., non Crantz, *T. serpyllum* subsp. *carniolicus* (Borbas) Lyka, subsp. *chamaedrys* (Fries) Vollman, subsp. *effusus* (Host) Lyka, subsp. *montanus* Arcangeli & subsp. *parviflorus* (Opiz ex H. Braun) Lyka, *T. ucrainicus* Klokov & Schost. (Very variable)

T. alpestris Tausch ex A Kerner (*T. chamaedrys* auct., non Fries, *T. serpyllum* subsp. *alpestris* (Tausch ex A. Kerner) Lyka; incl. *T. subalpestris* Kolokov).

T. oehmianus Ronniger & Soska

T. alternans Klokov

T. serpyllum L

T. serpyllum subsp. *serpyllum* (*T. serpyllum* subsp. *augustifolius* (Pers.) *Arcangeli* and subsp. *rigidus* (Wimmer & Grab.) Lyka)

T. serpyllum subsp. *tanaensis* (Hyl.) Jalas

T. talijevii Kolokov & Schost (incl. *T. hirticaulis* Klokov, *T. paucifolius* Klokov).

APPENDIX IV

Labiates native to Britain.

(Ross-Craig, 1967)

KEY FOR APPENDIX IV

A: upper part of flowering stem

B: calyx

C: corolla opened out

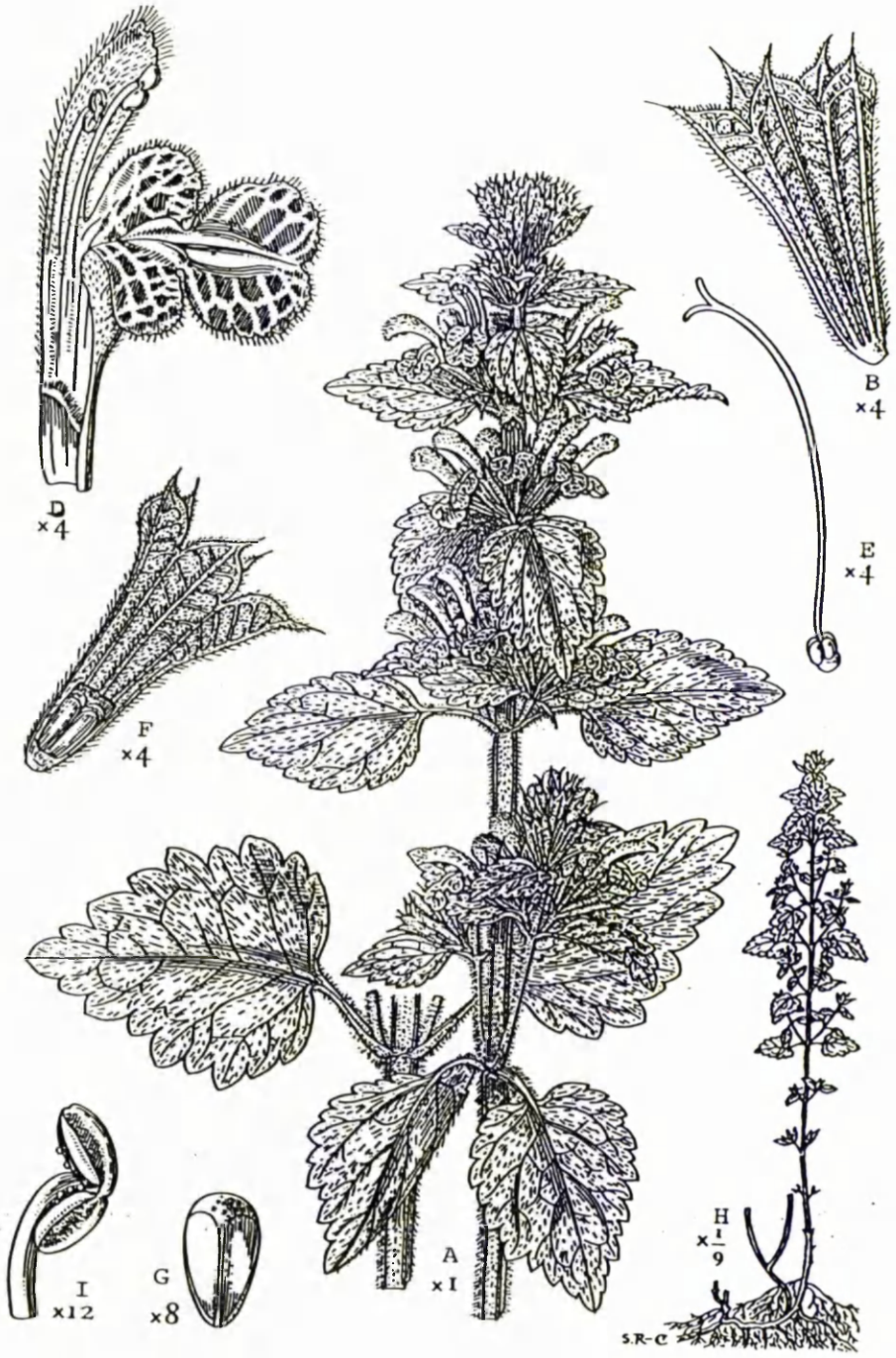
D: corolla cut away to show 2 stamens

E: gynoecium

F: calyx with nutlets

G: nutlet

H: plant



Ballota nigra L.

Black Horehound

GLECHOMA HEDERACEA

A - H: see key at start of appendix IV

I: part of hermaphrodite plant

J: part of female plant

K: calyx of female flower

L: corolla opened out showing vestigial stamens of female flower.

Glechoma hederacea L.
(syn. *Nepeta glechoma* Benth.; *N. hederacea* [L.] Trev.)

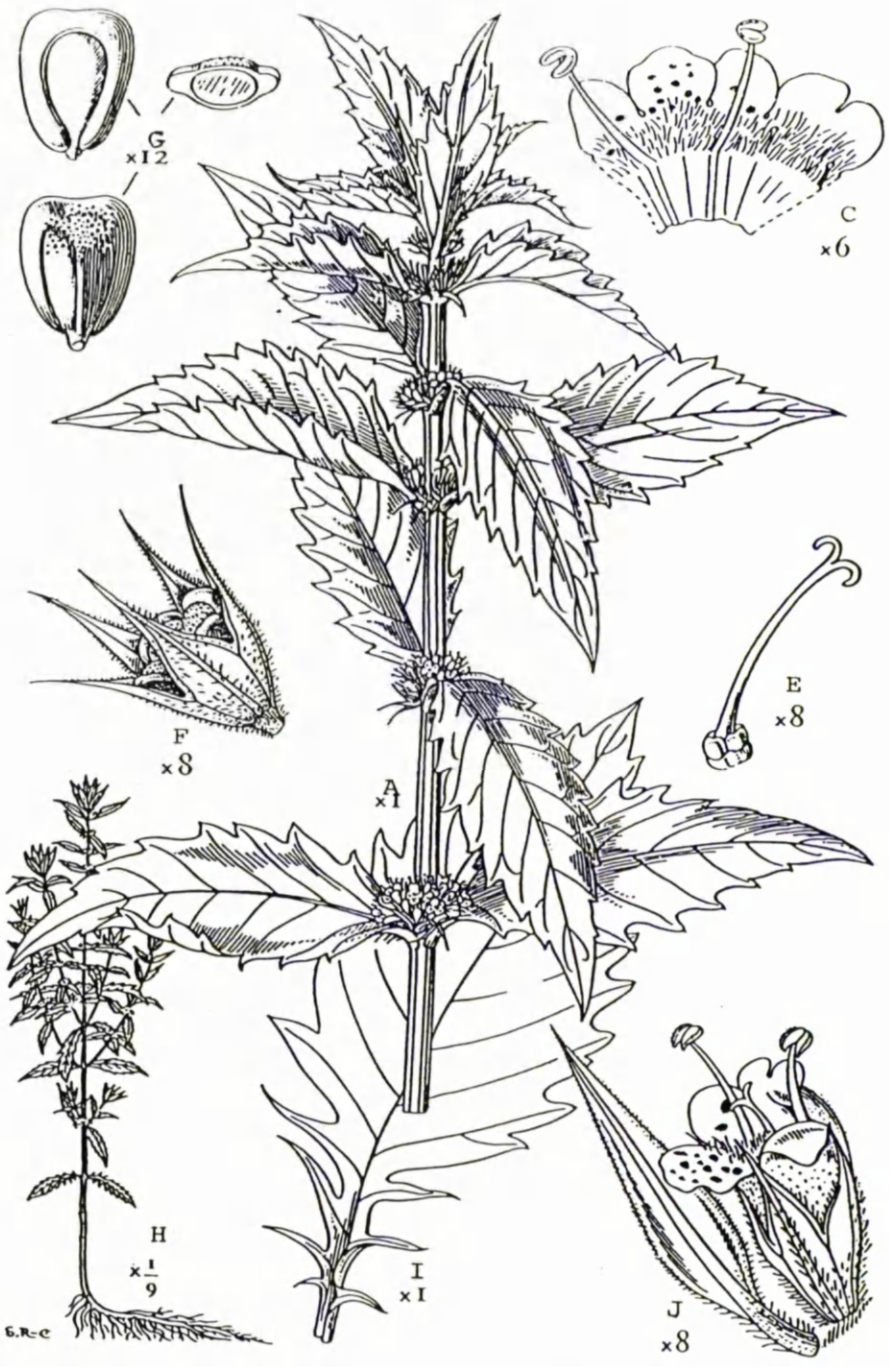
Ground-Ivy

LYCOPUS EUROPAEUS

A - H: see key at start of appendix IV

I: lower leaf

J: bractlet and flower



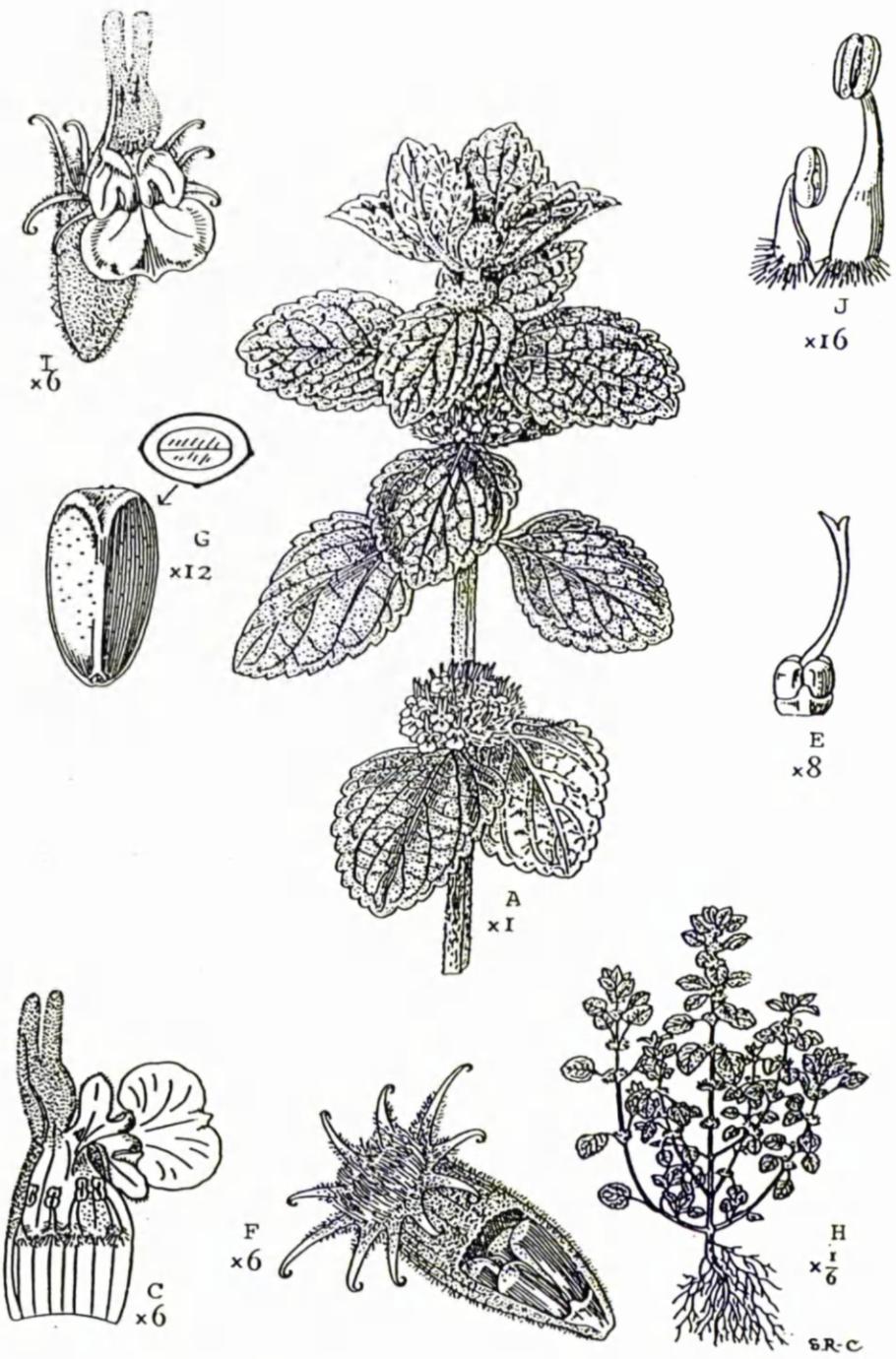
Lycopus europaeus L.

Gypsy-wort, Water Horehound

MARRUBIUM VULGARE

A - H: see key at start of appendix IV

I: flower J: stamens



Marrubium vulgare L.

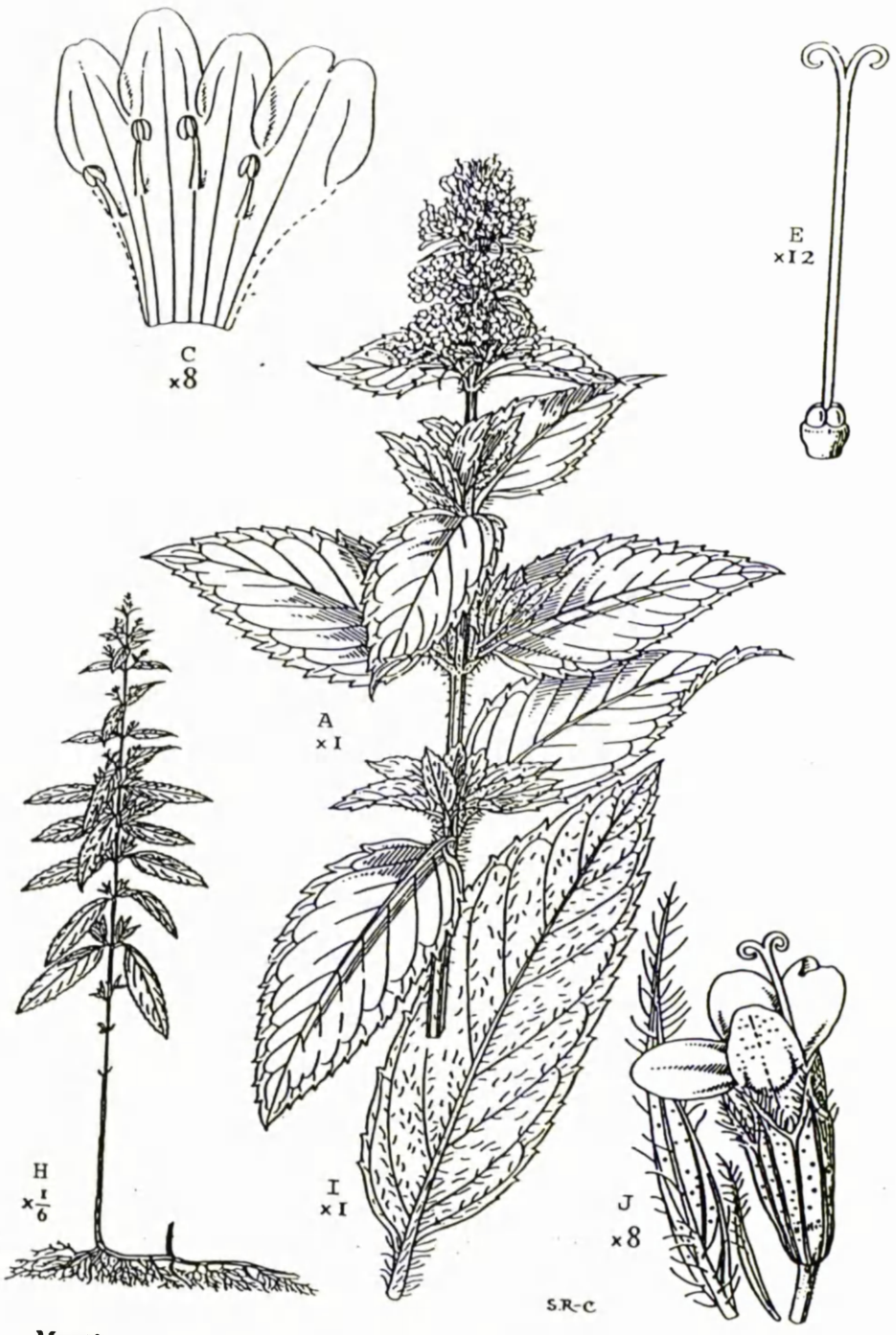
White Horehound

MENTHA PIPERITA

A - H: see key at start of appendix IV

I: leaf from lower stem

J: bract, bractlet and flower



Mentha × piperita L. (*M. aquatica* × *M. spicata*) Peppermint

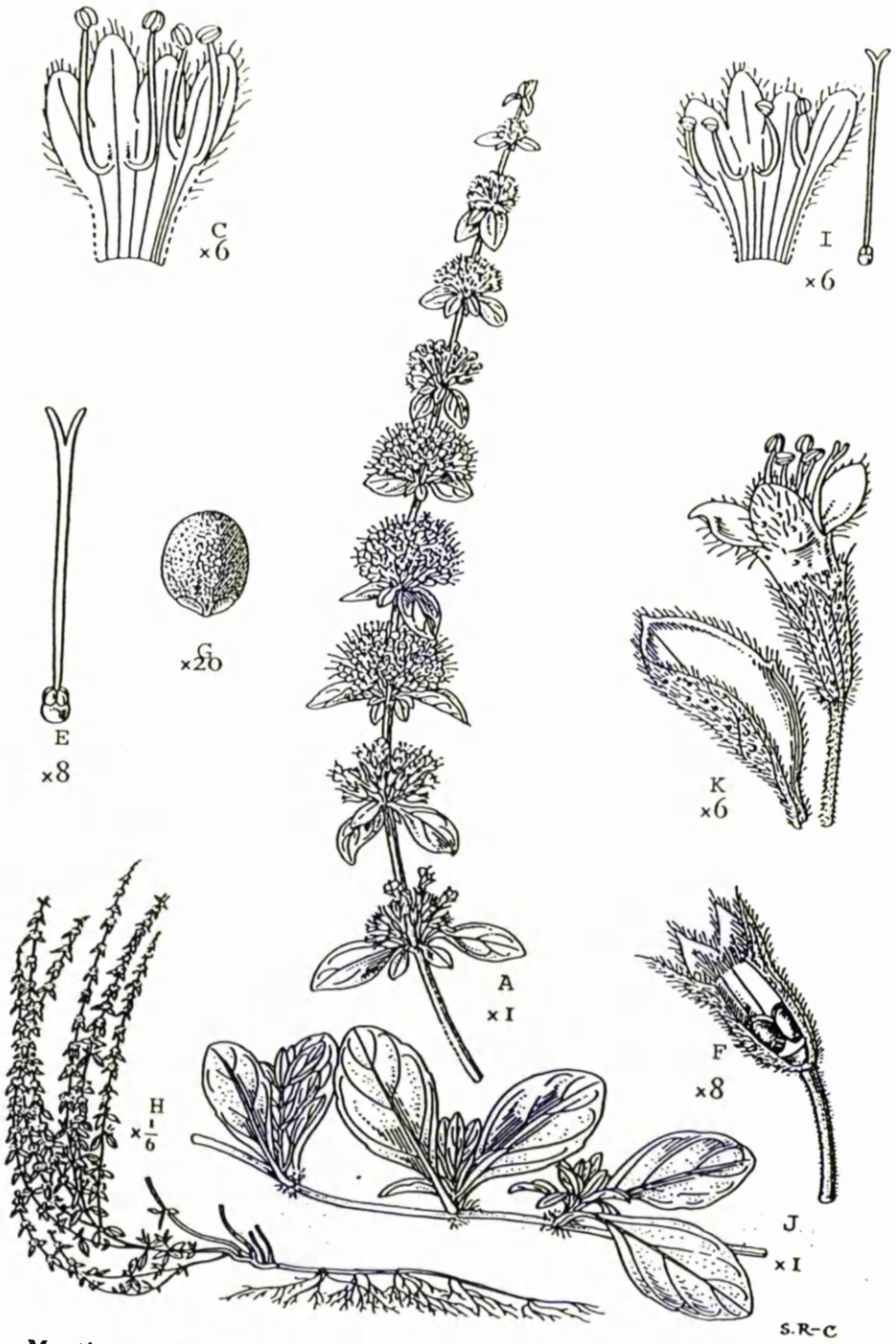
MENTHA PULEGIUM

A - H: see key at start of appendix IV

I: corolla opened out to show stamens and gynoecium from short-stamened plant

J: lower part of young shoot

K: bractlet and flower



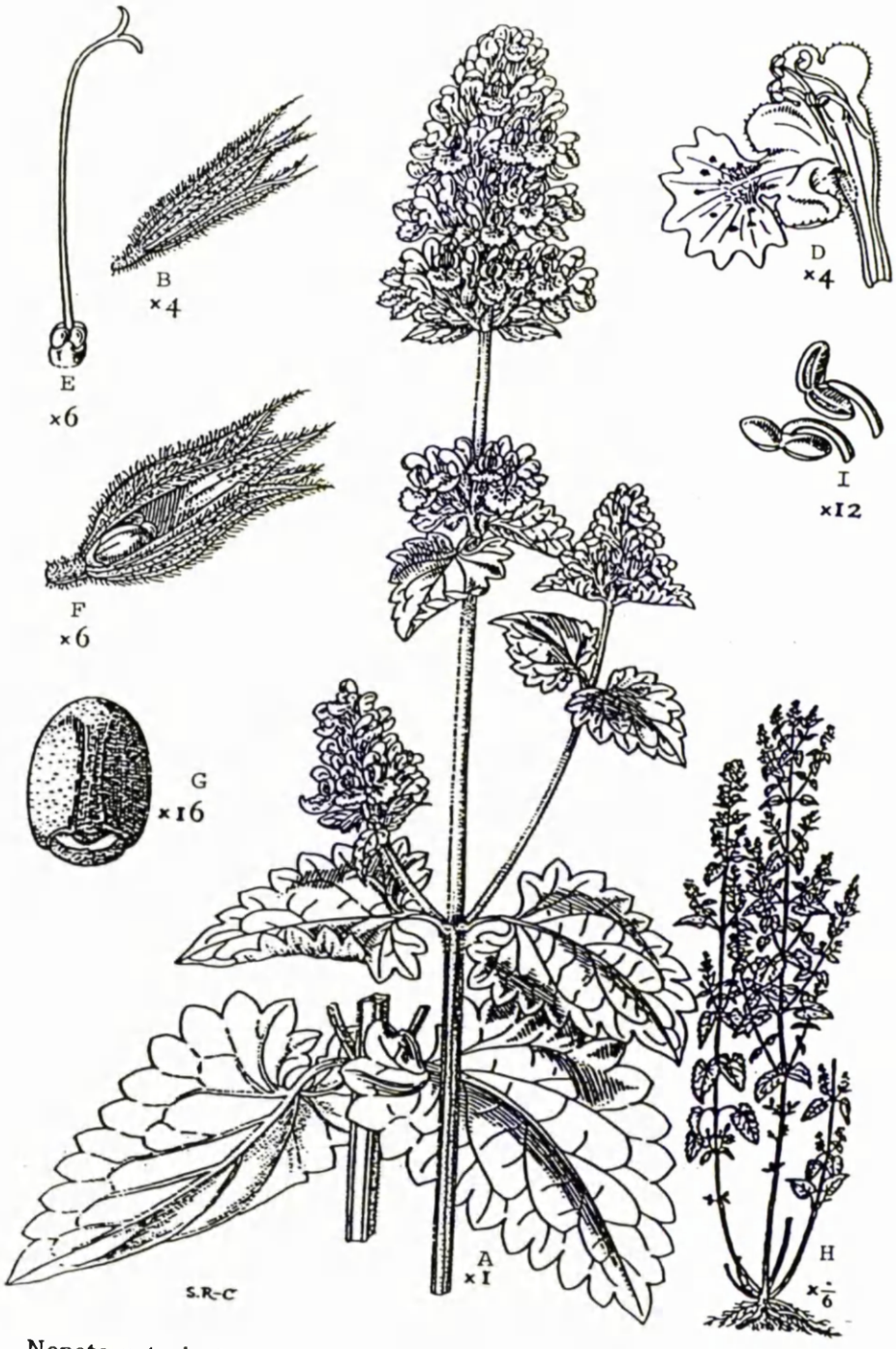
Mentha pulegium L.

Penny-royal

NEPETA CATARIA

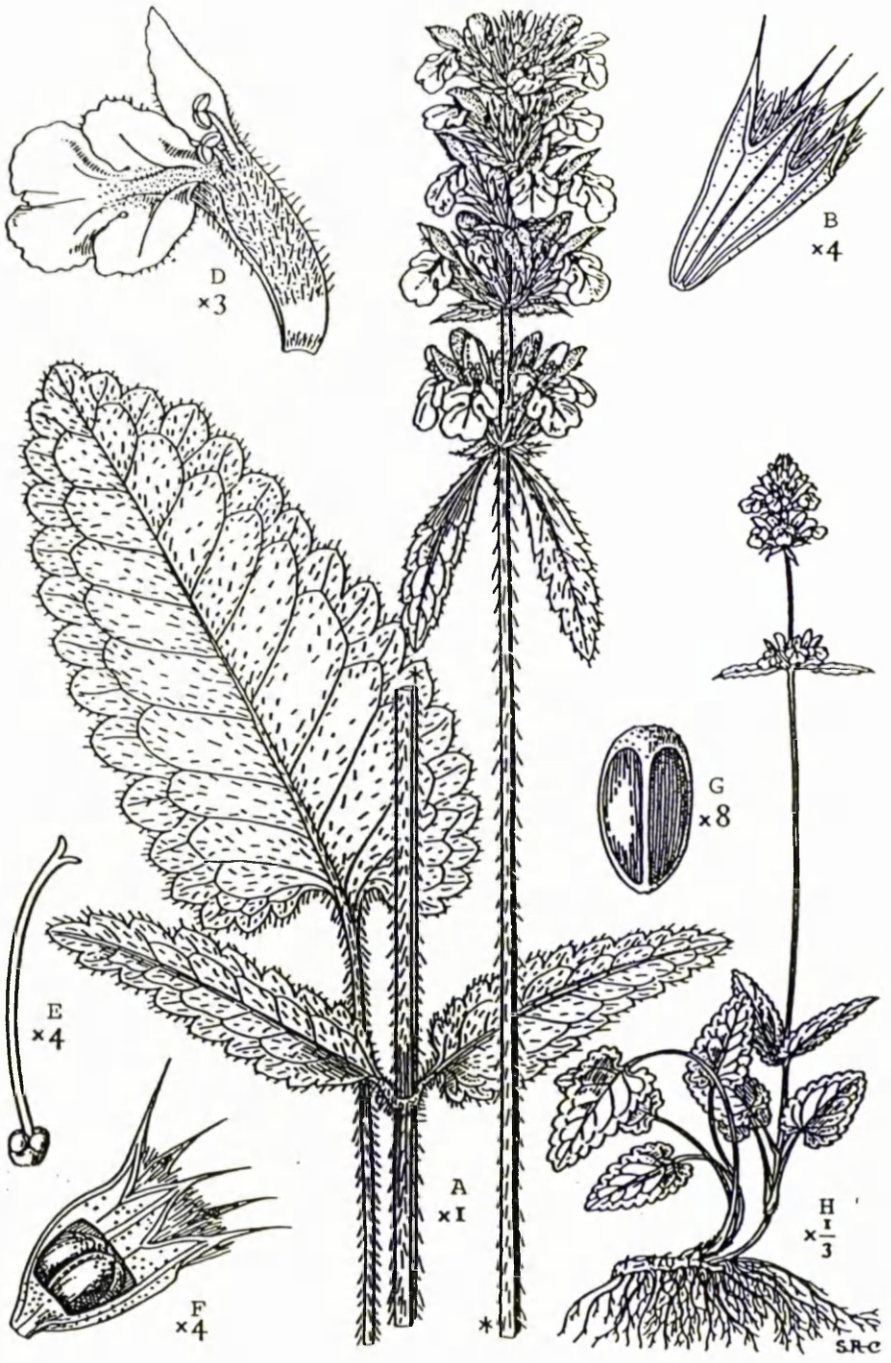
A - H: see key at start of appendix IV

I: anther and part of filament



Nepeta cataria L.

Catmint



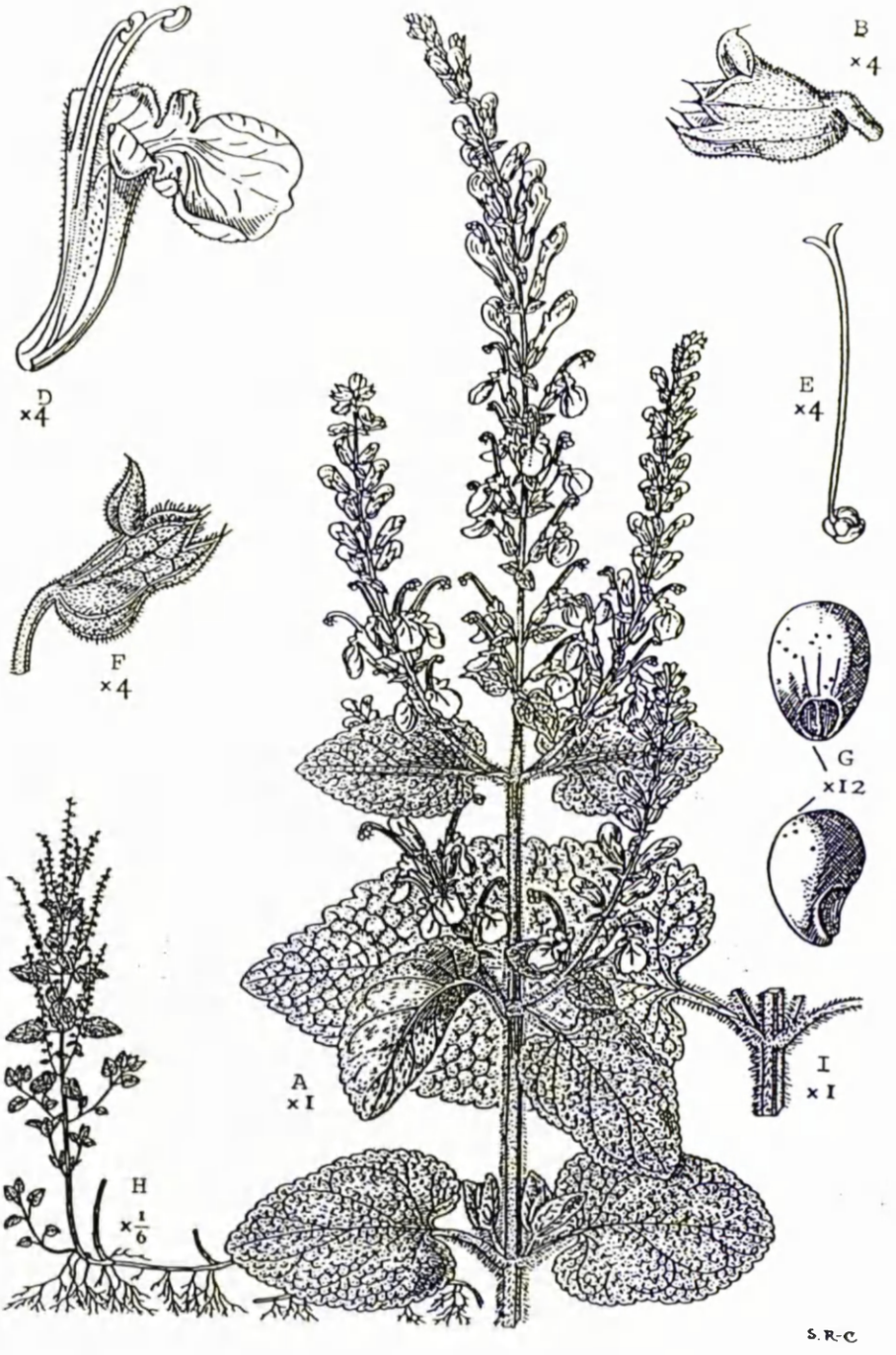
Stachys officinalis (L.) Trev.
(syn. *Betonica officinalis* L.; *Stachys betonica* Benth.)

Wood Betony

TEUCRIUM SCORODONIA

A - H: see key at start of appendix IV

I: lower stem from stronger plant



Teucrium scorodonia L.

Wood Germander

THYMUS SERPYLLUM

A: hermaphrodite plant

B: leaf of hermaphrodite plant - upper surface

C: leaf of hermaphrodite plant - lower surface

D: part of stem of hermaphrodite plant

E: corolla, stamens and gynoecium of hermaphrodite plant

THYMUS DRUCEI

F: female and hermaphrodite plants

G: upper surface of leaf

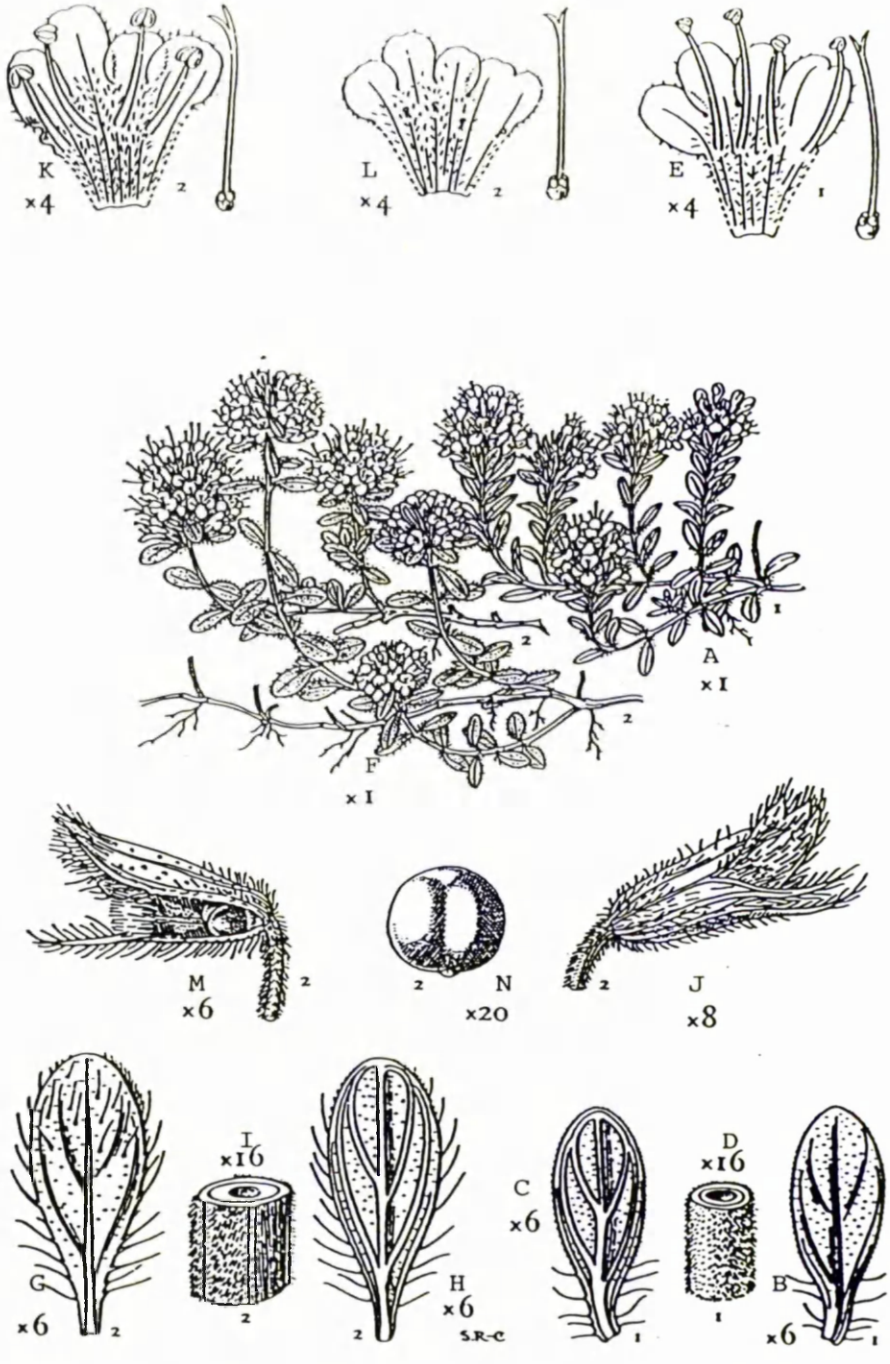
H: lower surface of leaf

I: part of stem

J: calyx

K: corolla, stamens and gynoecium of hermaphrodite flower

L: corolla, stamens and gynoecium of female flower



Thymus serpyllum L.
(sens. lat. incl. *T. drucei* Ronn. em. Jalas)

Wild Thyme

APPENDIX V

British labiate species containing both female and hermaphrodite plants.

(Ross-Craig, 1967)

GLECHOMA HEDERACEA

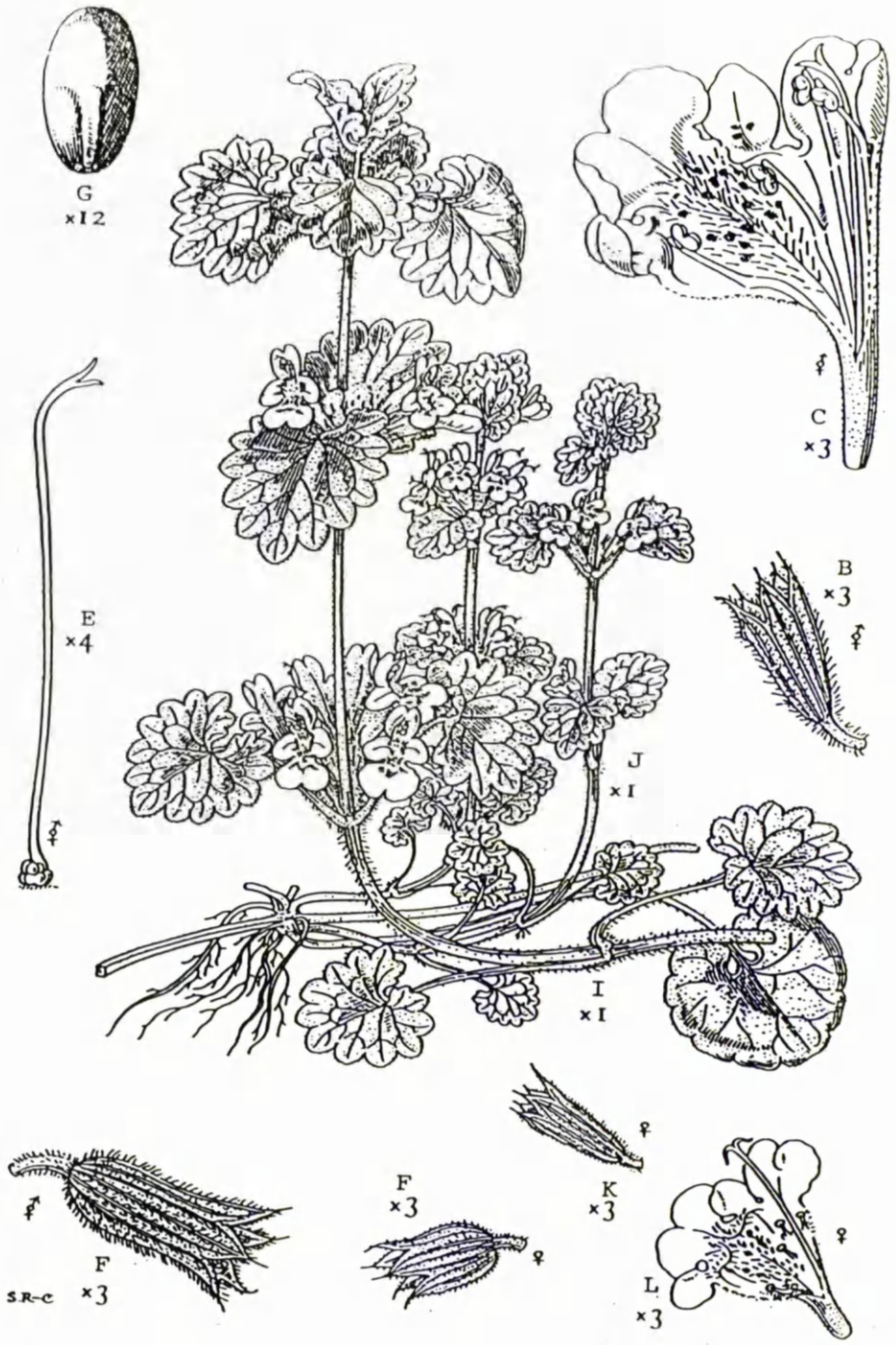
A - H: see key at start of appendix IV

I: part of hermaphrodite plant

J: part of female plant

K: calyx of female flower

L: corolla opened out showing vestigial stamens of female flower.



Glechoma hederacea L.
(syn. *Nepeta glechoma* Benth.; *N. hederacea* [L.] Trev.)

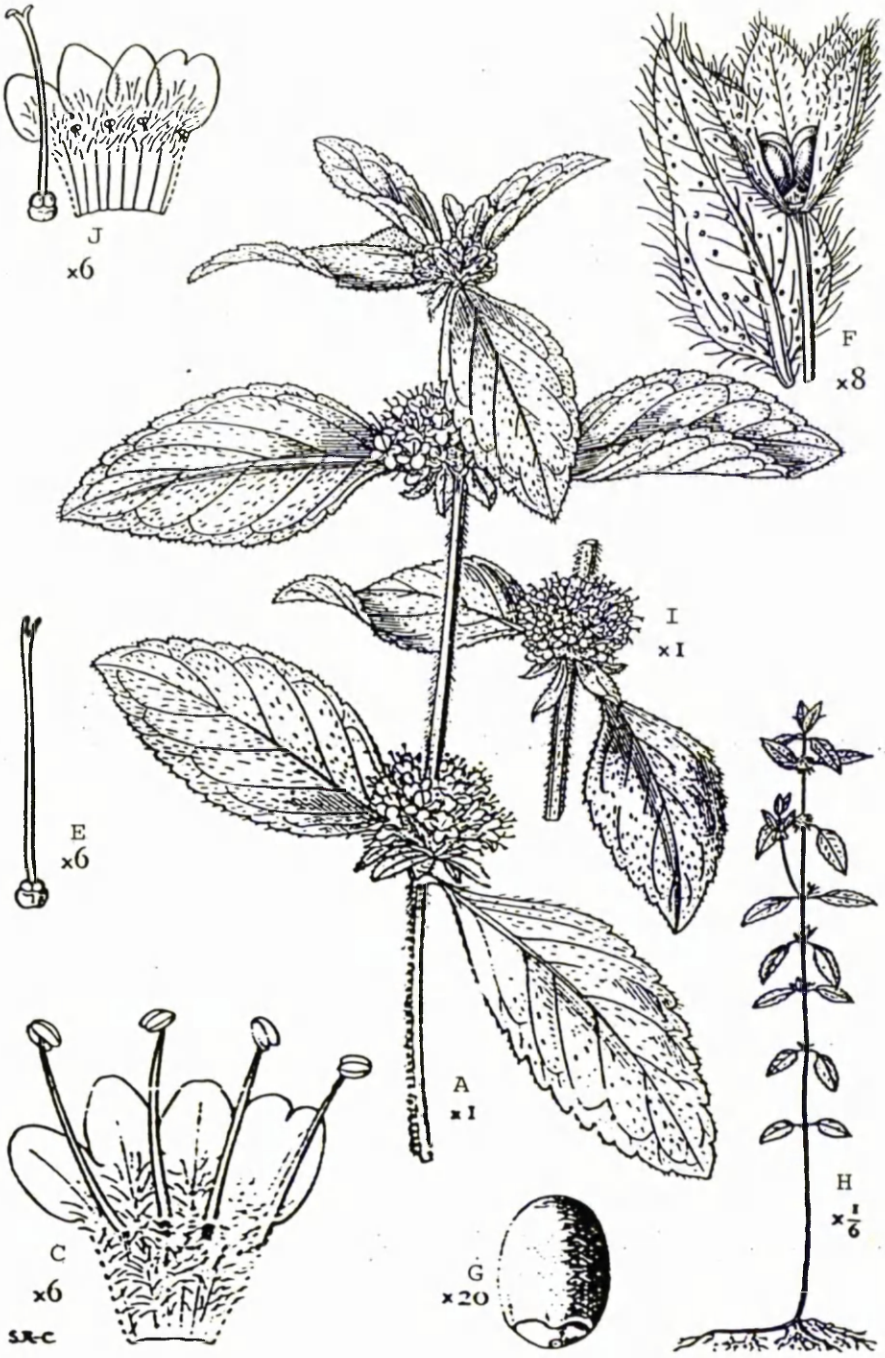
Ground-Ivy

MENTHA ARVENSIS

A - H: see key at start of appendix IV

I: part of flowering stem from female plant

J: flower and gynoecium from female plant



Mentha arvensis L. — Corn Mint

MENTHA AQUATICA

A - H: see key at start of appendix IV

I: leaf from lower part of stem

J: flower from plant with short stamens

K: part of stem from plant with short stamens

Mentha aquatica L. Water Mint

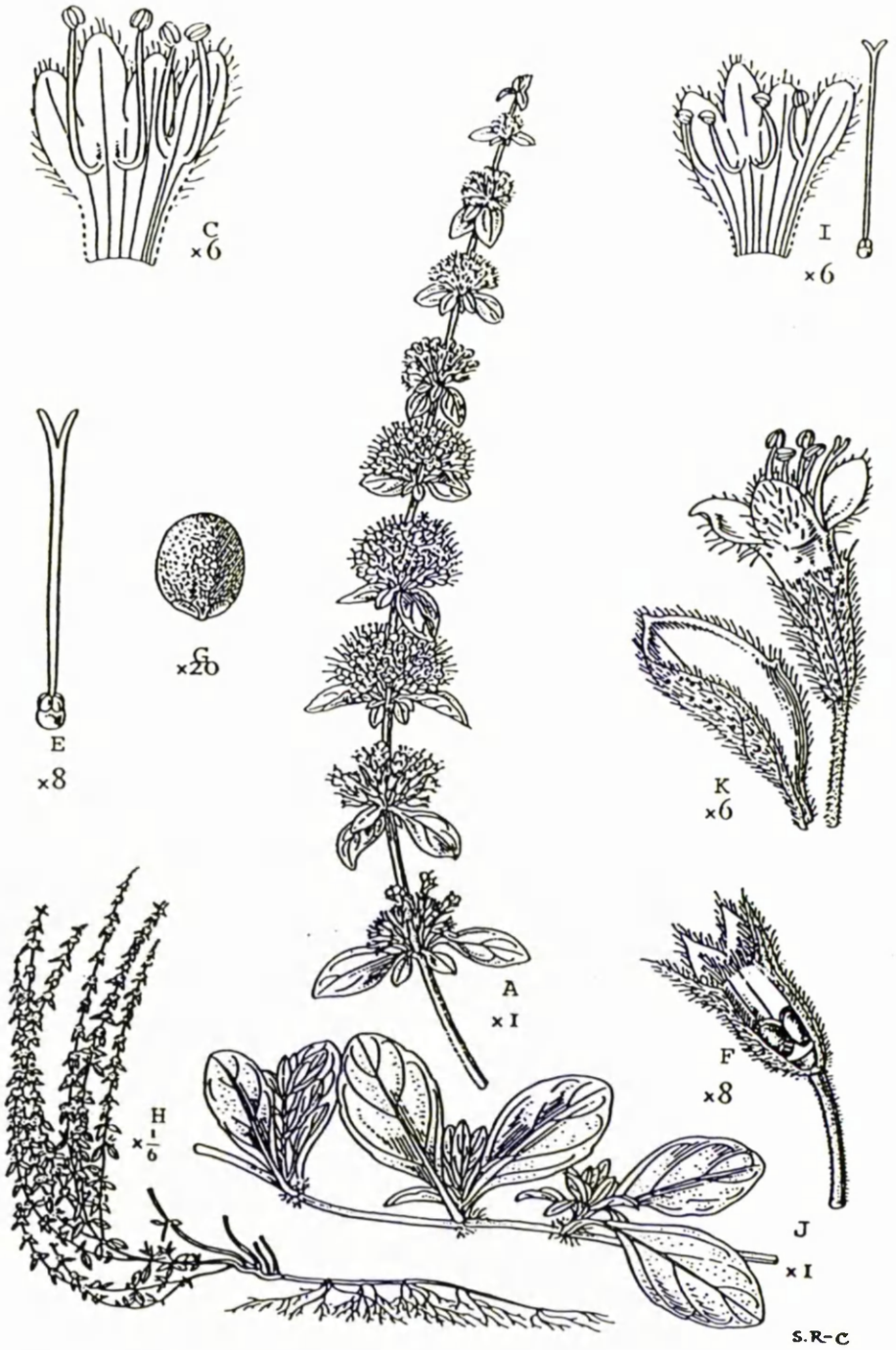
MENTHA PULEGIUM

A - H: see key at start of appendix IV

I: corolla opened out to show stamens and gynoecium from short-stamened plant

J: lower part of young shoot

K: bractlet and flower



Mentha pulegium L.

Penny-royal

ORIGANUM VULGARE

A - H: see key at start of appendix IV

I: corolla and gynoecium of hermaphrodite flower

J: corolla and gynoecium of female flower

Origanum vulgare L.

Common Marjoram

THYMUS PULEGIOIDES

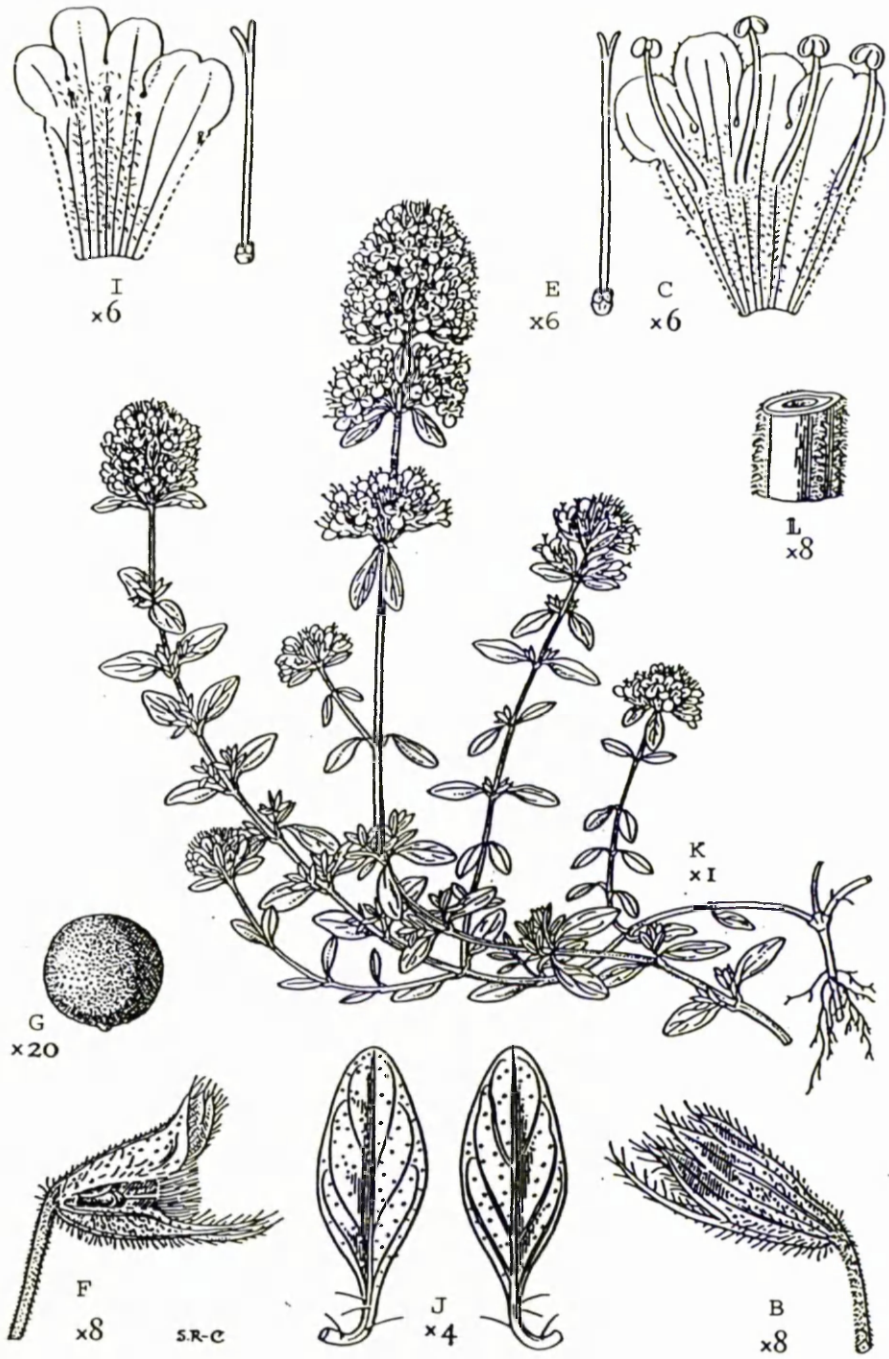
A - H: see key at start of appendix IV

I: corolla and gynoecium of female flower

J: upper and lower leaf surfaces

K: female plant and hermaphrodite flower stem

L: part of stem



Thymus pulegioides L. Wild Thyme
(syn. *T. glaber* Mill.; *T. ovatus* Mill.; *T. chamaedrys* Fr.)

THYMUS SERPYLLUM

A: hermaphrodite plant

B: leaf of hermaphrodite plant - upper surface

C: leaf of hermaphrodite plant - lower surface

D: part of stem of hermaphrodite plant

E: corolla, stamens and gynoecium of hermaphrodite plant

THYMUS DRUCEI

F: female and hermaphrodite plants

G: upper surface of leaf

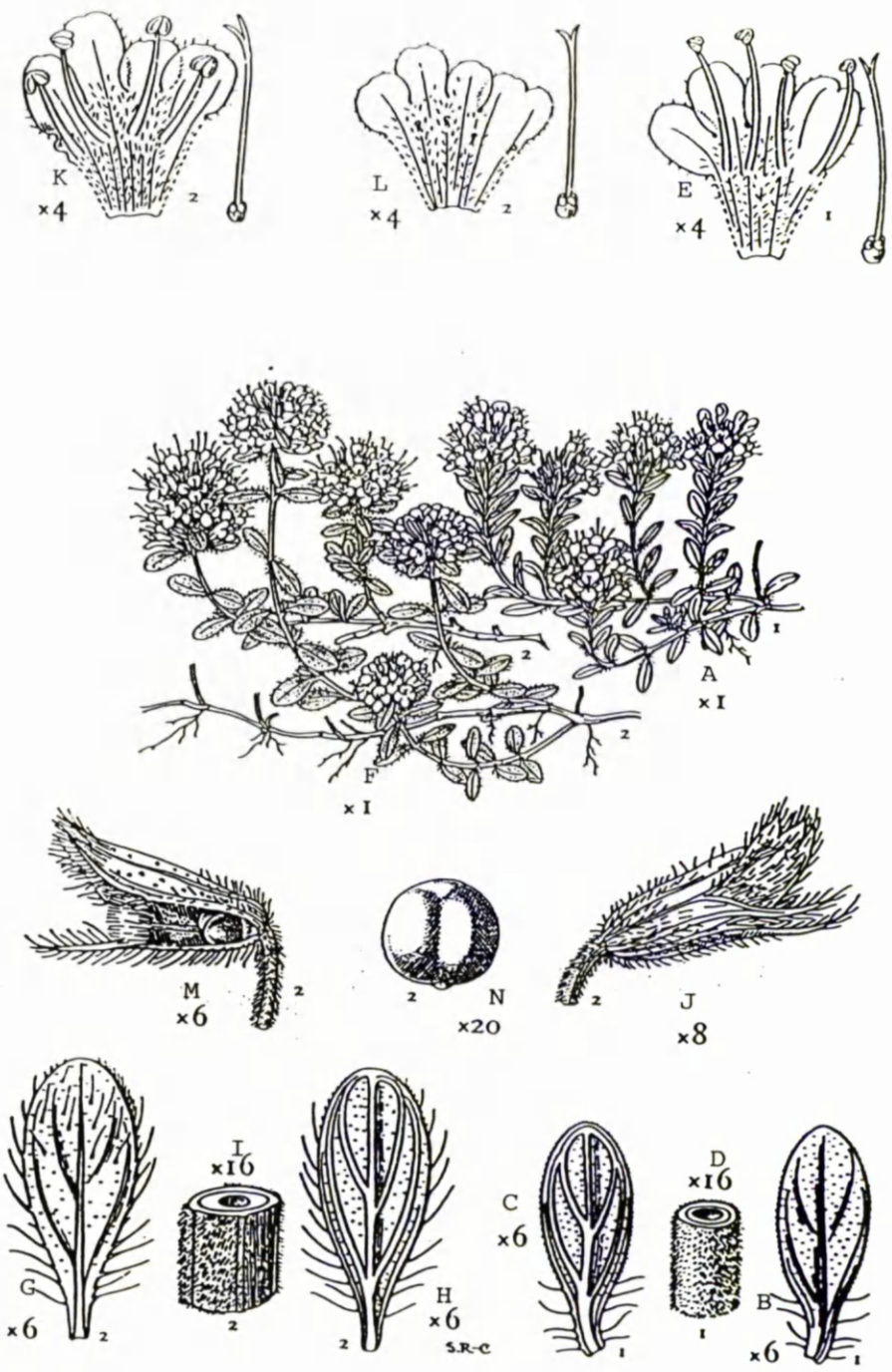
H: lower surface of leaf

I: part of stem

J: calyx

K: corolla, stamens and gynoecium of hermaphrodite flower

L: corolla, stamens and gynoecium of female flower



Thymus serpyllum L.
(sens. lat. incl. *T. drucei* Ronn. em. Jalas)

Wild Thyme

APPENDIX VI

T. VULGARIS VARIETIES (Titterington, 1989)

T. v. **Archers Gold** - 15 cm tall, bright green leaves, becoming gold later in the season. Dense, bushy growth with pink flowers.

T. v. **Aureus** - 12 cm tall, leaves turning bright gold, pink flowers.

T. v. **Variegata** - 15 cm tall, silver-green variegated leaves, pink flowers in June - July.

T. v. **Alba** - white flowers.

CROSS SPECIES

T. vugaris **x** *T. pulegioides* = *T. citriodorus* **(Pers.) Schreb. ex Scheigg & Korte** - similar habit to common thyme with richer green leaves lemon-scented leaves. This is the second most popular commercial thyme after *T. vulgaris* (Clapham *et al.* 1972).

T. citriodorus **var. Silver Queen** - variegated lemon thyme.

Appendix VII

Scottish Agricultural Colleges
SOIL SCIENCE UNIT
West of Scotland Agricultural College
Auchincruive, Ayr KA6 5HW

HORTICULTURAL SUBSTRATES ANALYSIS SERVICE

EDUCATION

Date Reported: 16/5/88 To: Samantha Jackson, Plant Science.

Field Name:	Temple Field Plot 1 (E)	Plot 2 (F)	Plot 3 (G)	Plot 4 (H)
Lab. Ref. No.	880072	880073	880074	880075
Soil Texture				
DETERMINATION				
% Moisture	1.8	1.5	1.3	1.5
% Loss on Ignition	7.6 M	7.1 M	7.3 M	7.0 M
pH (water)	5.88	6.31	6.63	5.88
Lime Req. to pH 6.5	434	133	-	434
Available P (mg l^{-1})	4.5 VL	26 M	12 L	20 L
Available K (mg l^{-1})	73 L	70 L	60 L	100 M
Extractable Mg (mg l^{-1})	290 H	197 M	214 H	143 M
Extractable B (mg l^{-1})				
pC	3.96 M	3.94 M	3.91 M	3.92 M

Lime requirement given in g/m^2.

COMMENTS:

Scottish Agricultural Colleges
SOIL SCIENCE UNIT
West of Scotland Agricultural College
Auchincruive, Ayr KA6 5HW

HORTICULTURAL SUBSTRATES ANALYSIS SERVICE

EDUCATION

To: S. Jackson, Plant Sciences Dept.

Date Reported: 25/5/88

Field Name:	Coylton Road 5	6		
Lab. Ref. No.	880078	880079		
Soil Texture				
DETERMINATION				
% Moisture	1.9	1.7		
% Loss on Ignition	8.0 M	7.9 M		
pH (water)	5.15	5.11		
Lime Req. to pH 6.5	945	973		
Available P (mg l^{-1})	73 M	63 M		
Available K (mg l^{-1})	169 M	166 M		
Extractable Mg (mg l^{-1})	94 M	96 M		
pC	3.78 M	3.76 M		

Lime requirement given in g/m^2.

Appendix VIII

WEATHER DATA, 1988 - 1990

MONTH	TEMPERATURE (°C) MEAN MAX.	MEAN MIN.	TOTAL SUNSHINE HOURS	TOTAL RAINFALL	30 cm SOIL TEMP.
1988					
MAY	16.0	5.9	237.8	42.3	11.6
JUNE	18.3	9.3	232.8	28.4	14.9
JULY	16.9	11.0	123.3	119.4	15.2
AUG	17.3	11.1	133.5	108.1	15.5
SEPT	15.4	9.5	107.9	118.9	13.8
OCT	13.2	6.6	95.5	93.0	10.7
NOV	9.5	2.7	86.5	68.2	7.3
DEC	9.6	5.9	20.4	101.5	7.4
1989					
JAN	9.5	5.4	26.7	105.5	7.4
FEB	8.3	3.4	58.3	103.0	6.5
MAR	9.1	3.0	84.2	125.9	6.2
APR	9.5	2.8	133.5	53.5	7.3
MAY	15.5	6.8	224.2	26.9	10.8
JUNE	16.3	8.7	208.1	54.7	13.1
JULY	20.4	11.4	269.2	51.7	15.4
AUG	17.1	11.2	105.1	144.1	14.5
SEP	15.5	8.9	77.9	39.5	13.1
OCT	13.1	7.9	69.3	97.0	11.5
NOV	8.8	3.3	82.0	31.4	8.2
DEC	6.2	0.7	51.1	55.7	5.0
1990					
JAN	8.5	3.7	38.6	157.1	5.9
FEB	8.6	3.3	35.4	180.0	5.8
MAR	10.0	5.0	76.0	94.2	6.9
APRIL	10.7	3.7	148.2	75.8	7.9
MAY	15.8	7.1	215.8	69.2	11.8

(the weather data from May to November, 1988 was supplied by rainfall station number 645590, Prestwick. Latitude 55.5 N, longitude 4.6 W and altitude 16 m [Crown copyright data, 1988])

SOLAR RADIATION (mWHr cm^2)

	TOTAL	MEAN	MAXIMA	MINIMA
1988				
DECEMBER	886.9	28.6	65.1	6.4
1989				
JANUARY	1286.4	41.5	123.4	8.4
FEBRUARY	2895.8	103.4	187.4	19.4
MARCH	5788.2	192.9	394.2	55.1
APRIL	9214.8	329.1	644.6	48.1
MAY	16277.0	525.1	770.1	196.3
JUNE	15998.0	533.3	798.2	172.7
JULY	16303.5	562.2	770.9	85.8
AUGUST	9680.5	312.3	550.3	79.3
SEPTEMBER	6518.8	217.3	460.9	74.7
OCTOBER	3424.1	110.5	305.2	8.7
NOVEMBER	1847.7	68.4	125.9	11.5
DECEMBER	1127.6	36.4	109.7	6.0
1990				
JANUARY	1418.5	45.7	112.2	6.9
FEBRUARY	2202.3	78.7	176.2	12.4
MARCH	4987.8	178.1	440.4	19.4
APRIL	10176.7	350.9	646.2	157.1
MAY	15299.4	493.5	803.4	206.0

Since the weather station at Auchincruive was out of commission from May to November, 1988 (inclusive) solar radiation figures for that period are not available.

Appendix IX: I.C.I. 'Dulux' colour cards used in classification of flower colour in *T. vulgaris*.

Chilterns	1131	Lambswool	1286
Vapour	1544	Honesty	1263
Thistledown	1521	Daybreak	1186
Fantasy	1211	Chiffon	1130
Cachou	1087	Heliotrope	1256
Paramour	1391	Colette	1146
Cobweb	1142	Wistful	1572
Largo	1289	Valentine	1540

Vesper	1549	Enchantment	1205
Veronica	1548	Cyclamen	1178
Barcarole	1046	Dollypink	1196
Wisteria	1571	Gigi	1236
Rhapsody	1434	Peony	1402
Galliard	1233	Camelot	1094
Gypsy	1252	Phlox	1407
Ballade	1039	Purslane	1427

5 Dulux Colour Collections by Matchmaker 5

Dulux