A Study of Some Factors Affecting the Yield and Composition of Peppermint Oil (*Mentha piperita* L.)

by

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Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

UNIVERSITY OF TASMANIA

HOBART

JULY 1980

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge, contains no copy or paraphrase of material previously published or written by any other person, except where due reference is made in the text of the thesis.

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ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr. R.C. Menary, Senior Lecturer in Horticultural Science of the Faculty of Agricultural Science, for his advice, constructive criticism and encouragement throughout this project. His efforts attracted the support of the Rural Credits Development Fund of the Reserve Bank of Australia to the project and this financial backing is acknowledged and appreciated.

I express my thanks to Mr. R.K. Lowry and Mr. D.A. Ratkowsky, C.S.I.R.O., Division of Mathematics and Statistics, Tasmanian Regional Laboratory, for their assistance with statistical analyses, and to the staff of the Central Science Laboratory, University of Tasmania, for assistance with Scanning Electron Microscopy and Gas Chromatography-Mass Spectrometry.

I wish to thank Mr. R.H. Cruickshank and fellow postgraduate students for helpful discussion and advice on various aspects of this project, the staff of the Faculty of Agricultural Science, Mr. G. Richardson and Mr. W. Peterson for their technical advice and assistance, and Mrs. S. Jones who typed the manuscript.

I would like to thank the Tasmanian Mint Farmers Association, in particular Mr. B. Brain, "Rotherwood", Ouse, for allowing me access to commercial plantings and for providing assistance with field experiments.

Additional financial support was provided by Bush Boake Allen, Australia, Ltd., Tasmanian Mint Farmers Association and a Commonwealth Extension Service Grant made to Dr. R.C. Menary.

Finally, I extend my appreciation to my wife, Sandra, for encouragement and assistance with all aspects of this thesis.

Summary

This study attempted to define some of the factors or groups of factors which together determine the yield and composition of peppermint oil. By investigating the effect of these factors on plants under glasshouse-growth room conditions, an attempt was made to understand the factors influencing oil yield and composition under field conditions. The manipulation of the field situation to increase oil yield without adversely affecting oil composition was investigated.

The oil content of peppermint leaves increased from basal to midstem leaves and decreased from midstem to apical leaves. Oil accumulation corresponded to the period of leaf expansion, during which glandular trichomes were observed to fill with oil. Midstem leaves accumulated maximum amounts of oil at the time inflorescences were observed on plants growing under long day-low night temperature (LD x LNT) conditions. Basal and apical leaves reached their maximum oil content prior to and following the appearance of inflorescences, respectively. Oil accumulation was favoured by LD x LNT conditions relative to SD x HNT (short day-high night temperature) conditions. The decreased oil accumulation under SD x HNT conditions did not appear to be associated with a deficiency of photosynthate, since oil maturation occurred to the same extent under both LD x LNT and SD x HNT conditions.

The results presented support previous reports of a true photoperiodic effect on dry matter, oil yield, growth habit and flowering. Furthermore, it appeared that there exists a true photoperiodic effect on the monoterpene composition of peppermint oil. Daylength, night

temperature, day temperature and light intensity were also important interacting factors determining oil yield and composition, under glasshouse-growth room conditions. The photosynthate model proposed by Burbott and Loomis (1967) explained the effect of environmental factors with respect to pulegone, menthone and menthofuran. Factors favouring the maintenance of high levels of photosynthate resulted in high concentrations of menthone and low concentrations of pulegone and menthofuran. The photosynthate model did not explain the effect of environmental factors on several other monoterpenes of peppermint oil.

An investigation of the net CO_2 exchange characteristics of peppermint indicated that light saturation occurred between 400 and 500 $\mu\Sigma m^{-2}s^{-1}$ in attached fully expanded leaves of peppermint. Maximum rates of 'apparent' photosynthesis occurred at 20°C. The important determinants of 'apparent' photosynthesis were an increase in 'true' photosynthesis when temperature was increased to 25°C, a steady increase in dark respiration with increased temperature, and a rapid increase in photorespiration between 15°C and 30°C. Such net CO_2 exchange characteristics of peppermint support the photosynthate model proposed to explain environmental effects on oil composition.

With respect to the field situation in Tasmania, provided that areas with reasonably high plant densities were considered, oil yield per unit area reached a maximum early in the growing season. Oil yield per unit area remained at the maximum level for a considerable period (5 to 6 weeks) with the only significant change being a final decrease in yield towards the end of the growing season. During the period of maximum oil yield, the percentage menthol increased from approximately 40% to 45%. Delaying harvest once the percentage menthol reached the required 45%, resulted in further increases in the percentage menthol, but at the expense of increased percentage menthofuran and decreased oil yields.

In addition to the above study of harvest date, the relationship between nitrogen application and irrigation rate and timing, on the yield and composition of peppermint oil and the possibility of obtaining two harvests of peppermint in one season, were investigated. High yields of oil were associated with high applications of nitrogen and high levels of irrigation, particularly throughout the last half of the growing season. The composition of oil extracted from herb at the commercial harvest date (approximately 45% menthol) was not significantly affected by either nitrogen or irrigation treatments. The oil yield from regrowth within the same growing season was significantly affected by irrigation and nitrogen treatments applied prior to the first harvest. When 300kg N/ha and 50mm of irrigation weekly (during the last half of the growing season) were applied, the oil yields from regrowth approached the yield normally obtained at the commercial harvest date. Oil from regrowth contained high concentrations of menthol, menthyl acetate, menthofuran and limonene, and low concentrations of menthone and cineole, relative to peppermint oil typical of Tasmanian production areas.

In a subsequent trial involving the manipulation of harvest date, nitrogen and irrigation, the first harvest was timed to coincide with maximum oil yield per unit area (40% menthol) and the second harvest occurred when the concentration of menthol exceeded 50%. In this way the total yield of oil per unit area was increased significantly without adversely affecting oil quality. By comparing the composition and yield potential of peppermint oil under Tasmanian conditions with that reported for other world production areas, it is concluded that Tasmania is well suited to the production of high yields of high quality peppermint oil.

Notes

Where possible, the abbreviations used in the bibliography are in accordance with 'Bibliographic Guide for Editors and Authors', published by the American Chemical Society, 1974.

The radiation environment of plants is referred to as 'light intensity' and indicates photon flux density measurements ($\mu\Sigma m^{-2}s^{-1}$).

The term photoperiod refers to the daily duration of continuous darkness. Daylength refers to the daily duration of light. That is, a short photoperiodic effect is an inductive response to a 'long night'.

Oil maturity is dependent on oil composition. Increased maturity is reflected by increased concentrations of menthol and menthyl acetate and decreased concentrations of menthone.

Oil quality refers to oil composition and the generally recognised indicators of high quality oil are outlined in Section II 1.2.

Guide of Appendices. Data presented in the appendices are organised under sectional headings which correspond with headings used in Sections III and IV (e.g. raw data and analysis of variance for Section IV A 3 is included in Appendix IV A 3).

I. INTRODUCTION

1. Tasmanian Peppermint Oil Industry

Peppermint oil production trials commenced in Australia in the 1920's when a four year trial was conducted in Western Australia. Although the oil extracted from this area was reputed to be of high quality (Marr, 1925), production did not continue.

Production in Australia only advanced past the experimental stage when small commercial areas were established in the Derwent Valley area of Tasmania in 1972. In 1976 the area planted to peppermint in Southern Tasmania was estimated at 30ha (Brain, 1976). At present the total area planted with peppermint is approximately 70ha; with 40ha being in the Derwent Valley, 10 to 15ha in the Huon Valley and the remainder in the north of the State, including King Island. From the estimated 50ha in the Derwent and Huon Valley areas, approximately 1 tonne of oil was produced in 1979 and slightly more than 1 tonne is expected in 1980. Therefore, although yields in excess of 50kg/ha have been recorded on individual farms and from trial plantings, considerably lower yields are associated with larger scale production.

The main factor contributing to the low average yields (20kg/ha) as compared with yields obtained from several individual farms (40-45kg/ha) appears to be associated with the decline in vigour of plantings in several established areas after approximately 4 years of production. Several pest and disease problems as well as several cultural problems such as late ploughing, late flaming and inadequate irrigation and fertilisers, have been implicated in this decline. However, it is likely that no single factor is completely responsible and that an interaction between a combination of these factors may be causing the observed decline.

During its establishment phase, the Tasmanian industry adopted many overseas techniques of production. For example, weed control, rust

control and harvest prediction were based on U.S.A. experience. In other respects the industry established its own production techniques, including irrigation and fertiliser practices. Several such techniques, in particular the low fertiliser regime and non-post harvest irrigation, have since been questioned.

An example of the general lack of knowledge which was associated with this industry during its establishment phase, involves the prediction of harvest date. Initially harvesting commenced when the plants were observed to flower, even though yield and quality characteristics of the oil at this stage of growth were not known. Subsequently, trial distillations were conducted and harvesting was timed to coincide with 45 percent menthol in extracted oils. Although the latter method provided some indication of the likely acceptability of the final product, little information was available on the changes in oil composition and oil yield per unit area during the growing season, under Southern Tasmanian conditions.

The general lack of understanding of this crop and the final product also lead to numerous difficulties associated with quality control; an essential requirement for the successful establishment of any new industry. For example, the nature of management practices which required manipulation to combat quality problems was unknown (e.g. did the loss of lower leaves caused by rust adversely affect quality and yield?).

2. Factors Affecting Yield and Composition of Peppermint Oil

Considerable information relating to all aspects of monoterpene metabolism, biosynthesis and accumulation is available in the literature (Loomis, 1967). With respect to peppermint, Loomis and associates, through their investigations, have made a very significant contribution

to the understanding of these processes. For example, Burbott and Loomis (1967) conducted the only controlled study of the effects of several environmental factors on the yield and composition of monoterpenes of peppermint. By combining the results of this study with other observations made by this group of workers, a model was proposed to explain the interacting effects of many factors on monoterpene metabolism.

With respect to numerous other factors affecting yield and composition of peppermint oil, the observations that have been made are somewhat less generally applicable. For example, the observed changes in composition and yield of oil with time and the effect of moisture stress and fertilisers may only be applicable within the environment in which such observations were made.

Despite the extensive research which has been conducted, several apparent disagreements are evident (e.g. photoperiodic effect on monoterpene composition).

3. Aims of the Present Study

(i) Investigate the interacting effect of several environmental factors on the yield and composition of peppermint oil, thereby adding to the model proposed by Burbott and Loomis (1967).

(ii) Undertake a preliminary study of the accumulation and interconversion of monoterpenes in peppermint oil both within individual plants and with increasing plant maturity, under different environmental conditions.

(iii) Follow changes in oil composition and oil yield throughout the growing season in Southern Tasmania, in an attempt to optimise oil yield per unit area and oil composition at harvest.

(iv) Manipulate factors such as irrigation rate and timing and nitrogen application, in an attempt to increase oil yield without adversely affecting oil composition.

(v) Evaluate the suitability of Southern Tasmania for the production of high yields of high quality peppermint oil.

(vi) Utilize information available in the literature as well as that obtained in (i)-(iv) above to manipulate the commercial yield and composition of peppermint oil in Southern Tasmania.

II. LITERATURE REVIEW

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1. Introduction

1.1 Peppermint Oil

Peppermint oil is extracted by steam distillation from the above ground portions of the plant *Mentha piperita* L. (Unless otherwise stated, all discussions will relate to the plant *Mentha piperita* L. var. Black Mitcham.) This oil occurs in minute glands on the upper and lower surfaces of leaves; stems contain little oil (Guenther, 1949b; Crane and Steward, 1962).

The volatile oil from peppermint comprises primarily monoterpenes, with less than 2 percent sesquiterpenes (Croteau *et al.*, 1972a). Sesquiterpenes will not be included in the current discussions or investigations.

Baslas *et al.* (1973) considered peppermint the most important commercial essential oil-bearing plant from the standpoint of number of acres grown for distillation. Unlike the oil obtained from *M. arvensis* L., the complete oil from *M. piperita* is incorporated into flavours. Peppermint oil is used in the flavouring of dentifrices, confectionery, pharmaceutical preparations and chewing gums (Ellis and Stevenson, 1950). Green (1975) reported that the peppermint variety *M. piperita* L. var. Black Mitcham, has existed in its present form since at least 1696. The modern flavouring industry is dependent on the unique and uniform flavour qualities of this variety and industrial users are reluctant to change without assurance of the same high degree of uniformity and acceptance of the product (Green, 1975).

1.2 Oil Quality

Since the complete oil is utilised by the flavouring industry, quality is of utmost importance. Although official criteria do exist for quality appraisal of peppermint oils (e.g. British Pharmacoepia, 1968),

the final quality assessment is usually based on organoleptical testing. However, there exists several generally recognised indicators of high quality in peppermint oils:

- high menthol (> 45%)
- low menthofuran (< 1-3%)
- low menthone (15-25%)
- high menthyl acetate (4-9%)

Lincoln and Murray (1978) considered that an increase in menthofuran above the preferred level of 1-3 percent, lowered oil quality and thus the market value of the oil. Nelson *et al.* (1971a)also reported menthofuran to be an ill-smelling and ill-tasting compound. Hocking and Edwards (1955) considered that menthyl acetate was a desirable component of high quality oil since it added an aromatic odour and flavour to the oil. On the other hand, menthone was considered to have a bitter flavour and a harsh odour (Hocking and Edwards, 1955; Manning, 1970).

In addition to the major constituents of peppermint oil, very many of the minor constituents may be of great importance in determining the final flavour and odour.

2. The Biosynthesis and Accumulation of Monoterpenes in Peppermint

An integration of biochemical, physiological and morphological observations.

2.1 The Pathways of Monoterpene Biosynthesis in Peppermint

A detailed discussion of the chemical, biochemical and *in vitro* studies on which the pathways of monoterpene biosyntheses are based is not the purpose of this review. However, a knowledge of the pathways leading to the various monoterpenes that are accumulated in peppermint is essential to the understanding of the effects of various cultural and

environmental factors on oil composition. In particular, it is the section of the pathway involving the conversion of pulegone to menthone and menthol, or pulegone to menthofuran which is of primary concern, since these conversions are of utmost importance in determining oil quality.

In the scheme of monoterpene biosynthesis outlined by Croteau and Loomis (1975), isopentenyl pyrophosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP) are considered to undergo condensation to form various open chain terpene pyrophosphates, in particular geranyl and neryl pyrophosphates. These workers considered that although geranyl pyrophosphate could function as the direct precursor of cyclopentanoid monoterpenes, neryl pyrophosphate was the immediate precursor of such terpenes. It was proposed that neryl pyrophosphate undergoes cyclization to form a hypothetical intermediate from which α-terpineol and several bicyclic cyclohexanoid monoterpenes such as the pinanes are formed. Such interconversions leading to α-terpineolwere considered common to both the biosynthesis of the C-2-oxygenated carvone series of monoterpenes found in spearmint and the C-3-oxygenated piperitenone series found in peppermint (Croteau and Loomis, 1975). In peppermint and spearmint α-terpineol was considered to be dehydrated to give mainly terpinolene or limonene, respectively. In peppermint, the next step in this proposed scheme was reported to be the hydroxylation of terpinolene to piperitenol and dehydrogenation to piperitenone. The final interconversion of monoterpenes to yield those commonly accumulated in peppermint is thought to involve the reduction of double bonds in both the ring and side chains of piperitenone, followed by reduction of the so-formed carbonyl (Croteau and Loomis, 1975).

In contrast to the suggestion of von Schantz and Norri [1968; Cited by Hefendehl and Murray (1976)], that terpenes occurring together are

largely formed independently, Battaile and Loomis (1961) considered that these terpenes were formed by a series of interconversions from an initial precursor terpene. Such a sequential biosynthetic series infers that each conversion of one compound to the next must be controlled by one or several genes which in turn control the formation of the necessary enzymes (Hefendehl and Murray, 1976). Croteau and Loomis (1975) suggested that these enzymes were highly specific. The diversity in peppermint oil composition may result from the operation of different gene controlled enzyme systems under different conditions. In this way, the effect of factors such as environmental conditions and plant maturity on oil composition may result from an effect on the activity of the various enzyme systems controlling monoterpene metabolism.

2.2 Monoterpene Interconversions

Reitsema (1958) proposed a biosynthetic sequence for the monoterpenes accumulated by peppermint. This sequence commenced with the unsaturated ketone piperitenone and proceeded in the direction of the saturated alcohol menthol.

That is, Piperitenone → Pulegone → Menthofuran

↓ ↓

Piperitone → Menthone → Menthol

Within this scheme it was proposed that any one of the reductions may occur to different degrees. For example, a failure of pulegone reduction may result in an accumulation of pulegone (as is the case with *M. pulegium*), or oxidation of pulegone to menthofuran.

Subsequently, Reitsema *et al.* (1961) demonstrated the incorporation of radioactive label from $^{14}CO_2$ into various peppermint oil monoterpenes. When exposure to $^{14}CO_2$ was short (3 min) the predominant labelled monoterpene was piperitone. Longer exposures (15 min) resulted in label appearing in several of the early components in Reitsema's scheme, as

well as numerous monoterpenes commonly accumulated in peppermint oil. Menthone, menthol and pulegone were identified. Therefore, it appeared that 15 minutes exposure to $^{14}CO_2$ was sufficient to allow synthesis of the monoterpenes reported to occur towards the end of Reitsema's scheme. This suggestion is in agreement with the findings of Hefendehl *et al.* (1967). These workers reported that peppermint shoots harvested immediately after 5 minutes exposure to $^{14}CO_2$, contained oil in which appreciable amounts of label was observed in all terpenes investigated, including menthol and menthofuran. In contrast to the above findings, Battaile and Loomis (1961) reported that peppermint shoots exposed to $^{14}CO_2$ for 17 hours in the light, did not incorporate label into either menthol or menthofuran when harvested immediately after exposure. Approximately 3 to 8 days were required before such compounds were labelled. Hefendehl *et al.* (1967) attributed this apparent disagreement with the findings of Battaile and Loomis (1961) to the insensitivity of the autoradiography techniques employed by the latter workers.

Further evidence in support of Reitsema's biosynthetic scheme has been provided by the results of numerous infiltration experiments using radioactively labelled oil components as reaction substrates. In this way, Battaile and Loomis (1961) demonstrated the conversion of piperitenone to piperitone and pulegone to menthone and menthofuran by leaf tissue of peppermint. Similarly, Reitsema *et al.* (1961) demonstrated the conversion of menthone to menthol, pulegone and several hydrocarbons. The infiltration of leaf tissue with labelled limonene and pinanes resulted in the appearance of five chromatographic spots, some in the areas of menthol, menthone and pulegone (Reitsema *et al.*, 1961). Whether such conversions were those typical of normal plant pathways or were the result of the conditions of the experiment (e.g. autooxidation of limonene and pinanes) was not determinable in these infiltration experiments

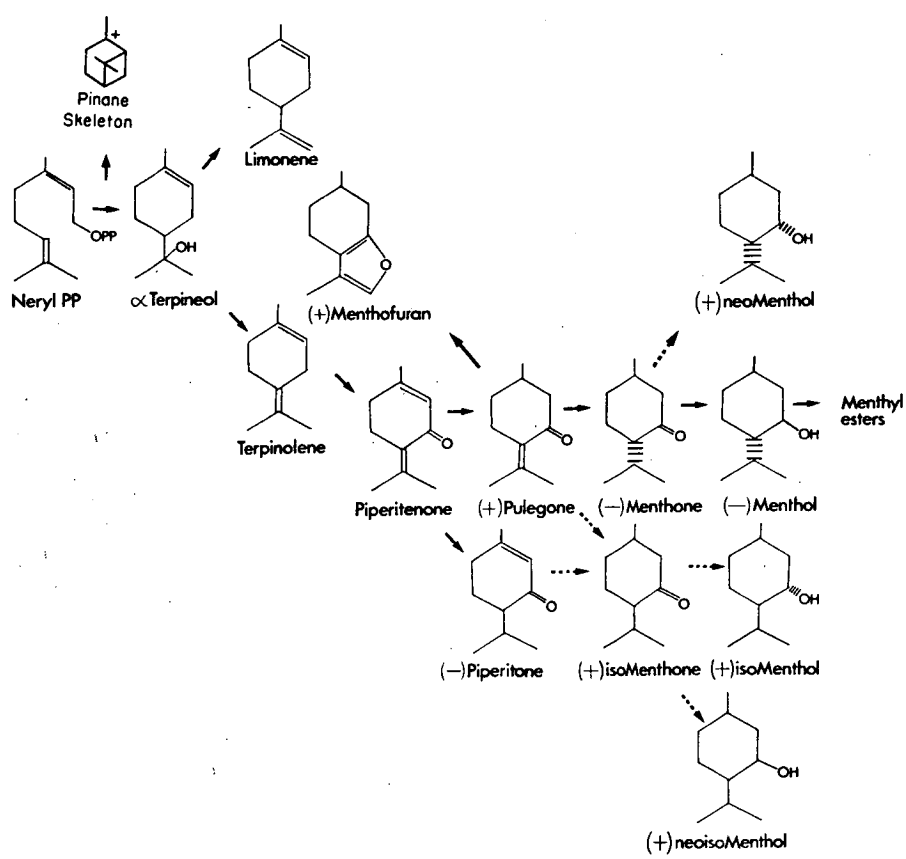
(Reitsema *et al.*, 1961).

Additional supporting evidence for the biosynthetic scheme has been provided by analysing leaves of increasing age on individual plants. This work has shown that menthofuran and pulegone are predominantly found in very young tissue. As older leaves were considered, the following sequence was observed; menthone, menthol, menthyl acetate (Reitsema *et al.*, 1957; Battaile and Loomis, 1961). The change in composition of peppermint oil with plant maturity has also been considered to reflect a time course of terpene synthesis and interconversions.

Recently developed techniques involving cell-free preparations from peppermint have allowed workers to demonstrate several *in vitro* conversions of monoterpenes. For example, Croteau and Hooper (1978) demonstrated the acetylation of menthol by a soluble enzyme preparation from peppermint leaves.

Figure II, 2.1 shows the known and postulated interconversions of the principal monoterpenes of peppermint. (Adapted from Croteau and Loomis, 1975.)

Fig. II 2.1.



2.3 Metabolic Turnover of Monoterpenes

Traditionally, monoterpenes and many other secondary plant products have been considered end-products of metabolism and as such, "metabolically inert". Contrary to this view, there is an ever increasing amount of information which suggests that monoterpenes are capable of undergoing rapid metabolic turnover. This in turn may suggest that these compounds have some metabolic function to serve within the plant.

An understanding of conditions which favour metabolic turnover as opposed to accumulation of monoterpenes in peppermint is essential in any attempt to rationalise the processes controlling oil accumulation and hence oil yield per plant.

Two main experiments have been reported which support the metabolic turnover of monoterpenes in peppermint. These experiments have involved kinetic studies using $^{14}CO_2$ and periodic analyses of monoterpenes in peppermint plants.

Kinetic Studies Using $^{14}CO_2$

Burbott and Loomis (1969) selected visually matched peppermint shoots from plants growing under short photoperiods, high day and night temperatures and low light intensity. These plants were exposed to $^{14}CO_2$ for 20 hours in closed vials, with alternating light and dark. Shoots were then sampled at intervals for 3 days after exposure. It was concluded from this work that the monoterpenes of peppermint gained label in the light and lost label in the dark, without any corresponding change in the total amount of monoterpene present (Fig. II, 2.2). These workers considered that since the experiment was conducted in a closed system, a large proportion of the respiratory $^{14}CO_2$ released during the dark would be available for fixation during the following

light period. As a result the subsequent increase in labelled monoterpenes during the second light period was not unexpected. The failure of monoterpenes to gain label during the third light period was attributed to the conversion of the $^{14}CO_2$ into metabolically inactive materials as well as the observed wilting of cuttings, which had occurred by this stage.

Cuttings used in the above experiment were reported to contain approximately equal amounts of menthones and menthofuran. Such an observation was not unexpected since these plants were taken from conditions reported to favour the accumulation of approximately equal amounts of menthones and menthofuran (Burbott and Loomis, 1967). It was also reported that the bulk of monoterpene label was divided equally between menthones and menthofuran. This is an apparent disagreement with a previous report from this laboratory (Battaile and Loomis, 1961) in which several days were necessary to label end-products of biosynthesis, such as menthofuran, which in this case would appear to have been labelled effectively after 20 hours.

In a similar experiment involving a shorter period of exposure to $^{14}CO_2$, monoterpenes gained label for 6 hours and almost lost this entire labelling during the subsequent 3 hour period (Burbott and Loomis, 1969) (Fig. II, 2.3). Unlike the previous experiment described by these workers, the latter experiment was conducted in continuous light. Therefore, it was concluded that the loss of label was not a direct result of the dark period.

With respect to experimental techniques, Burbott and Loomis (1969) outlined several difficulties associated with the selection of identical plants for their time course experiments. These workers reported that when visually matched cuttings from clonal material were harvested, extracted and analysed simultaneously, it was not uncommon to find

twofold differences in the total amount of essential oil per cutting. This problem was reported to result in considerable variation in labelling patterns. For this reason it was considered necessary to select the results for the time course study from cuttings uniform in the amount of monoterpenes and which were visually matched. In these time course experiments it is of utmost importance that selected shoots were identical in all respects. In this context, it should be noted that shoots uniform in total amount of monoterpene per cutting need not necessarily exhibit similar rates of oil synthesis and thus incorporation of radioactive label, during the experimental period. Secondly, in the short time course experiment (Fig. II, 2.3), it was suggested that the first two values should be rejected since these cuttings contained much less monoterpenes than the others and were therefore indicating physiological non-uniformity. Burbott and Loomis (1969) also considered that the variation in the amount of monoterpene although existing, did not parallel the variations in labelling. However, in many respects increased amounts of essential oil were paralleled by increased labelling and *vice versa* (e.g. Fig. II, 2.3). These variations in the total essential oil content were discounted because they were considered to be of a much lower magnitude than the five to tenfold fluctuations of label that were commonly observed. However, if the total essential oil of a shoot is considered as being comprised of two pools of oil, a large non-labile pool and a smaller metabolically active pool, and if the fluctuations in oil content between shoots is a reflection of changes in the latter pool, then fluctuations within this pool may well be of a similar magnitude as those presented for the incorporation of label. Finally, it should be noted that the metabolic turnover observed may be a characteristic of unrooted cuttings and may not occur to the same extent in rooted plants.

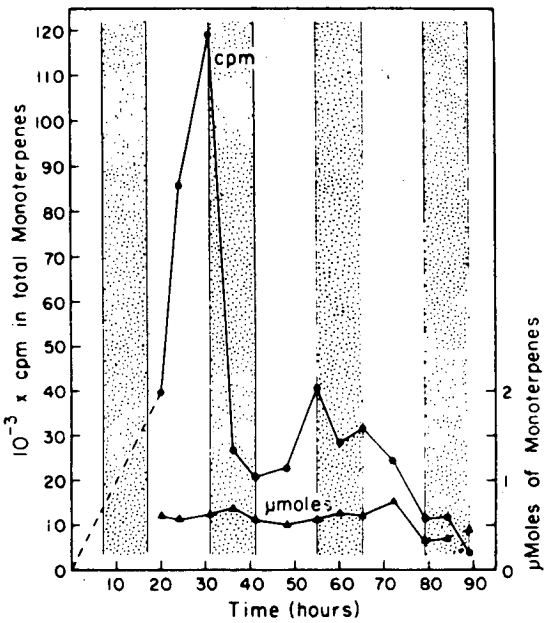
Therefore, results from experiments such as those outlined above, should be interpreted with consideration of the problems and possible limitations involved.

Periodic Analyses of Monoterpenes

In a second series of experiments, Burbott and Loomis (1969) grew visually matched peppermint cuttings in a controlled environment and the monoterpenes were analysed periodically, node by node, during the course of plant development. The data included in Table II, 2.1 and Fig. II, 2.4 are from plants grown with a 16 hour day at $24^{o}C/8^{o}C$ (day/night) temperature, under growth cabinet light intensities.

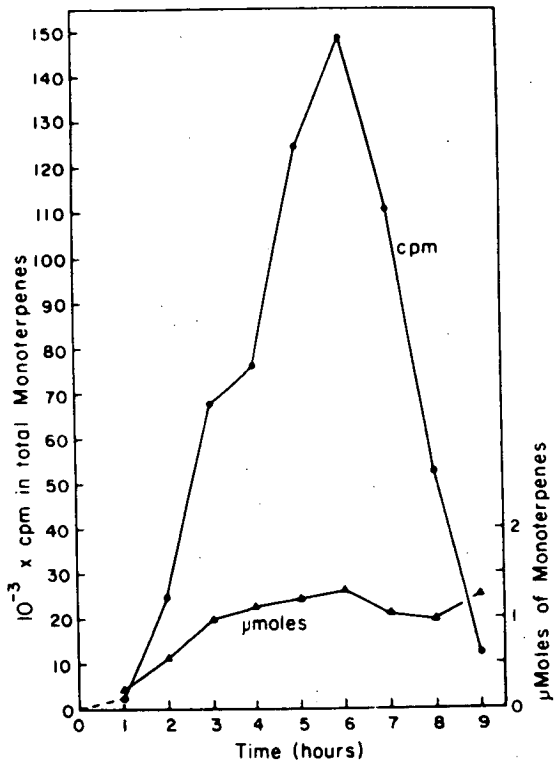
From the data presented, Burbott and Loomis (1969) concluded that the intermediate and lower leaves reached their highest essential oil content at the time when floral initiation could be observed macroscopically. The monoterpene content of these leaves decreased rapidly after this stage. The peak amount of essential oil was associated with a rapid increase in the amount of menthone present. Menthone also decreased during the period when oil yield was observed to decrease. Although the decrease in menthone was associated with a small increase in menthol, the latter increase was not considered sufficient to account for the rapid decrease in menthone.

The upper leaves completed their development after floral initiation. These leaves were reported to have a menthone peak at the time of first bloom, followed by accumulation and subsequent loss of menthone. The lowest leaf pairs were reported to accumulate very little monoterpenes. This observation was considered consistent with the previous report of Burbott and Loomis (1969) in which unrooted cuttings were reported to synthesise but not accumulate monoterpenes. The lowest leaves were observed to expand during a stage when the shoot was forming roots.



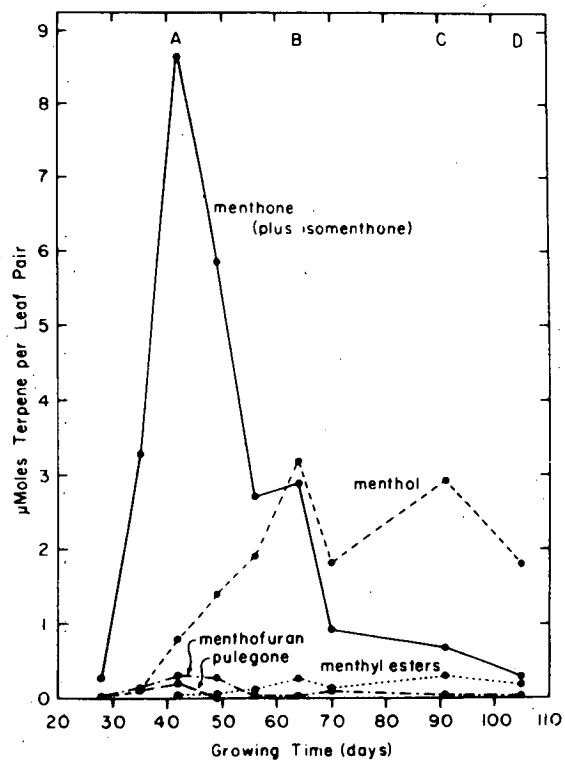
Time course of labeling of peppermint monoterpenes from $^{14}CO_2$ in alternating light and darkness. Stippling indicates dark periods. Twenty-six cuttings were exposed to 400 μc of $^{14}CO_2$ for 20 hr before sampling was started. Each sample consisted of 2 cuttings, and the values given are per 2 cuttings.

Figure II 2.2



Time course of labeling of peppermint monoterpenes from $^{14}CO_2$ in continuous light. Twenty cuttings were exposed to 800 μc of $^{14}CO_2$ for 1 hr and then sampled hourly. Each sample consisted of 2 cuttings, and the values given are per 2 cuttings.

Figure II 2.3



Individual monoterpenes of leaf pair number 9 of peppermint plants grown on a 16-hr photoperiod with a 24° day and 8° night. (Average of triplicate analyses; same experiment as table I. For explanation of A, B, C, and D, see footnote 2 of table I.)

Figure II 2.4

Table II 2.1. Development of essential oil in peppermint (data = mμmoles of monoterpenes per leaf pair) (taken from Burbott and Loomis, 1969).

Leaf Pair[1]	Days										
					A[2]			B		C	D
	16	21	28	35	42	49	56	64	70	91	105
Inflorescence						810	4560	5960	21250	*24500*[3]	7180
15	4							*550*	470	110	50
14							200	*3380*	1520	300	330
13						940	2720	*3780*	3220	2290	840
12					1530	3600	3990	*5170*	4830	4120	2510
11				530	3320	*6840*	5400	4520	5590	4220	1330
10				1040	*8560*	7040	4760	5640	3250	4530	2350
9			330	3930	*10300*	7740	4520	5950	2960	4080	2720
8		40	970	4960	*9210*	3610	3720	4120	2570	3420	1160
7		180	2270	3770	*5680*	3120	2240	3120	2070	2280	920
6	80	450	2520	2910	*4360*	2070	1520	2220	1190	1310	550
5	330	760	1470	1460	*2980*	1560	1710	1100	610	1020	420
4	400	570	740	760	*1230*	570	480	550	800	520	320
3	410	550	660	*800*	750	400	660	450	710	580	330

[1]Leaf pairs are numbered from the base of the plant.

[2]A = time at which floral initiation could be recognised macroscopically; B = time at which first flowers opened; C = full bloom; D = end of bloom.

[3]Italicized values indicate the maximum monoterpene content reached in the respective leaf pairs.

Given that leaves on unrooted cuttings do not accumulate monoterpenes, then it is plausible that the lower leaves in the above experiment should contain lower amounts of monoterpenes. However, in the periodic analyses of monoterpenes described above, the synthesis and accumulation of oil was observed to continue long after the leaves had fully expanded. In this case, it might be expected that once conditions within the plant had changed (i.e. formed roots) accumulation rather than metabolic turnover of monoterpenes would occur in lower leaves.

Secondly, if it is assumed that essential oil is accumulated only in glands and the number of glands on a fully expanded leaf remains constant, then it follows that an extremely large increase in gland size must occur immediately prior to floral initiation. For example, in leaf 10 an eightfold increase in gland size in a 7 day period would be required to accommodate the peak amount of essential oil(Table II 2.1).

When plants were grown under shorter days (14 hours) and warm nights ($24^{o}C$), neither the large peak nor the rapid decrease in oil content were observed (Burbott and Loomis, 1969). Instead, changes in oil content were reported to be more gradual. Burbott and Loomis (1969) reported that during the period of decreasing oil yield,an increasing number of empty or partially empty oil glands were observed. These glands were reported to have an appearance suggesting metabolic depletion rather than external injury.

Croteau and Martinkus (1979) also conducted periodic analyses of monoterpenes from midstem leaves of flowering peppermint. Consistent with the above findings, menthone turned over rapidly at the onset of flowering. These workers reported that when radioactively labelled menthone was incubated with leaf discs of flowering peppermint, labelled menthol was the major steam-volatile product (10% of incorporated label). However, the major portion of the incorporated tracer (86%) resided in

the non-volatile metabolites of the labelled menthone; (+)-neomenthyl glucoside appeared to be the major non-volatile metabolite. This conversion of menthone into non-volatile metabolites would account for the rapid decrease in the volatile oil content of peppermint leaves following floral initiation, reported by Burbott and Loomis (1969). Croteau and Martinkus (1979) suggested that,if during turnover, the monoterpenes are utilized at sites other than the oil glands, a means of transporting these lipophilic materials would be required. Monoterpenyl glucosides were suggested to represent such a transport form (Croteau and Martinkus, 1979).

Metabolic turnover of monoterpenes arising from MVA-^{14}C, glucose-^{14}C and sucrose-^{14}C, has also been well documented (Scora and Mann, 1967; Banthorpe *et al.*, 1970; Croteau and Loomis, 1972; Croteau and Loomis, 1975). According to Loomis and Croteau (1973) storage pools such as oil contained in glandular secretory spaces, probably turnover quite slowly. The rapid turnover observed in the previously described kinetic studies most likely represented the turnover of a more metabolically active pool.

Croteau *et al.* (1972b) reported that turnover was dependent on environmental conditions (e.g. light and temperature) and on the physiological condition of the plant. Generally, most of the labelled terpenes produced in short term experiments were metabolised and not stored. Loomis and Croteau (1973) concluded that the variation in turnover period with the time of the day that cuttings were taken, suggested that terpene biosynthesis and accumulation was dependent on the amount of endogenous photosynthate available. In particular, terpene storage was reported to be enhanced by an abundance of photosynthate.

In conclusion, Loomis and Croteau (1973) stated that "evidence suggests that synthesis, turnover and storage of essential oils are

controlled by the balance between photosynthesis and utilization of photosynthate. Catabolism of essential oil components during times of photosynthate deficiency does not seem unreasonable, as such compounds represent a considerable amount of potential metabolic energy".

2.4 Site of Oil Synthesis

The accumulation of essential oil in peppermint has been associated with the filling of specialised glandular structures (Loomis and Croteau, 1973). These glandular structures appear during early leaf development and at least during these early stages of development oil synthesis is rapid. The extent to which oil synthesis continues in the expanding leaf has been the subject of several investigations.

$^{14}CO_2$ Tracer Studies

Battaile and Loomis (1961) exposed peppermint shoots to $^{14}CO_2$ and reported that only young expanding leaves contained labelled terpenes, when these shoots were subsequently analysed. These workers concluded that only these young expanding leaves were capable of synthesising monoterpenes. A more correct conclusion would be that only these leaves were capable of synthesising monoterpenes from exogenous $^{14}CO_2$.

Reitsema *et al.* (1961) and Hefendehl *et al.* (1967) supported the above findings that young tissue rapidly incorporated radioactive label into monoterpenes, but neither group of workers commented on the ability of older leaves to synthesise monoterpenes. Hefendehl *et al.* (1967) suggested that the radioautography techniques used by Battaile and Loomis (1961) were too insensitive to detect low concentrations of several labelled monoterpenes formed after exposure to $^{14}CO_2$. If such a criticism was justified then it is also plausible that this technique may have been unable to detect low levels of incorporation of label into monoterpenes in older leaf tissue.

Periodic Analyses of Monoterpenes

In subsequent work, Burbott and Loomis (1969) periodically analysed monoterpenes during the course of plant development. From this work it was concluded that monoterpene synthesis continued longer after the leaves had reached full size, than suggested by evidence based on the incorporation of label from $^{14}CO_2$. This apparent disagreement in results was suggested to arise from either differences in environmental conditions under which the plants were grown, or the fact that after a certain stage of development,secretory cells were cut off from outside carbon sources but continued to produce monoterpenes from stored substrates.

Oil Gland Morphology

Additional information on the site of oil synthesis and accumulation has been provided by observations of the glandular structures in which the oil accumulates. These oil glands have been studied in detail by several workers (Ameluxen, 1964; Ameluxen, 1965; Ameluxen, 1967; Ameluxen *et al.*, 1969). These workers observed that peppermint had two types of glandular structures; three-celled glandular hairs with one secretory cell and ten-celled glandular trichomes with eight secretory cells (Ameluxen *et al.*, 1969). From detailed studies, it was concluded that peppermint oil glands were unique in the degree of degeneration of internal membrane structures at a very early stage of leaf development (Ameluxen, 1965). In a review of this subject, Loomis (1967) stated that the glandular cells have a very dense cytoplasm with no large central vacuole. The intracellular organisation in very young glands was observed to be similar to adjacent epidermal cells except that the endoplasmic reticulum was more highly developed in glands. Loomis (1967) concluded that as the glandular secretory space developed, the strongly

osmophilic material previously contained in many small vacuoles disappeared and the cells commenced to degenerate; "In the trichomes the cell organelles shrink, the osmophilic material appears in the subcuticular space and the intracellular membrane structure degenerates". Similar processes were observed in the glandular hairs, although degeneration was not observed to proceed to the same extent (Loomis, 1967). In the glandular hairs, the essential oil remained in cytoplasmic vacuoles (Loomis and Croteau, 1973).

Ameluxen (1965) considered that all of the above changes in glandular structures occurred at a very early stage of leaf development. In the glandular hairs these changes were reported to be completed by the time the leaf was 1.0 to 1.5mm in length and in the trichomes by the time the leaf was 4 to 5 mm in length (Loomis, 1967). That is, the observed degeneration of structure in the oil gland cells and the filling of glands with oil, reported by Ameluxen (1964, 1965), occurred while the leaves were still very young and had hardly commenced expansion (Loomis and Croteau, 1973).

It appears from Ameluxen's observations that oil synthesis only occurred in the extremely young leaves since cellular contents of secreting glands degenerate at an early stage of leaf development; the assumption being that degenerate cells are not capable of synthesising oil. Contrary to this view, $^{14}CO_2$ tracer studies and periodic analyses of monoterpenes (Battaile and Loomis, 1961; Burbott and Loomis, 1969) suggested that synthesis and accumulation of oil continued long after the above stage of leaf development had been reached.

Lemli (1963) observed that oil glands required 2 to 3 weeks to fill with oil, after their formation. Furthermore, Lemli (1963) considered that the maximum capacity of glands (0.07 to 0.08µg) occurred 4 to 6 weeks after leaf formation, at a stage when leaf expansion had ceased.

No further increase in oil content was observed. Lemli's comments were based on light microscopic examination and were apparently confined to glandular trichomes, since this worker did not recognise the existence of two types of glands. Lemli (1963) also reported that the final number of glands per leaf were present on the very young leaf and that this number did not change during leaf development. However, the oldest and youngest leaves were reported to have the smallest number of glands.

Bullis *et al.* (1948) reported that the size of glands increased rapidly until full bloom after which a very slow increase was observed. These workers also reported that the number of glands increased until full bloom. However, it should be noted that the above observations were based on gland counts and measurements from a random sample of leaves taken periodically during the growing season, and therefore do not refer to changes in gland number or size on an individual leaf basis.

Gas chromatographic analysis of glands isolated from young leaves (less than 1.5cm in length) by Ameluxen *et al.* (1969),indicated that the ten-celled glands contained a very "mature oil" in which menthol and menthyl acetate were the predominant monoterpenes. In contrast, the three-celled hairs contained an "immature oil", high in menthone (cited by Loomis and Croteau, 1973). Loomis and Croteau (1973) suggested that this observation was related to the fact that the ten-celled trichomes "mature" and lose their internal membrane structure earlier than hairs.

There is direct evidence to suggest that essential oils accumulate in glandular structures in peppermint (Ameluxen, 1964, 1965). It also seems likely that oil is synthesized in these glands. However, there are several indications that oil glands are not the only site of oil synthesis and accumulation. Ameluxen (1967) observed numerous osmium-staining "filament bundles" in young leaf cells of peppermint. He suggested that these structures represented essential oil precursors.

Loomis and Croteau (1973) suggested that the apparent disagreement in results obtained from $^{14}CO_2$ tracer studies and periodic analyses of monoterpenes, with observations made by Ameluxen (1965), could represent a further indication that synthesis and accumulation occurs in areas other than oil glands. That is, either the oil gland cells continue to function longer than they appear to, or that synthesis occurs in other parts of the plant (Loomis and Croteau, 1973).

2.5 Biosynthetic Sites

Although it is generally accepted that mevalonic acid (MVA) is the precursor of monoterpenes, most plants are unable to efficiently utilize exogenous MVA for the biosynthesis of monoterpenes (Croteau and Loomis, 1975). Typically, only 0.01 to 0.1 percent of MVA or acetate label was incorporated into monoterpenes, even when optimum dose rates and method of administration were employed (Battu and Younken, 1966; Loomis, 1967; Banthorpe *et al.*, 1972).

Croteau and Loomis (1975) concluded that monoterpenes labelled from $^{14}CO_2$ or MVA-^{14}C, in almost all cases, contained the bulk of the label in the portion of the molecule which was derived from IPP. Therefore, it was suggested that the IPP derived from labelled precursor combined with DMAPP that was present in a metabolic pool at the site of synthesis. That is, a compartmentation with respect to monoterpene synthesis was suggested.

Hefendehl *et al.* (1967), Loomis (1967) and Burbott and Loomis (1969) concluded that $^{14}CO_2$ in the light was a relatively good monoterpene precursor; much better than MVA-^{14}C. Therefore, it was suggested that the site of monoterpene synthesis was isolated from the rest of the plant and that the bulk of MVA utilized in monoterpene synthesis must arise at the site of synthesis from translocated photosynthate, probably

sugars (Loomis, 1967; Croteau *et al.*, 1972b). The high incorporation of glucose - ^{14}C observed by Loomis and Croteau (1973) suggested a preferential transport of sugars to the terpene-producing cells.

Burmeister and von Guttenburg (1960) studied the accumulation of essential oil under low O_2 conditions and with metabolic inhibitors. On the basis of their findings, it was reported that the biosynthesis of essential oil is a partially anaerobic process, which occurs as an adaptation to limited O_2 supply. Furthermore, the morphology of glands was considered to be such as to suggest a degree of isolation both from the rest of the plant and from the atmosphere (i.e. 'single stalk cell, heavily cutinized') (Croteau *et al.*, 1972b).

Therefore, Croteau *et al.* (1972b) suggested that the biosynthetic sites in peppermint are not readily accessible to either carbon substrates or O_2. In addition, the early membrane degeneration suggested by Ameluxen (1965) may result in a deficiency of functional mitochondria in these glands. If the above conditions do exist at biosynthetic sites,and if at the same time the supply of photosynthate is limited, then Croteau *et al.* (1972b) concluded these glandular cells would be very energy-deficient. The following hypothesis was forwarded by Croteau *et al.* (1972b). The *in vivo* biosynthesis of acetyl-CoA from sugars yields ATP and reduced pyridine nucleotides, both of which are required in the utilization of acetyl-CoA for monoterpene synthesis. Therefore, when exogenous MVA is introduced to such glands, the above co-factors still need to be generated endogenously if terpene synthesis is to proceed. It was suggested that such a requirement may present a problem for monoterpene biosynthesis within an isolated oil gland where photosynthate may not be readily available and where primarily fermentative mechanisms may be operative. In this way, oil glands may

be very sensitive to the type and amount of fermentable substrates available to them from adjacent cells.

In an attempt to test the above hypothesis, Croteau *et al.* (1972b) investigated the effect of unlabelled glucose on the incorporation of MVA-^{14}C into monoterpenes. Glucose was observed to enhance the incorporation. Similarly, increasing the concentration of CO_2 to 500 ppm during incorporation in the light, significantly increased monoterpene labelling from MVA-^{14}C. Both glucose and 500 ppm CO_2 were considered to have their effect by increasing the supply of photosynthate to the terpene-producing cells.

A lack of co-factors such as $NADPH_2$ in oil-producing cells would not only have an effect on oil synthesis, but also on maturation and monoterpene interconversions, since $NADPH_2$ has been showed to be a necessary co-factor in the conversion of pulegone to menthone and isomenthone, and menthone to menthol (Battaile *et al.*, 1968). Therefore, any factors having an effect on net carbohydrate balance within the plant could be expected to effect both synthesis and accumulation of monoterpenes.

3. Environmental Effects on the Yield and Composition of Peppermint Oil

There are many indications that the biosynthesis and metabolism of monoterpenes in peppermint are influenced by environmental factors (Burbott and Loomis, 1967). Environmental factors such as day temperature, night temperature, daylength and light intensity have been reported to affect the yield and composition of peppermint oil (Burbott and Loomis, 1967).

3.1 Geographic Areas of Production

Although peppermint oil of acceptable composition (containing menthol, menthone and menthyl acetate and little pulegone and menthofuran)

can only be produced in certain geographic areas, it is obvious from the literature that no one factor such as daylength, is the sole determinant of these production areas. Chandra *et al.* (1968) reported the production of high quality oil in India (60.6% menthol, 7.5% menthyl acetate and 0.7% menthofuran). Gupta *et al.* (1971) and Ghosh and Chatterjee (1976) concluded that although production of oil having an acceptable composition was possible in India, limitations existed due to the agroclimatic requirements of the crop, especially as it affects oil composition. These workers reported that oils produced at high altitudes had high concentrations of menthol whereas oils produced at lower altitudes had an optical rotation of $+6^{o}15'$ (Gupta *et al.*, 1971). (Positive values of optical rotation are indicative of high menthofuran concentrations.) Higher temperatures and/or lower light intensities would be expected at lower altitudes and these factors may have resulted in increased levels of menthofuran. In contrast to the findings of most workers, plants observed by Virmani and Datta (1968) at Lucknow ($26'52^{o}N$) flowered and produced oil of acceptable composition under conditions of short days, high day temperatures and high night temperatures. Peppermint oil produced in Florida ($29^{o}40'N$) was low in menthol and generally of poor quality (Hocking and Edwards, 1955). Fahney *et al.* (1955) reported total menthol concentrations ranging from 46.32 to 58.0 percent and menthyl acetate concentrations ranging from 6.82 to 15.6 percent in oils produced in Egypt ($30^{o}N$). In contrast, oils produced in Israel ($33^{o}N$) contained only 12.9 percent total menthol and had an odour reminiscent of pennyroyal (Hocking and Edwards, 1955). Pennyroyal contains high concentrations of pulegone (Battaile *et al.*, 1968). Therefore, it is not possible to impose strict geographical boundaries on the production of peppermint oil of acceptable composition, since many environmental

factors interact to determine the final oil composition from any production area.

With respect to production areas, Guenther (1961) reported that although peppermint grew luxuriantly in tropical or subtropical countries, the essential oil yield was low. As a result, this worker suggested that production of peppermint oil should be restricted to the northern latitudes.

3.2 Daylength

Effect on Plant Growth and Oil Yield

Ellis (1960) considered daylengths of at least 16 hours essential for high yields of peppermint oil. Allard (1941), Langston and Leopold (1954) and Stewart (1962) indicated that peppermint was daylength-sensitive. This was demonstrated by Langston and Leopold (1954) to be a true photoperiodic effect. Short days gave rise to decumbent plants with small leaves and a profusion of stolons. Long days resulted in erect plants with large leaves, flowers and high yields of essential oil. In the work of Langston and Leopold (1954) daylengths of 10, 12 and 14 hours failed to bring about floral initiation. Although a daylength of 16 hours produced a long day plant, floral initiation was not observed. In contrast, Allard (1941) reported minimal flowering under 14 hour daylengths. Burbott and Loomis (1967) reported that temperature influenced the time of flowering and the critical daylength. According to Langston and Leopold (1954), light intensity did not affect the initiation of flowering; floral development was favoured by increased intensity.

In the experiment of Langston and Leopold (1954), all cuttings with the exception of one group of plants (designated as continuous 18 hour days) were grown under short day conditions (10:14) for 30 days before

commencing the photoperiod treatments. The continuous 18 hour day plants were grown under 18 hour days from the time all cuttings were planted. Thus, the only difference between 18 hour day plants and continuous 18 hour day plants was the pre-treatment growing conditions. The effect of pre-treatment growing conditions were observed in plants even after 49 days under the treatment conditions. For example, Langston and Leopold (1954) reported that the continuous 18 hour day plants differentiated flowers more rapidly. From this it was concluded that peppermint plants became photoperiodically receptive during early stages of growth. The 18 hour day plants were reported to accumulate only one half the amount of essential oil relative to continuous 18 hour plants. This may have resulted from the fact that long day plants were observed to have more glands per unit area on the lower leaf surface, than short day plants. That is, leaves on the 18 hour day plant produced during the pre-treatment period would have differentiated the number of oil glands characteristic of short day plants. In contrast, all leaves on the continuous 18 hour day plants (with the possible exception of those differentiated prior to planting) would have experienced long day conditions during their formation.

Therefore, the importance of pre-treatment effects on the subsequent treatment response should be emphasised. This is particularly the case in photoperiod experiments. For example, because plants do become photoperiodically receptive at an early stage of growth, those leaves differentiated prior to commencement of the treatment, which are observed to expand during the treatment, may in fact be more characteristic of the pre-treatment growing condition than of the treatment growing conditions.

Effect on Oil Composition

Several workers have studied the effects of photoperiod on oil composition. Grahle and Holtzel (1963) found that leaves of *M. piperita* grown at 20°C constant temperature and subjected to long days (18:6), contained relatively small amounts of menthofuran and large amounts of menthol and menthone. Plants subjected to short days (12:12) contained relatively small amounts of menthone and menthol and large amounts of menthofuran. In order to differentiate between photosynthetic and photoperiodic effects, Grahle and Holtzel (1963) conducted night interruption studies with peppermint. The data obtained indicated that the observed differences in oil composition were a consequence of a true photoperiodic effect. These workers found that short days (12:12) resulted in oil, high in menthofuran (85%) and low in menthol (10%) and menthone (1%). Plants subjected to a photoperiodic treatment of (12:12) but with one hour of interrupting light in the middle of the dark period, yielded oil which was low in menthofuran (9%) and relatively high in menthol (56%) and menthone (25%), thus resembling plants grown under a (18:6) long day photoperiod, with respect to oil composition. A possible criticism of the technique used by Grahle and Holtzel (1963) is that these workers did not completely separate the effects due to photoperiod from those due to photosynthesis. That is, the extra hour of light introduced into the middle of the dark period increased the time available for photosynthesis by one hour. However, it is unlikely that the extra hour of light would have such pronounced effects on composition, if a photosynthetic mechanism were responsible.

Hefendehl *et al.* (1967) referred to data obtained by Holtzel (1964; Cited by Hefendehl *et al.*, 1967). Hefendehl *et al.* (1967) reported that the results of Holtzel suggested that two different biosynthetic pathways existed in peppermint, one of which was dependent on the length of

photoperiod. The sequence piperitenone → piperitone → menthone → menthol was reported to be operative only during long exposures to light (18 hour day), whereas the transformation piperitenone → pulgeone → menthofuran was apparently independent of photoperiod.

Such a report by Hefendehl *et al.* (1967) is not consistent with the reported findings of Grahle and Holtzel (1963). Firstly, there appears to be a degree of confusion with respect to the use of the term 'photoperiod'. Although it was suggested that the first pathway was dependent on photoperiod, it was a long daylength rather than a long photoperiod (short night) which was considered necessary by Hefendehl *et al.* (1967). In fact, Grahle and Holtzel (1963) did report that long photoperiods (in the true sense) were needed for the conversion to menthone. Secondly, Grahle and Holtzel (1963) reported that the conversion to menthofuran occurred only under short photoperiodic conditions and therefore was not independent of photoperiod.

Subsequent reports by Burbott and Loomis (1967) are in apparent disagreement with the findings of Grahle and Holtzel (1963). Burbott and Loomis (1967) included experiments with interrupted nights and low light intensity and concluded that photoperiod as such did not directly influence the composition of monoterpenes in peppermint oil. Plants grown under conditions of 8 hours light per day at 25°C constant temperature and plants grown under identical conditions with a 15 minute light flash in the middle of the dark period produced oils which were considered typical of short day plants. Both oils were reported to contain principally menthofuran. These workers found that when the light intensity was reduced, plants grown under daylengths of 18 hours at 25°C constant temperature, also produced oil with a composition typical of short day plants.

It appears from the above discussion that there exists an apparent disagreement between the conclusions of Burbott and Loomis (1967) and Grahle and Holtzel (1963), with respect to the existence of a true photoperiodic effect on the composition of peppermint oil.

3.3 Interaction Between Daylength, Night Temperature and Light Intensity

In an investigation of the effects of night temperature and daylength on monoterpenes of peppermint, Burbott and Loomis (1967) concluded that with either an 8 or 14 hour day there was a striking effect of night temperature on oil composition, when plants were grown at 25°C day temperatures. Warm nights (25°C) favoured the relatively oxidized compounds menthofuran and pulegone, while cool nights (8°C) favoured accumulation of the more reduced compound menthone. When daylength was increased to 18 hours, Burbott and Loomis (1967) concluded that night temperature had little effect on the composition of oil. Menthone was the predominant monoterpene under both warm and cool night conditions. However, an 18 hour photoperiod at low light intensity gave very poor growth and produced predominantly menthofuran under warm nights and menthone under cool nights. The light intensity used in this latter experiment was considered adequate for photoperiodic effects but provided little energy for photosynthesis. With a 12 hour day and a cooler day temperature regime (15°C days), menthone predominated with either 8°C or 15°C night temperatures.

Burbott and Loomis (1967) reported that inflorescences, whenever they appeared, contained high levels of menthofuran and pulegone, even under cold nights. Inflorescences developed on the 18 hour day plants exposed to full light intensity after 21 days in the growing conditions, and after 63 days on the 14 hour day plants (25°C/25°C).

Therefore, from the experimental work presented, Burbott and Loomis

(1967) concluded that there were clearly photoperiodic effects on flowering and vegetative growth in peppermint, both of which were promoted by either long light periods or by interruption of the dark period. However, photoperiod was not considered to have any direct effect on monoterpene metabolism. The increased amount of essential oil formed under long day conditions was considered to be largely a reflection of increased growth (Burbott and Loomis, 1967).

In an attempt to explain their results, Burbott and Loomis (1967) advanced the following model. "It is possible that the oxidation-reduction level of the monoterpenes reflect the general oxidation-reduction state of the respiratory co-enzymes of the terpene-producing cells, and that this depends on the balance between daytime photo-synthesis and night time utilization of photosynthate." That is, in the light photosynthesis would produce reducing conditions and in the dark the products of CO_2 fixation would serve as respiratory substrates. Burbott and Loomis (1967) considered that as long as these respiratory substrates were available in abundance, the respiratory co-enzymes would remain in a relatively reduced state. Depletion of these substrates resulting in oxidizing conditions would be envisaged as resulting in depletion of reduced respiratory co-enzymes. In particular, strongly oxidizing conditions might be expected during the latter part of a long warm night (Burbott and Loomis, 1967).

Several conditions under which Burbott and Loomis (1967) conducted their experimental work warrants discussion at this stage. Firstly, although several references were made to the fact that experiments were conducted at "full light intensity", it would appear that this only referred to full light intensity within controlled environment rooms. Such light intensities are typically much lower than natural light intensity. That is, all experiments were conducted at low light

intensity relative to natural light intensity. When Burbott and Loomis (1967) reduced light intensity further, the monoterpene composition became more sensitive to changes in factors such as night temperature. For example, plants grown under 18 hour days at "high light intensity" produced predominantly menthone under both warm and cool night conditions. A reduction in light intensity resulted in these 18 hour day plants producing menthofuran under warm night conditions. In this way, the sensitivity of monoterpene composition to changes in daylength and night temperature, may only be characteristic of plants growing under the relatively low light intensities of controlled growth rooms. In addition, the relatively high day temperature regime used ($25^{o}C$) may have increased the sensitivity of monoterpene composition to changes in other factors. This was suggested by the observed insensitivity of monoterpene composition to changes in daylength and night temperature when plants were grown at cooler day temperatures ($15^{o}C$).

Secondly, plants were subjected to the treatment growing conditions for a variable and relatively short period of time, prior to obtaining the results reported. Plants from which cuttings were taken were grown in the greenhouse under photoperiods of 14 hours or longer (i.e. high light intensities and intermediate to long day conditions; 14 hour day plants were reported to flower in the subsequent experiment). Cuttings, consisting of the tuft of youngest leaves at the growing tip, plus the next three leaf pairs, were rooted in the greenhouse for 7 days. Following this 7-day rooting period, plants were transferred to the treatment growing conditions for varying periods to obtain the results reported.

That is, 14 hour day for 10 days

8 hour day for 13 days

8 hour day with interrupted night for 12 days

18 hour day for 21 days

18 hour day with low light intensity for 26 days

12 hour day for 19 days

Significant pre-treatment effects on peppermint were outlined in a discussion of results presented by Langston and Leopold (1954). Similar effects may have occurred in the present study. For example, plants exposed to 8 hour days at a $25^{o}C/25^{o}C$ temperature regime were observed to have nine leaf pairs after 13 days in the growth cabinet. Burbott and Loomis (1967) reported that leaves below the fourth pair had developed (expanded)before the cuttings were rooted and placed in the treatment conditions, and were therefore not analysed. An initial analysis indicated that these leaves contained predominantly menthol. The high menthol content of these leaves was considered a consequence of leaf age rather than environmental conditions under which they were produced. However, it could be argued that high menthol levels reflected the long-intermediate daylengths and high light intensity under which these leaves were produced. Secondly, although leaves above the fourth leaf pair were reported to develop (expand) during the treatment, several of these pairs would have been formed prior to placement into the treatment conditions (i.e. tuft of youngest leaves plus next three leaf pairs were planted). Leaves within this 'tuft' would have formed under the pre-treatment conditions which were long to intermediate in daylength and high light intensity. As a result, at least some of the leaves analysed in the 8 hour day plants would have been formed under 14 hour (or longer) days. That is, the only "true short day" leaves that existed at the time analyses were conducted, were those that had been produced and subsequently expanded during the 13 day period. Therefore, it could be suggested that only the uppermost leaf pairs

were typical of those produced under the treatment conditions, and that a significant pre-treatment effect existed in lower leaf pairs that were analysed. If the above criticism is valid then only the upper leaf pairs should have been considered when evaluating such effects as those caused by introducing a light flash to the 8 hour day plants. Results taken from the graphs of Burbott and Loomis (1967) for this set of treatments are as follows:

Results taken from graphs presented by Burbott and Loomis (1967)	8 hour days (25°C/25°C)				8 hour days + light flash (25°C/25°C)			
Leaf Pair	Tip	9	8	7	Tip	9	8	7
µmoles of terpenes per leaf pair								
Menthone	0	0	0	0.01	0.15	0.27	0.55	0.65
Menthol	0	0	0	0	0	0	0.03	0.05
Pulegone	0.02	0.12	0.13	0.17	0.25	0.38	0.52	0.32
Menthofuran	0.03	0.13	0.17	0.27	0.28	0.41	0.68	0.78

Although it would appear that menthofuran and pulegone were the predominant monoterpenes under both of the above growing conditions, the introduction of a light flash in the middle of the 16 hour night resulted in a significant increase in the amount of menthone. Therefore, it would not appear possible to reject a photoperiodic effect on monoterpene composition, on the basis of the above results. Furthermore, Burbott and Loomis (1967) reported their results as µmole terpenes/leaf pair. Although such a method is valid, it tends to confound changes in oil composition with changes in the total amount of monoterpenes per leaf. From the results presented, it is possible to remove this confounding effect by expressing the results as percentages that the individual

monoterpenes represent of the total. [This method was used by Grahle and Holtzel (1963).]

Results recalculated from graphs presented by Burbott and Loomis (1967)	8 hour day (25°C/25°C)				8 hour day + light flash (25°C/25°C)			
Leaf Pair	Tip	9	8	7	Tip	9	8	7
% mole composition								
Menthone	0	0	0	2.2	22.1	25.5	30.9	36.1
Menthol	0	0	0	0	0	0	1.7	2.8
Pulegone	40	48	43	38	36.8	35.8	29.2	17.8
Menthofuran	60	52	57	60	41.2	38.7	38.2	43.3

If the composition of oil obtained from leaf pair 7 is considered, menthone increased from 2.2% to 36.1%, menthofuran decreased from 60% to 43.3% and pulegone decreased from 38% to 17.8%, when a light flash was introduced to the 8 hour day plants of Burbott and Loomis (1967). On the basis of the above results, the conclusion of Burbott and Loomis (1967) that photoperiod probably has no effect on monoterpene metabolism, seems questionable. Therefore, since the results presented are from individual plants (other consistent results were reported to exist) and no indication of variability was provided (statistical significance was not indicated), it would seem difficult to draw many soundly based conclusions from the results presented. That is, although the addition of a 15 minute light flash to the 8 hour day treatment did not convert the oil composition to that of 18 hour day plants under the conditions of this experiment, some effect of the photoperiodic treatment was apparent.

With the possible exception of some photoperiodic treatments, conclusions drawn by Burbott and Loomis (1967) are substantiated by

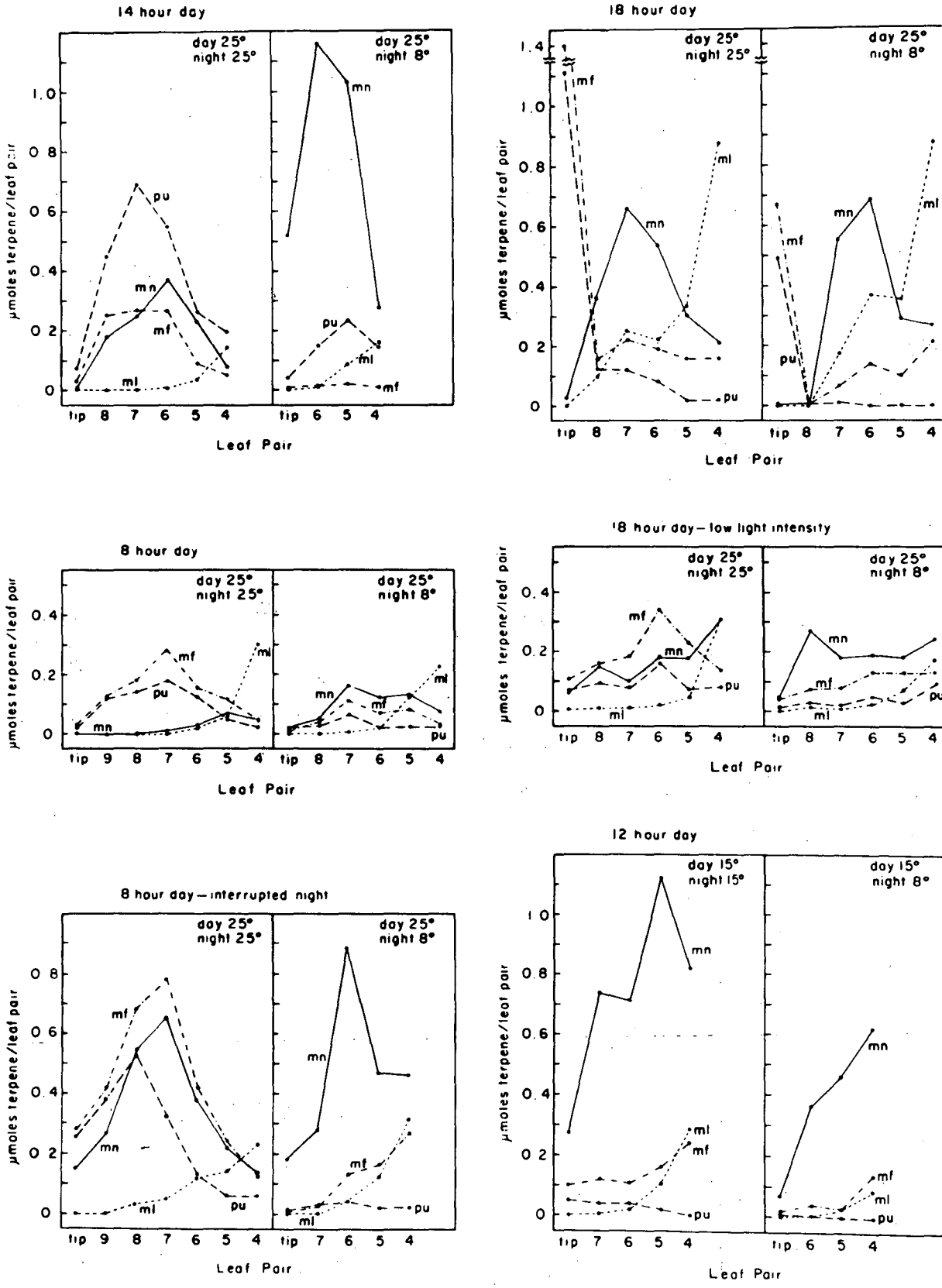
the results presented. However, in some cases there is a tendency to over-simplify the conclusions. For example, it would appear that 8 hour day and 14 hour day plants did respond to changes in night temperature in a similar manner, yet the oil compositions that resulted were substantially different. The results of Burbott and Loomis (1967) are included in Figure II 3.1.

Despite the possible limitations outlined above, the investigations of Burbott and Loomis (1967) provide the major evidence on which the current understanding of the effect of environmental factors on monoterpene metabolism is based. The proposed model represents a good working hypothesis, and is capable of explaining the effects of environmental factors on oil composition, in relation to the effect on photosynthate balance within the plant. The findings of Croteau *et al.* (1972b) which were discussed in detail in Section 2, support the model of Burbott and Loomis (1967). Croteau *et al.* (1972b) considered the supply of photosynthate to terpene-producing cells of utmost importance with respect to biosynthesis, metabolism and interconversion of monoterpenes.

Within the above model, it is possible to rationalise the interacting effects of environmental factors on monoterpene metabolism. For example, it was reported by Virmani and Datta (1968) that high quality peppermint oil was produced at Lucknow (26'52^{0}N). Although the relatively short days and high temperature regime existing at this location would favour depletion of photosynthate, it is possible that factors such as high light intensity were responsible for allowing the plant to maintain reducing conditions and thus favour production of oil having high menthol and menthone rather than high pulegone and menthofuran.

Figure II 3.1. Effect of light and temperature on the monoterpenes of peppermint.

(Taken from Burbott and Loomis, 1967)



3.4 Other Environmental Effects

There are numerous reports in the literature of the effect of individual environmental factors on oil yield and composition. However, few reports relate the observed effects to the overall interaction between environmental factors. With respect to the effect of temperature, Crane (1969) reported that at temperatures below 21°C the highly volatile constituents α- and β- pinene and limonene were reduced relative to higher temperatures. At temperatures above 21°C an increased proportion of menthol was transformed to menthyl acetate. Biggs and Leopold (1955) considered a temperature of 20°C optimal for leaf development, lateral branching, initiation of flower primordia and development of flowers after they had been initiated. Hotin (1968) observed an increase in the amount of oil accumulated, when temperatures were increased to 23-25°C, and a corresponding decrease in menthol content. Borkowski and Chochlew (1959) reported that low humidity and high temperatures increased the essential oil content of peppermint. Nelson *et al.* (1971a) reported that evaporative cooling peppermint by sprinkler irrigation, when the ambient temperature exceeded 30°C, resulted in lower concentrations of menthofuran and pulegone. These workers suggested that evaporative cooling had the same effect as the cool night treatments, outlined by Burbott and Loomis (1967). A reduction in temperature would be expected to alter the photosynthate balance within the plant by decreasing utilization of photosynthate by respiration. The maintenance of temperatures below 30°C may also increase CO_2 fixation, if it is assumed that the optimum temperature for photosynthesis in peppermint is below 30°C. Loomis (1977a)reported that reduction of moisture stress in peppermint affected oil composition by affecting plant growth habit. The extent of branching, leaf loss and flowering were

all influenced by moisture stress. Therefore, the effect of evaporative cooling on oil composition may have been associated with the alleviation of moisture stress, in the plants observed by Nelson *et al.* (1971a).

4. Cultural Factors Affecting the Yield and Composition of Peppermint Oil

4.1 Harvest Date

Changes in Oil Yield and Oil Composition with Plant Maturity

Changes in the composition of peppermint oil have been associated with plant maturation. Numerous workers have observed that menthols and menthyl esters increased while menthones decreased with increased plant maturity (Rabak, 1916; Chiris, 1925; Rutovskii and Travin, 1929; Ellis and Gaylord, 1944; Ellis, 1945; Bullis *et al.*, 1948; Watson and St. John, 1955; Laughlin, 1960; Baslas, 1970; Manning, 1970; Lammerink and Manning, 1973; Duhan *et al.*, 1975). Duhan *et al.* (1975) reported that menthone increased and menthol decreased after full bloom. According to Nelson *et al.* (1970) the concentration of pulegone, menthyl acetate and menthofuran was highest in the middle of the growing season. Manning (1970) noted that menthofuran increased up until the time of full bloom, after which it decreased.

These observed changes in oil composition with plant maturation would appear to be a reflection of leaf age. Loomis (1977a)reported that mature leaves contained menthol and menthyl esters, immature leaves contained menthone and inflorescences contained menthofuran and pulegone. In addition, Burbott and Loomis (1969) and Croteau and Martinkus (1979) reported a rapid synthesis of menthone in midstem leaves of peppermint at the time of floral initiation. Croteau and Martinkus (1979) suggested that much of this pre-blooming peak in menthone was metabolised to non-volatile, neomenthyl glucoside, soon after flowering, at a time when oil yield from these leaves was observed to decrease.

Embong *et al.* (1977) observed that herb harvested at 20 percent bloom in Southern Alberta yielded oil of best quality (from the standpoint of oil composition). Oil extracted from herb at 5 percent bloom yielded immature oil (high menthone, low menthol), whilst at 50 percent bloom oil contained high concentrations of menthofuran and had reverted to immature quality due to the commencement of secondary growth.

In addition to changes in oil composition with plant maturation, oil yield has been reported to vary throughout the growing season. Numerous workers have reported that oil yield per unit area increased throughout the season and was at a maximum during the period of full bloom (Chiris, 1925; Bullis *et al.*, 1948; Fahney *et al.*, 1955; Watson and St. John,1955; Virmani and Datta, 1970; Embong *et al.*, 1977).

One of the initial problems encountered when commencing production of an essential oil crop in a new area is the timing of harvest. Such a problem was encountered during the establishment of the peppermint oil industry in Southern Tasmania. Generally, information relating to changes in oil composition and yield during the growing season within the Southern Tasmanian environment was not available. Harvesting in the above area was initially timed to correspond with the full bloom stage, even though yield and quality characteristics of the oil at this time were largely unknown. Subsequently, sample distillations were conducted and harvest was timed to coincide with 45 percent menthol in extracted oils. This criteria of harvest timing, based on 45 percent menthol in oil extracted from sample distillations, allowed the production of oil acceptable to industry with respect to menthol concentrations. However, it did not provide any indication of either overall quality or yield per unit area. Information relating to the possible increase and decrease in yield per unit area and changes in oil composition preceding and following the 45 percent menthol stage

have not been previously investigated in Southern Tasmania. In addition, the importance of correct timing of harvest on oil composition and oil yield and the period over which harvest could safely be spread, was not known.

Timing of Harvest

Timing of harvest has been reported to be of utmost importance for both yield and quality of oil extracted from *Mentha piperita* L. (Manning, 1970). A desirable time to harvest might coincide with maximum oil yield per unit area and optimum oil quality. In practice these requirements may be in conflict. For example, Embong *et al.* (1977) reported that in Southern Alberta, maximum oil yield per unit area corresponded to full bloom, whereas the most acceptable oil quality was associated with herb harvested at 20 percent bloom. These workers suggested a compromise between yield and quality which involved harvesting prior to the stage of maximum yield to avoid high concentrations of menthofuran.

Numerous workers have found that for optimum oil and menthol yields plants should be harvested at the full bloom stage (Chiris, 1925; Fahney *et al.*, 1955; Watson and St. John, 1955; Virmani and Datta, 1970). Ellis and Gaylord (1944) considered this method of harvest prediction unreliable and too dependent on environmental conditions. These workers quoted instances where meadow mint did not flower even though maximum oil and menthol content had been reached. Ellis *et al.* (1941) found the stage of maturity more difficult to judge under field conditions as compared with small trial plots, since plants of all degrees of maturity were found in the larger areas. These workers also reported that the above problem was more difficult in meadow mint than row mint because the latter matures more evenly.

Ellis and Gaylord (1944) investigated the relationship between menthol content and oil yield and found that the oil content of plants

increased to a stage at which the oil contained 45 percent menthol. If plants continued growing, the yield of oil per plant decreased. This decrease was accompanied by an initial increase in menthol, followed by a decrease in menthol. Within 10 to 15 days the decrease in oil yield amounted to 30 percent of the total oil yield. An increase of similar magnitude was observed to occur in the period which preceded the time of optimum harvest. This increase and decrease in oil yield was reported to be much greater in some seasons and that under some conditions oil yield was maintained at a plateau value for a considerable period (Ellis and Gaylord, 1944). Embong *et al.* (1977) reported that maximum yield was only possible over a very short period of time in Alberta.

Ellis (1968) considered that "most producers in the U.S.A., used a 'rule of thumb' to determine when to commence harvesting. Samples of herb are harvested and distilled when flowering commences, to determine oil yield. If satisfactory yields are obtained, harvesting is continued regardless of the menthol content in the hope that the blend of oil from the total crop will produce an acceptable quality product."

Hoelscher and Bacon (1930), Hocking and Edwards (1943) and Schroeder (1963) investigated the relationship between the dimensions and number of oil glands and the yield of oil. These workers found a very poor correlation between the unit area production of oil and gland counts and measurements. In contrast, Paun (1970) reported that the density of oil glands and their volume per unit area of leaf were good indicators of oil quality and were positively correlated with oil yield per area. Apart from the obvious laborious nature of conducting gland counts and measurements for harvest date determination, the findings of Paun (1970) appear somewhat questionable when observations made by other workers are considered.

Therefore, it would appear that oil yield and composition vary throughout the growing season and that these variations depend to some extent on the area concerned. Maximum yield of oil and optimum quality may or may not coincide, the rise and fall in yield per area may or may not be rapid, and the timing of harvest may or may not be critical, to produce satisfactory yields of good quality oil. However, such observations need to be made in any new area before the successful production of high yields of high quality oil can be ensured. Furthermore, it appears that the most satisfactory technique of establishing this information would be to follow oil yield per unit area and oil composition with time in the area of production, during several seasons.

Multiple Harvesting of Peppermint

Guenther (1949b)reported that during some seasons in the U.S.A. it was possible to obtain a second harvest of peppermint. However, the second harvest, known as "clippings",was reported to produce an inferior oil, generally of poor quality. This worker concluded that a second harvest of peppermint was not advisable unless the field was to be abandoned, because two harvests ruined the stand and vigour of the planting, in subsequent seasons. Watson and St. John (1955) considered that a second harvest of peppermint was possible if the first crop was harvested substantially earlier than was customary. When a second harvest was conducted the plants harvested were observed to be at a much earlier stage of maturity than those of the first harvest. The resultant oil was not considered to have a good odour or flavour and a poor stand of peppermint was reported in the following season.

4.2 Irrigation and Nitrogen

In the commercial peppermint oil production areas of Southern

Tasmania, an annual application rate of 35kg N/ha, 15kg P/ha and 40kg K/ha represents the current fertiliser practice (T.M.G.A., *pers. comm.**). In some areas, minimal amounts of additional nitrogen are applied during latter stages of crop growth. Irrigation is commenced in late November and the equivalent of 25mm is applied weekly through overhead sprinklers. This irrigation regime is continued until harvest (late February); no post-harvest irrigation is applied.

Oil yields obtained from these areas are typically 35 to 40kg/ha. Such yields are considerably lower than obtained from West Coast areas of the U.S.A., but are comparable with yields obtained in the Mid-West areas of the U.S.A. (Ellis, 1960).

Ellis (1960) ascribed the higher yields obtained from the West Coast region to a slightly longer photoperiod, more hours of sunlight and higher light intensity. This worker suggested that the upper limit of oil yield was controlled by these environmental factors. If 35 to 40kg/ha represents the upper limit to oil yield under Southern Tasmanian conditions, then increasing nitrogen and/or irrigation may not substantially increase oil yield per unit area.

The effect of environmental conditions on oil yield and composition may be either direct or indirect. Direct effects include those effects discussed in Section 3. For example, it was reported that daylength had a direct effect on both oil yield and oil composition, with long days favouring high yields of high quality oil. Environmental factors may also effect oil yield and composition through an effect on plant growth (i.e. indirect effects). For example, Loomis (1977a)reported that conditions favouring the production of inflorescences, loss of lower leaves, leaf expansion and formation of lateral branches are important determinants of oil yield and composition. Unlike the direct environmental effects on plant metabolism, it may be possible to modify

[*Tasmanian Mint Growers Association]

indirect effects on plant growth through the manipulation of cultural factors. Within the Southern Tasmanian environment, it may be possible to increase the yield of oil per unit area above 35 to 40kg/ha by manipulating such cultural factors as harvest date, nitrogen and irrigation. Investigations involving the manipulation of these cultural factors have not previously been reported in this area.

With respect to the effects of irrigation and nitrogen on oil yield and composition, investigations have adopted two main approaches. The most frequent approach has been rather "empirical" in nature. That is, factors such as the level, timing and form of applied nitrogen and/or irrigation have been varied and the effects on oil yield and composition recorded. The important consideration in these experiments has been the final treatment response and little emphasis has been placed on understanding the system in which the effect was produced. More recently, several workers have adopted an integrated approach to understanding the effects of cultural factors. Within this approach, manipulation of nitrogen and irrigation is considered a means of modifying the overall system.

The Effect of Irrigation on Oil Yield and Oil Composition

The effect of irrigation on peppermint oil yield depends on the amount and distribution of natural rainfall and environmental conditions such as temperature (Krupper *et al.*, 1968). As a result, any specific findings obtained from an irrigation trial should only be considered to apply under the environmental conditions in which the trial was conducted. This limitation exists in all reported effects of irrigation on oil yield and composition, since irrigation represents only one of many interacting factors involved.

From a review of the literature, Kerekes and Hornok (1973) considered that irrigation increased herbage and essential oil yields

and that the critical time was between bud stage and first harvest. Krüpper *et al.* (1968) reported that irrigation of peppermint should be arranged so that the soil moisture is maintained within the range of 65 to 80 percent of field capacity. These workers also reported that irrigation should not be applied within 2 weeks of harvest, since this resulted in plants having a higher water content and longer periods were necessary for drying prior to distillation. Schröeder (1963) suggested that the optimum soil moisture content for peppermint was between 80 to 90 percent of field capacity and that either a lack or excess reduced the volatile oil yield. Similarly, Hotin (1968) reported that an increased soil moisture deficit decreased the volatile oil yield. Schröeder (1963) attributed the high water requirements of peppermint to the small proportion of deep roots. Most roots were found within 7.5cm of the surface, hence under dry conditions the majority of roots would be rapidly deprived of water. Kerekes (1960) found that the moisture requirement of a peppermint crop increased to a maximum prior to full bloom. Lammerink and Manning (1971) noted that peppermint responded to high applications of nitrogen and irrigation, especially approaching harvest (January-February). Nelson *et al.* (1970) found no significant difference in oil yield per unit area when meadow mint was either rill irrigated every 4 days, 7 times during the growing season or 5 times during the growing season. Embong *et al.* (1977) reported that irrigation equivalent to 30 to 45mm was applied 4 times per season using furrows placed 90cm apart, in Southern Alberta.

The above reports are examples of the "empirical" approach, in that although they report valuable observations for the particular environments in which they were made, they are neither generally applicable nor contribute significantly to the understanding of how irrigation (or lack of irrigation - moisture stress) influenced plant

metabolism and/or plant growth.

In contrast, Loomis (1977a)adopted an integrated approach to the study of irrigation and moisture stress in peppermint. These studies correlated field measurements of temperature, humidity, light intensity and irrigation method with measurements of leaf diffusive resistance, plant moisture stress,and carbohydrate balance on yield and composition of oil. Loomis (1977a)considered that plant growth habit was determined by daily moisture stress patterns, which in turn were determined by atmospheric moisture conditions and by irrigation practices. Optimum quality oil was considered to require a balance of young and old leaves, with a minimum of bloom. Maximum oil yields per unit area were considered to demand small leaves. These smaller leaves were observed to contain almost as much oil per leaf as larger leaves, but as a consequence of shading, fewer larger leaves could be supported per unit area. Loomis (1977a)considered that leaf growth was regulated by moisture stress and night temperature, with moderate to high stress and/or cool nights giving rise to small leaves. The large difference in oil yield per unit area in several of the major oil producing areas of the U.S.A., were considered to result from such differences in plant growth. In the Mid-West, high humidity and warm nights resulted in large leaves and low oil yields. In the Yakima Valley and Eastern Oregon, low humidity and cool nights resulted in small leaves and high yields. However, associated with these high yields was a considerable loss of mature leaves and much bloom, which adversely affected oil quality. In the Madras and Willamette Valley areas,night temperature and moisture stress were reported to balance each other to produce leaves of intermediate size, moderate leaf loss, moderate bloom and good yields of oil. Loomis (1977a) suggested that the type of irrigation had an important effect on growth and metabolism in peppermint. Furrow

irrigated plants experienced high moisture stress even when furrows were filled with water. Sprinkler irrigation wetted the leaves and thereby reduced this stress.

According to Loomis (1977a)it may be possible to manipulate oil yield and composition under conditions in the U.S.A. by carefully controlling moisture stress in peppermint plants. It was suggested that moisture stress induced early in summer to produce small drought-tolerant leaves, followed by a reduction of stress towards the end of the season to prevent leaf loss and reduce the extent of flowering, may be advisable. Such procedures may include sprinkling only at night during the early summer and sprinkling and misting during the day, in the latter part of the growing season.

With respect to oil composition, Loomis (1977a)found little variation in the chemical composition of oil obtained from "moisture stressed" as compared with "non-moisture stressed" leaves at the same stage of development. However, differences existed due to the variation in types of leaves present in the two crops (i.e. loss of mature leaves decreased the menthol content of oil).

In conclusion, Loomis (1977a)stated that learning to manipulate and maintain a moderate plant moisture stress, may be the key to optimizing yield and quality in peppermint. Moisture stress and other factors interaction with it were considered to control the photosynthate-growth-differentiation balance and determine whether photosynthate is directed towards growth, flowering, or synthesis and maturation of essential oil (Loomis, 1977a).

Croteau (1977) observed that peppermint grown in a controlled environment under simulated sprinkler irrigation produced essential oil in 23 percent lower yields than identical plants grown under simulated furrow irrigation. The decrease in oil yield with sprinkler

irrigation was associated with an increased rate of oil evaporation which was attributed to hydration and swelling of the cuticle enclosing oil glands, and its affect on cuticular permeability. Oil from sprinkler irrigated plants contained more menthol (25%) and less menthone (53%) than oil from furrow irrigated plants (14% menthol, 58% menthone). Similarly, menthol and menthyl acetate increased and pulegone and menthofuran decreased with sprinkler irrigation (Nelson *et al.*, 1971a; Dow *et al.*, 1974). Nelson *et al.*, (1971a) associated these compositional changes to the evaporative cooling effect of the applied irrigation.

Studies by Kerekes and Hornok (1973) concluded that irrigation did not alter the composition of peppermint oil. Gilmore (1977) demonstrated that soil moisture had an important role in influencing the monoterpene composition of Loblolly Pine. Lammerink and Manning (1971) concluded that water stress at harvest, resulted in an increased concentration of menthofuran arising from flowers.

The Effect of Nitrogen on Oil Yield and Oil Composition

Significant increases in oil yield per unit area, have been observed as a result of high applications of nitrogen fertiliser (Ghosh and Chatterjee, 1976; Embong *et al.*, 1977). The high oil yields characteristic of the Washington area of the U.S.A. have been associated with high applications of nitrogen fertiliser (200 to 400 kg/ha) (Nelson *et al.*, 1970).

Schratz and Wiemann (1949) increased the application of nitrogen from 0.15 to 1.20g per plant and observed an increase in oil content from 1.4 to 2.6 percent and an increase in oil yield per plant from 35 to 315mg. Subsequent work by Baird (1957) found that although nitrogen increased herb and oil yield, there was no significant effect of the added nitrogen on percentage oil yield. Nelson *et al.* (1971b) reported

an increase in oil yield of approximately 70kg/ha when the nitrogen fertiliser application was increased from 50kg/ha to 300kg/ha.

Numerous workers have suggested the application of additional amounts of basal nutrients, especially phosphorus and potassium and to a lesser extent sulphur (Baird, 1957; Davis *et al.*, 1957; Franz, 1972; Pavlenko, 1972; Singh *et al.*, 1977). Baslas (1970) found that although both nitrogen and phosphorus increased oil yield, a combination of nitrogen, phosphorus and potassium resulted in a decreased oil yield.

Neubauer *et al.* (1974) recommended the application of 100 kg of urea per hectare in split applications, at the commencement of growth of both the first and second crop of peppermint, per season. Khotin (1950) reported large increases in oil yield as a result of applications of sodium nitrate and ammonium sulphate, early in the growing season. Latypov (1960) suggested the use of ammonium rather than nitrate, nitrogen as a means of increasing essential oil yields. In addition, sulphates were reported to be more effective than chlorides (Latypov, 1960). Subsequently, Matusiewicz and Madziar (1971) reported a preference for sodium and calcium nitrate as the form of fertiliser nitrogen. Crane and Steward (1962) considered peppermint intolerant to ammonium as the sole nitrogen source when peppermint was grown in water culture.

With respect to the effect of increased nitrogen application on oil composition, the results in the literature are varied. O'Connor (1965), Kirsnyte and Kavaliauskiene (1966), Baslas (1970) and Franz (1972) reported an increase in menthone and a decrease in menthol, as a result of increased applications of fertiliser nitrogen. In contrast, Hotin (1968) and Gretskaya *et al.* (1972) found an increase in menthol with increased nitrogen. Latypov (1960), Neubauer *et al.* (1974) and Mustyatsé and Grigorets (1975) considered that increased applications

of nitrogen had no adverse effects on oil quality.

Ellis *et al.* (1941), Green (1963) and Franz (1972) concluded that the reported effects of nitrogen on oil composition were not direct consequences of the fertiliser regime on essential oil metabolism. These changes were attributed to alterations in plant growth habit and maturation.

III GENERAL MATERIALS AND METHODS

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In this section the techniques and experimental materials common to experiments in more than one of the following sections will be discussed.

1. Plant Material

Peppermint (*Mentha piperita* L. var. Black Mitcham) was used in all glasshouse, laboratory and field trials. The initial selection of propagation material for glasshouse trials was obtained from a commercial planting of peppermint at "Rotherwood", Ouse, in the Derwent Valley area of Tasmania. Clonal material for glasshouse and laboratory trials was obtained by propagating material from one initially selected plant. The original material was obtained from the U.S.A., by Mr. E.F.K. Denny, 'Bridestowe Estate', Lilydale, Tasmania.

2. Harvesting, Drying and Storage

The procedures at harvest were dependent on the intended method of oil extraction:

Steam Distillation

All glasshouse and field material was harvested at ground level, weighed for fresh weight determination, subsampled to reduce the fresh weight of samples to approximately 2kg and dried in the glasshouse (20 to 25^{o}C) for approximately 24 hours, prior to storage or distillation. Drying in the glasshouse continued until the plant material had a moisture content of approximately 30 to 40 percent. Where possible, plant material was immediately steam distilled. However, due to the large number of samples involved in some trials and the time required for distillation, storage of samples was often necessary. For sample storage, plant material was placed in sealed polythene bags and stored

at -20°C. Prior to distillation, all samples were comminuted.

Solvent Extraction

Harvesting of samples commenced in the morning approximately 3 hours after the beginning of the light period. Leaf pairs were removed node by node from the stem, starting with the basal leaf pair. Very little time elapsed between harvesting and extraction (max. 5 min.), but when the number of extractions was large the harvesting-extraction period was unavoidably long (8 to 10 hours). However, all harvesting-extractions were completed on the same day. The above technique is in accordance with that outlined by Burbott and Loomis (1969). These workers did not observe any diurnal fluctuation in oil content and as a result the difference in time required to complete extractions was not considered to affect the final analyses. Treatments from within a complete block were harvested with minimal delay. Following extraction, samples were stored in sealed glass vials at -20°C, to await analysis.

3. Extraction

Two extraction techniques were used to obtain peppermint oil samples for analysis. The type of extraction used was dependent on the size of the sample (i.e. individual leaves and small plants were extracted by solvent extraction, whilst large samples of plant material were steam distilled).

Solvent Extraction

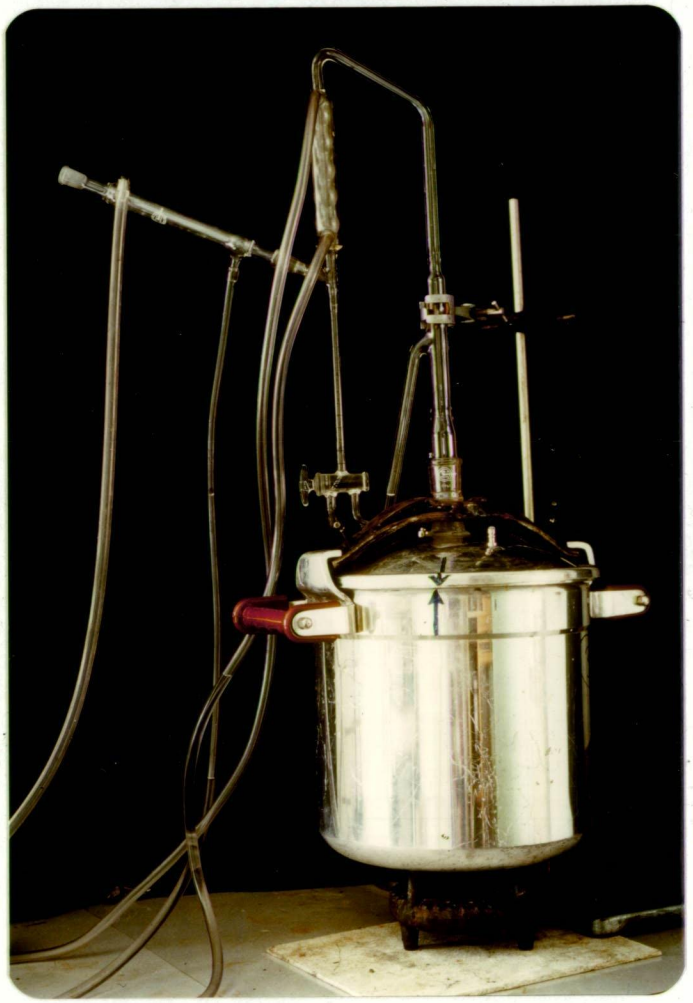
Tissue was extracted four times by grinding in a mortar with re-distilled n-hexane, in the presence of anhydrous sodium sulphate, resulting in a final extract volume of 10ml. The extracts were decoloured with charcoal, centrifuged at low speed to remove any charcoal, anhydrous sodium sulphate and plant material, and concentrated

under a stream of nitrogen at room temperature. These procedures were described by Burbott and Loomis (1967).

Steam Distillation

The apparatus used for steam distillation consisted of a modified 20ℓ (S.E.B.) aluminium pressure cooker. This pressure cooker was modified by blocking the pressure release outlet and fitting a glass condenser to the top of the lid. The type of condenser used was such that the condensed oil remained in the condenser unit and the distillation water returned to the pressure cooker. The interior of the pressure cooker was fitted with a stainless steel screen, supported approximately 10cm above the surface of the boiling water. This stainless steel screen functioned in holding the herb above the boiling water. The capacity of the unit was approximately 800g of partially dried plant material. In each distillation run 1ℓ of water was added to the unit and the distillation rate maintained at 6ml/min throughout the distillation period. Complete exhaustion of peppermint oil required 1-1.5 hours depending on the quantity of herb and its moisture content. In all cases the distillation was allowed to continue until no minute oil droplets could be observed passing over the surface of the condenser, since from previous experience this stage corresponded to complete exhaustion of oil from the material (Clark, 1976). During some distillation runs, using large quantities of high yielding herb, it was necessary to "run off" the peppermint oil collected in the arm of the condenser to prevent it from returning with the distillation water to the pressure cooker. The distillation apparatus is illustrated in Plate III 3.1.

Plate III 3.1. Steam distillation unit.



4. Analysis of Oil Samples

Gas Chromatographic Techniques

Gas chromatographic analyses of oil samples were conducted using a Pye Unicam Series 104 Chromatograph, fitted with a flame ionization detector (F.I.D.). The samples were injected using a Hamilton microlitre syringe (No. 7105, NCH) fitted with a churney adaptor. The column used for analyses was a 56m x 0.5mm I.D., F.F.A.P., SCOT capillary column. Operating conditions were as follows: carrier gas (N_2) flow rate 2ml/min, air flow rate 500ml/min and hydrogen flow rate 25ml/min. The column oven temperature was programmed from 80°C to 160°C at 2°C/min. No injector head heating was used.

The identification of peaks eluting from the SCOT column was made by comparing the retention times of peaks to a sample chromatogram provided by Dr. E.V. Lassak (Museum of Applied Arts and Sciences, Sydney, Australia), by standard additions of authentic samples of individual compounds known to occur in peppermint oil and by combined gas chromatography-mass spectrometry. Mass spectra of peppermint oil components were obtained with a VG-7070F Mass Spectrometer (V.G. Micromass Ltd., Winsford, England), interfaced to a Pye Unicam 204 Gas Chromatograph. The column used was a 56m x 0.5m I.D. Carbowax 20M, SCOT capillary column, with a helium flow rate of 2ml/min.

A sample chromatogram, indicating peak identity (based on the above methods) is included in Figure III 4.1. The retention times of compounds and the typical variability observed when repeated analyses were conducted on the same sample are indicated in Table III 4.1. Appendix III 4.1 illustrates the mass spectra obtained and compares these spectra with reference spectra (Willhalm and Thomas, 1965; Thomas and Willhalm, 1966; Stenhagen *et al.*, 1974).

Figure III 4.1. Gas Chromatogram
(Oil Sample: Fritzsche Single Rect. 492003)

Pye Unicam Series 104 Chromatograph fitted with F.I.D., F.F.A.P., SCOT capillary column 56m x 0.5mm I.D.

Carrier gas (N_2) 2ml/min

Chart speed 30cm/hr

Column Oven Temperature Programme $80^{o}C$ to $160^{o}C$ at $5^{o}C$/min.

Peak Area Determination using a Pye Unicam DP88 computing integrator. Integration parameters used:

PW = 8, SS = 30, BL = 30, TP = 30, T_1 = 200, T_2 = 600, DL = 500.

Component No.	Component Name	Retention Time (secs)	% Total Peak Area
1	α-Pinene	213	1.141
2	β-Pinene	250	2.185
3	Limonene	306	2.433
4	Cineole	314	6.670
5	Menthone	586	15.064
6	Menthofuran	604	5.335
7	Isomenthone	617	2.392
8	Menthyl Acetate	709	4.323
9	Neomenthol	742	2.646
10	Menthol	809	47.265
11	Pulegone	841	0.166

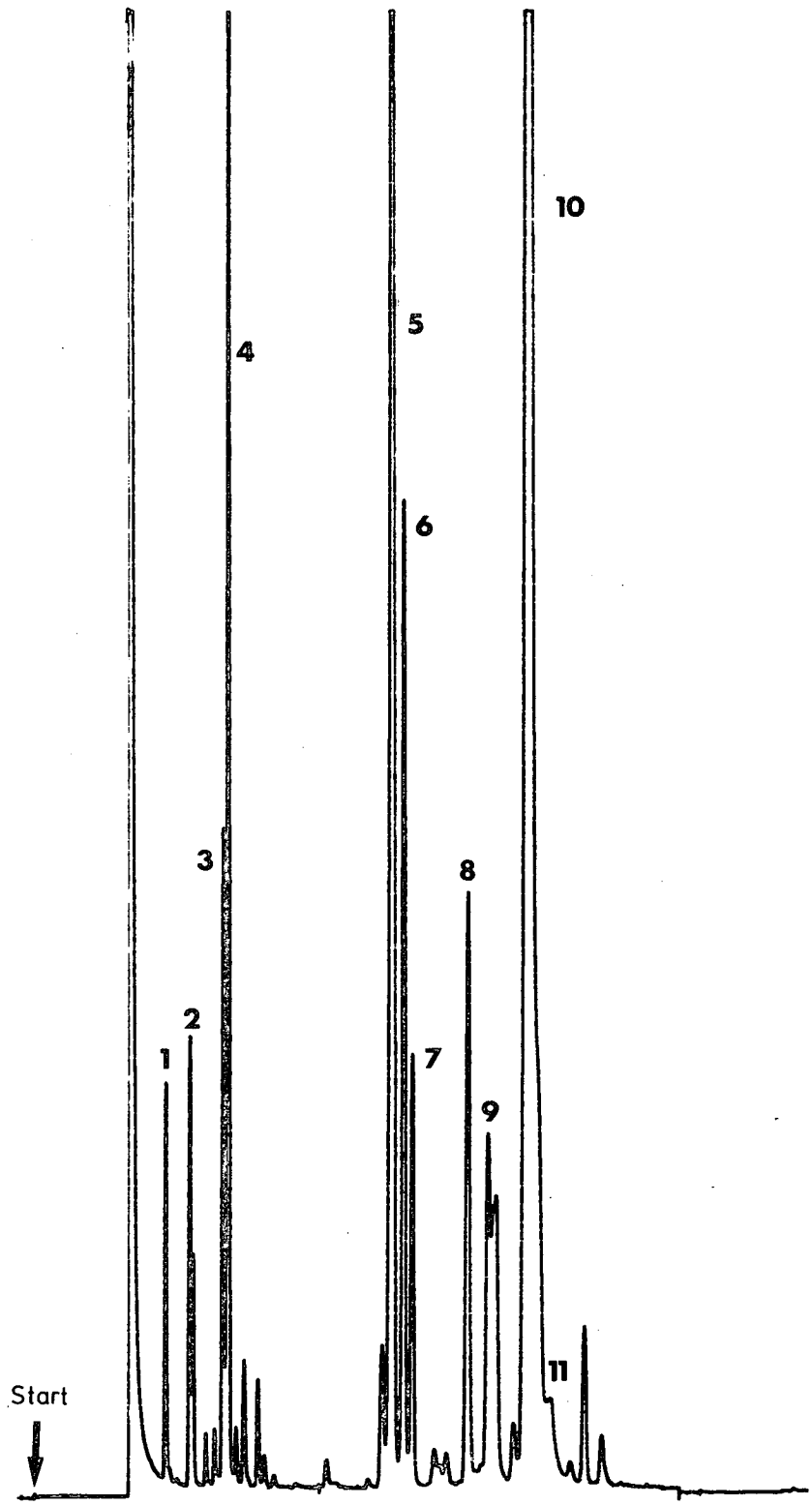

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Table III 4.1. Retention Times and Reproducibility of Peak Area Measurements - when the same oil was chromatographed on several occasions, using the F.F.A.P. SCOT capillary column.

Compound	Retention time (sec)	Percentage Peak Area(o)		
		1	2	3
α-pinene	213	0.59	0.59	0.67
β-pinene	250	1.21	1.22	1.22
Limonene	305	2.00	2.00	2.01
Cineole	315	4.82	4.85	5.23
Menthone	586	24.51	24.35	24.79
Menthofuran	604	1.16	1.16	1.16
Isomenthone	617	3.03	2.99	3.01
Menthyl Acetate	709	2.24	2.22	2.22
Neomenthol(+)*	742	3.99	3.93	3.34
Menthol	809	48.21	47.65	47.59
Pulegone	841	1.12	1.15	0.98

(o) Peak area and retention times determined by a Pye Unicam DP88 computing integrator.

*The identity of all compounds except neomenthol was confirmed by the addition of authentic samples, comparison with standard chromatograms on a similar column, and GC-MS fragmentation patterns.

The peak eluting after α-pinene, labelled β-pinene, was observed to be a combination of two peaks. As well as β-pinene it appeared that sabinene had a retention time of approximately 250 seconds, but these two peaks were not well resolved by the F.F.A.P. column. Therefore, any reference to β-pinene will infer β-pinene + sabinene Secondly, it was not possible to positively identify peak number 9 by any of the above methods. However, this peak appeared to be due to an isomer of menthol. Croteau and Hooper (1978) reported that peppermint oil contained 5% neomenthol and only traces of isomenthol and neoisomenthol. Therefore, it has been assumed that peak number 9 was neomenthol. A comparison of fragmentation patterns of this peak with those reported by Thomas and Willhalm (1966) is included in Appendix III 4.2.

Peak area was determined using a Pye Unicam DP88, computing integrator. Integrator factors used in area determinations were PW = 8, SS = 30, BL = 30 and TP = 30. Determination of these factors was in accordance with the supplied operations manual.

In addition, peak areas were calculated using triangulation and good agreement was obtained between the two methods.

Calibration of Gas Chromatography

Composition of peppermint oil samples (percentage w/w) was determined from the integrated peak areas, using the method outlined by Smith and Levi (1961). This method involved the computing of appropriate correction factors for each compound. Such a technique circumvented the introduction of exact volumes of standard substances and avoided the addition of weighed amounts of internal standard to each sample (Smith and Levi, 1961). Reference compounds available were chromatographed under conditions identical to those used for analyses of peppermint oil. Peak areas corresponding to each standard and its impurities were

calculated and expressed as percentages of the total. Mixtures of reference substances were then made up by weight and similarly assayed. Utilizing data obtained for both the individual reference compounds and their impurities, true weight percentages of the constituents making up a given mixture were calculated. Menthol was considered the primary standard and its correction factor set at 1.00. For all other compounds, correction factors were then established by bringing the relative areas of their peaks in line with the relative amounts originally weighed out. Peak areas were then converted to weight percentages by multiplying by the respective correction factors (Smith and Levi, 1961). As reported by Smith and Levi (1961) these factors, although representing specific criteria for the compounds when chromatographed in accordance with the procedures described, are not applicable to other columns or different experimental conditions. In subsequent experiments and calculations, the correction factors of compounds for which authentic samples were not available or the identity of which were not known, were set at 1.00 (same response as menthol). Correction factors and the chromatographic data from which these were calculated are included in Appendix III 4.3.

In addition to the calibration procedure described above, the weight percentage of menthol and menthone was determined in a standard peppermint oil sample using the technique described by Clark (1976). This technique involved standard additions of menthol or menthone to a sample of peppermint oil in the presence of known amounts of internal standard (1ml of peppermint oil, 1ml of 20% β - methylnaphthalene, made up to 5ml in a volumetric flask with redistilled n-hexane). These mixtures of oil, exogenous menthol or menthone, internal standard and hexane were chromatographed on a 165cm x 0.4cm glass column packed with 5% Carbowax 20M on Gaschrom Q (80-100 mesh) with a carrier gas (N_2) flow rate of 30ml/min. Peak heights of menthol or menthone and the

internal standard were determined, and the ratio of the peak height of the compound of interest to the peak height of the internal standard was plotted against the amount of exogenous compound added. By extrapolating this curve to the x-axis, the position on the x-axis when both exogenous and endogenous compound equals zero, was located. This x-intercept was then allowed to equal zero and a new x-axis was added to the graph, from which the endogenous content of either menthol or menthone in any oil sample could be determined. These calibration curves presented by Clark (1976) are included in Appendix III 4.4 and were used to compare the weight percentage of menthol and menthone in oil samples to those determined by the method of Smith and Levi (1961). Finally, the menthone and menthol concentration of oil samples was determined by titrimetric methods outlined by Guenther (1949a) and British Pharmacoepia (1968). The results obtained using the latter two methods were consistent with results obtained using the method of Smith and Levi (1961). Therefore, unless otherwise stated, the Smith and Levi (1961) calibration technique was used to convert peak areas to weight percentages, in all experiments.

5. Gas Exchange Measurements

Gas Exchange System

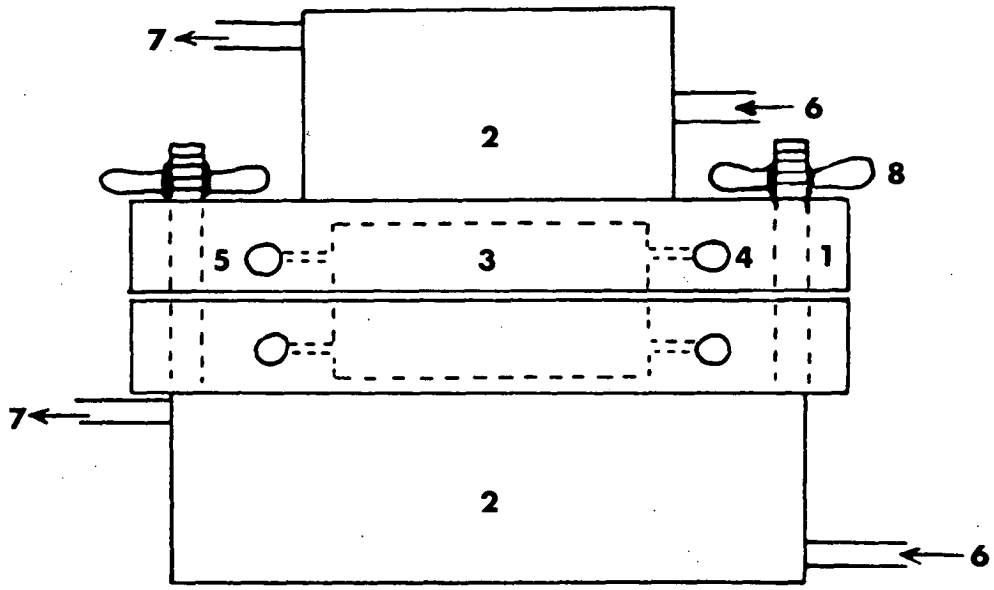
Rates of net CO_2 exchange were measured on attached leaves in a perspex leaf chamber placed inside a light cabinet. An open circuit system was used to monitor net CO_2 exchange within the leaf chamber. Details of the leaf chamber and open circuit CO_2 monitoring system are given in Figure III 5.1 and 2 respectively.

Leaf Chamber

The temperature of the leaf chamber was controlled by adjusting the temperature of the surrounding water jacket and was continuously monitored using a thermocouple placed inside the leaf chamber on the under surface of the leaf.

Figure III 5.1 (a) and (b).

Leaf chamber. 1, perspex block; 2, perspex water jacket; 3, leaf cell($1dm^2$); 4, gas inlet (900ml/ml); 5, gas outlet; 6, water inlet; 7, water outlet; 8, wing nuts and bolts to tighten chamber; 9, neoprene 'O' ring.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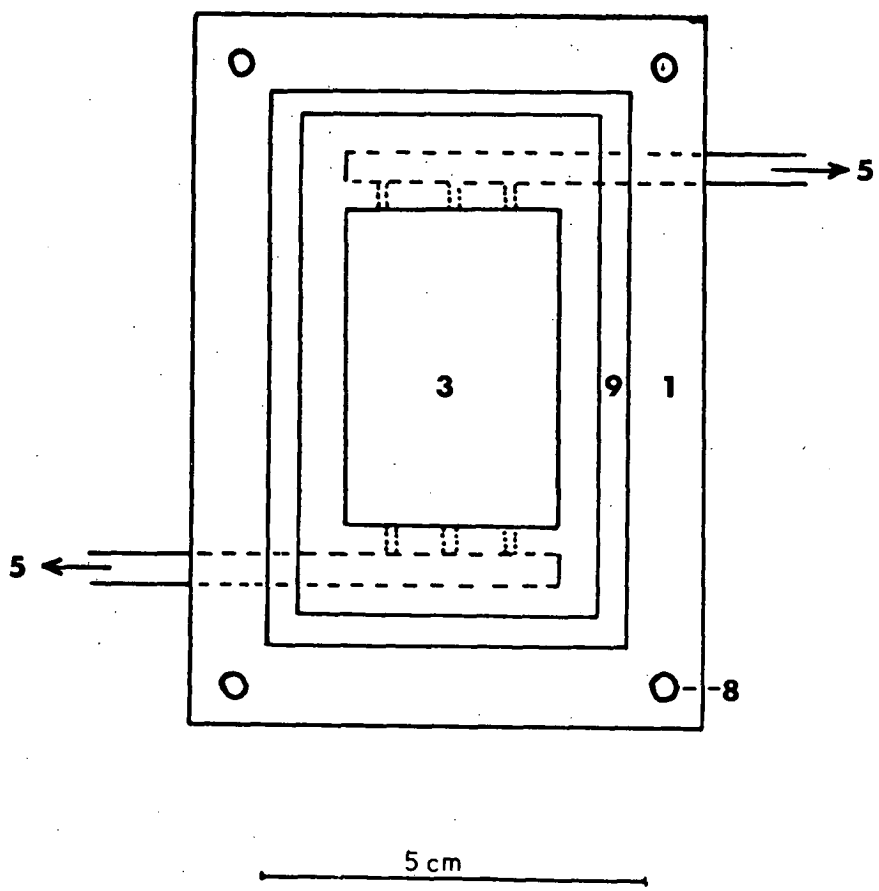
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5 cm

Figure III 5.2.

Diagrammatic representation of the open circuit CO_2 monitoring system.

A. Gas supply (compressed medical air or 2% O_2 in N_2, 310 ppm CO_2).

B. Pressure control gauges (100-1000ml/min).

C. Gas temperature control system and humidification system.

D. Tubes to remove excess water.

E. Light cabinet (lined with aluminium foil).

(i) Lighting. 4 x 150W Lugon bulbs, 4 x 250W Osram bulbs, 1 x 700W Philips HPLP lamp.

(ii) Light intensity control. Sarlon shade screens.

(iii) Water bath.

F. De-humidification system. Test tubes immersed in ice-salt mixture contained in vacuum flasks.

G. Drying tubes containing Drierite.

H. I.R.G.A., Grubb Parsons SB2.

I. Flowmeters (900ml/min^{-1}).

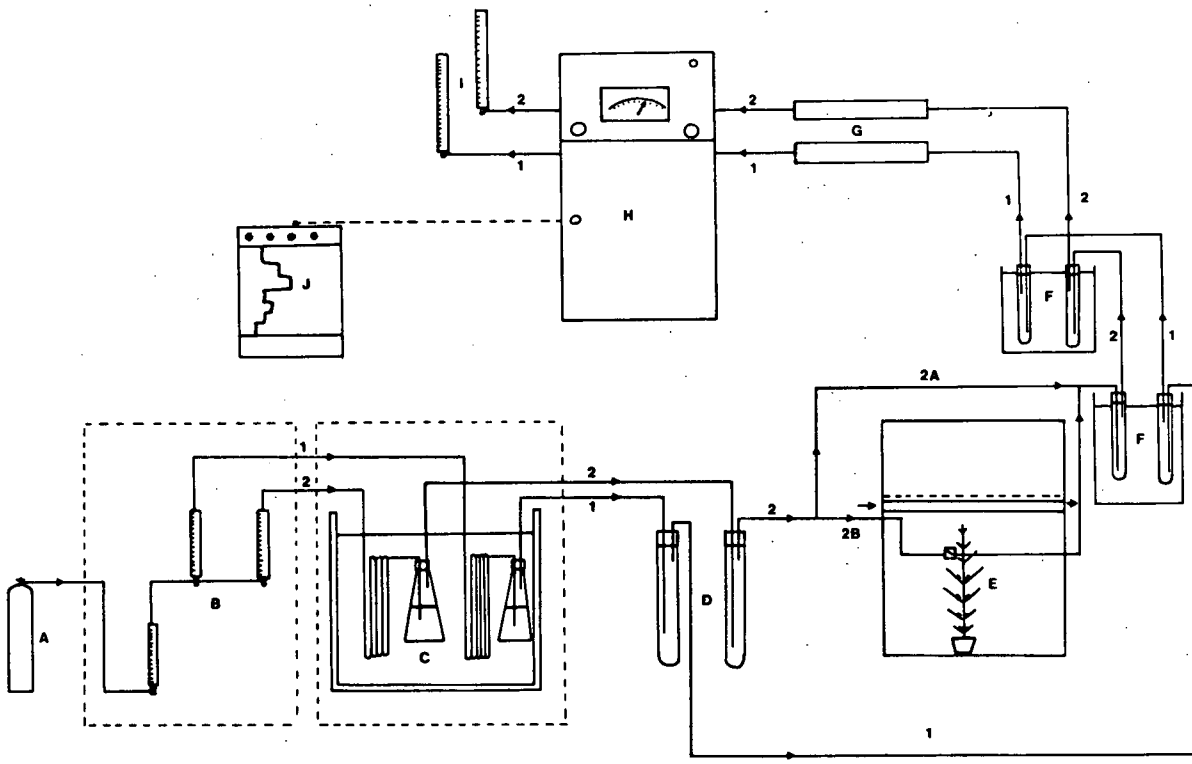
J. Chart recorder.

Gas supply lines (0.5cm O.D. copper tubing with flexible polythene joints).

1. Reference line.

2. a. By-pass line (allowing calibration and base line correction).

b. Chamber supply line.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After the leaf was in position, the petiole was placed in a groove on the lower perspex block, and the 'O' ring, petiole and thermocouple were covered with vaseline to ensure that the chamber remained air-tight during the experimental period.

Light intensity was controlled by inserting varying thicknesses of Sarlon shade cloth between the light source and the leaf chamber, and was measured using a Lambda L1-185 meter fitted with a quantum flux sensor. The quantum flux sensor measured photosynthetically active radiation (400-700nm) and results are reported in $\mu\Sigma m^{-2}s^{-1}$. All light intensity measurements were made above the chamber and were corrected for the light reduction caused by the water jacket and perspex chamber.

Open Circuit CO_2 Monitoring System

Several precautions were taken to ensure temperature and humidity control in the leaf chamber air supply.

- humidification was conducted in a water bath maintained at the leaf chamber temperature.

- room in which the system was located was provided with temperature control facilities and as far as possible, this temperature was maintained at the temperature of the leaf chamber.

- the length of tubing between the humidification system and the leaf chamber was minimised.

- to avoid differences in temperature and humidity between the leaf chamber and reference air supply (as well as any possible effect of the humidification system on CO_2 concentration) both reference and leaf chamber air supplies were subjected to the same treatment; except that the reference line did not pass through the chamber.

Calibration of Infra-Red Gas Analyser (I.R.G.A.) and Method of Determining Net CO_2 Exchange

The I.R.G.A. was calibrated using gas mixtures of known CO_2 concentration (supplied by C.I.G., Hobart). In this way the CO_2 concentration in the reference and leaf chamber by-pass line was varied to produce known concentration differences between the two lines (ΔCO_2). The chart response to changes in ΔCO_2 is provided in Appendix III 5.1. From this response it was possible to convert observed chart responses to ppm CO_2, differential between the two lines. That is, ΔCO_2 (ppm) = 0.6403 x (Chart Response) - 0.5665.
(At the commencement of each experiment, ΔCO_2 between two reference gases was re-checked.) Base line correction of the chart recorder was obtained by passing air with the same CO_2 concentration through both lines (i.e. $\Delta CO_2 = 0$).

Conversion of ΔCO_2 (ppm) to net CO_2 exchange (mg CO_2 $dm^{-2}hr^{-1}$) was by the following equation:

$$\text{mg } CO_2 \text{ dm}^{-2}\text{hr}^{-1} = \frac{4400}{22.4} \times \frac{54}{1} \times \frac{\Delta CO_2}{10^6} \times \frac{1}{0.1}$$

That is, mg CO_2 $dm^{-2}hr^{-1}$ = 1.061 x ΔCO_2.

6. Microscopy

Scanning Electron Microscopy (S.E.M.)

Tissue Preparation. Two preparative techniques were used to fix tissue prior to SEM examination.

a. Approximately $10mm^2$ sections of leaf tissue were exposed to osmium tetroxide vapour in the dark, overnight at 4^oC.

b. Approximately $10mm^2$ sections of leaf tissue were immersed in 5% glutaraldehyde in sodium phosphate buffer (0.1M, pH 7.3) for 2 hours,

rinsed twice with buffer (2 x 10 min), and post-fixed in 1% osmium tetroxide in buffer for 1 hour.

After fixation, tissue was rinsed with buffer (2 x 10 min) and dehydrated in a graded acetone series (10 → 25 → 50 → 75 → 80 → 85 → 90 → 95 → 97.5 → 100% x 3, using distilled water as the diluent, 15 minutes were allowed for each of the above solutions).

Whilst still immersed in the final 100% acetone, tissue was transferred to a Polaron E-3000 Critical Point Dryer (Polaron Equipment Pty. Ltd., Watford, England) and critical point dried from carbon dioxide. Dried tissue specimens were then glued onto brass SEM stubs with conductive paint (Dotite) and gold coated.

After coating, tissue was examined in a JEOL JXA 50-A scanning electron microscope. All micrographs were recorded on Polaroid types 52 or 107 Polaplan film.

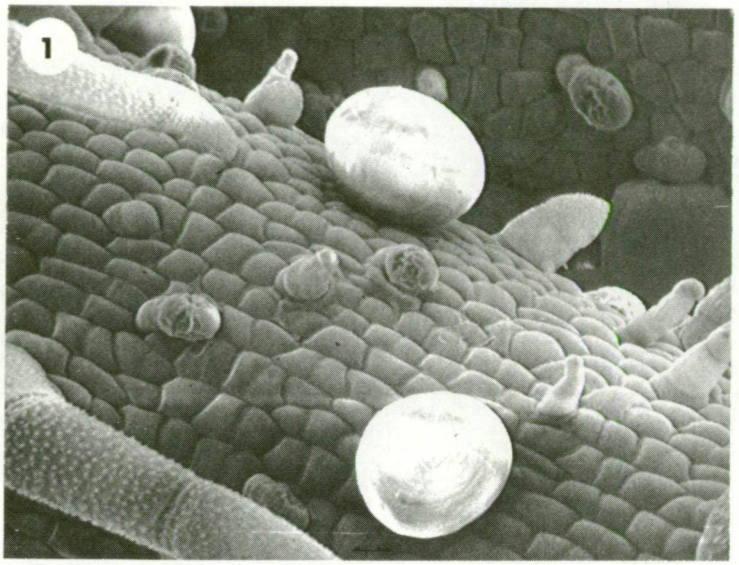
Comments on fixation techniques: Although fixation of leaf tissue with osmium tetroxide vapour resulted in preservation of the ten-celled glandular trichomes, the three-celled glandular hairs appeared very distorted (Plate III 6.1). Initial fixation in glutaraldehyde followed by post-fixation in osmium tetroxide resulted in preservation of both types of glandular structure (Plate III 6.2). During the initial investigation of fixation techniques, glutaraldehyde was used without post-fixation in osmium tetroxide and resulted in preservation of the three-celled glandular hairs but not the ten-celled glandular trichomes.

Light Microscopy

Approximately 1mm strips of leaf tissue were fixed according to technique b above. After fixation, tissue was rinsed with buffer (2 x 10 min) and dehydrated in a graded ethanol series[10 → 25 → 50 →

Plate III 6.1. Peppermint leaf tissue fixed in osmium tetroxide vapour overnight. (Note many of the three-celled glandular hairs have collapsed.) Bar = 30µm.

Plate III 6.2. Peppermint leaf tissue fixed in 5% glutaraldehyde for 2 hours followed by post-fixation in 1% osmium tetroxide solution for 1 hour. (Note three-celled glandular hairs appear well preserved.) Bar = 30µm.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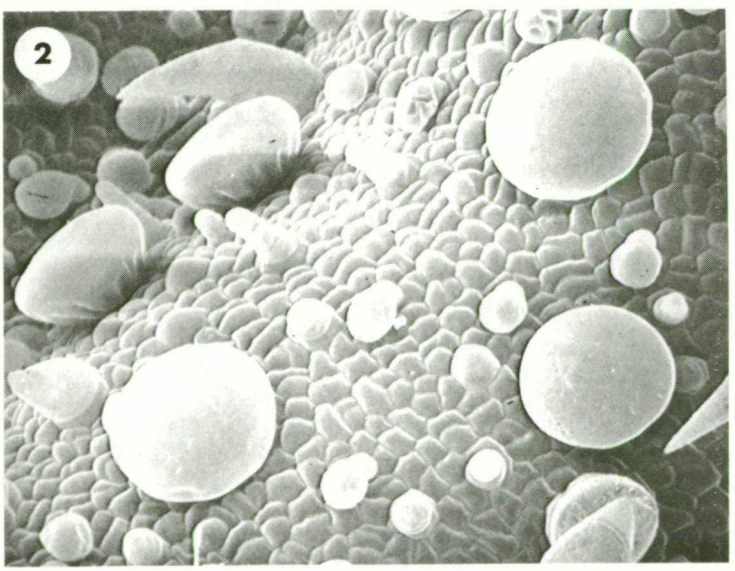
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75 (15 min each) → 80 → 85 → 90 → 95 [45 min each] → 97.5 → 100% x 2 (45 min each)].

Following dehydration the tissue was transferred through a graded series of ethanol / Spurr's medium to pure Spurr's medium over the period of one day. [(For detailed information concerning the composition of Spurr's medium, the reader is referred to Spurr (1969).]. Tissue remained in the Spurr's medium overnight and with two changes of the medium was transferred to small 'polythene vial caps' in pure Spurr's medium, and polymerised overnight at 70°C.

One micron sections of Spurr's embedded leaf tissue were stained with crystal violet and examined under the light microscope. Light micrographs were recorded on Kodak Plus X Pan A.S.A. 125 film.

7. Porometry

Leaf diffusive resistance measurements were made using a Lambda L1-65 Autoporometer fitted with a L1-20S Lambda sensor. Calibration of this instrument was conducted in accordance with the instruction manual.

The calibration curve and temperature conversion factors are included in Appendix III 7.1 and 2 respectively.

8. Glasshouse-Growth Room Experiments

Glasshouse

Plants were grown in an air conditioned glasshouse at the University of Tasmania, Hobart. The air flow within the glasshouse and the rate of air changes were controlled to provide a minimum of twenty changes of air per hour. The air stream was heated by an oil fired furnace or cooled by refrigeration as required. Temperature control within the

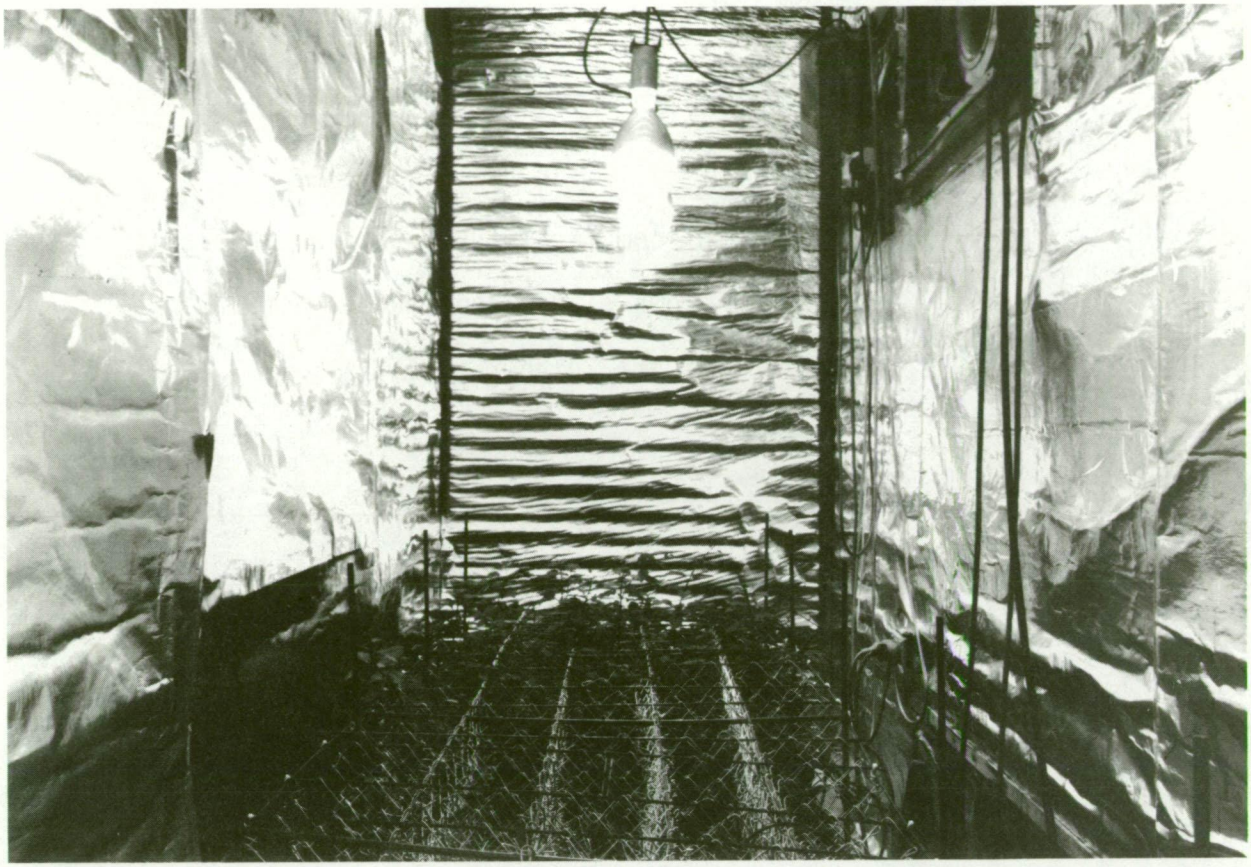
glasshouse was automatic so that temperatures were maintained above $15^{o}C$ at night and below $30^{o}C$ during the day. Glasshouse day temperatures varied from $18^{o}C$ to $30^{o}C$ from winter to summer. However, day time fluctuations in temperature within the glasshouse were much smaller than the fluctuations between seasons (approximately $\pm 3^{o}C$). Relative humidity was automatically controlled above 50 percent by injection of water sprays into the air stream. No artificial lighting was provided in the glasshouse. Glasshouse light intensities varied from $900\mu\Sigma\ m^{-2}s^{-1}$ to $1200\mu\Sigma\ m^{-2}s^{-1}$, when measured using a Lambda L1-185 meter fitted with a quantum flux sensor.

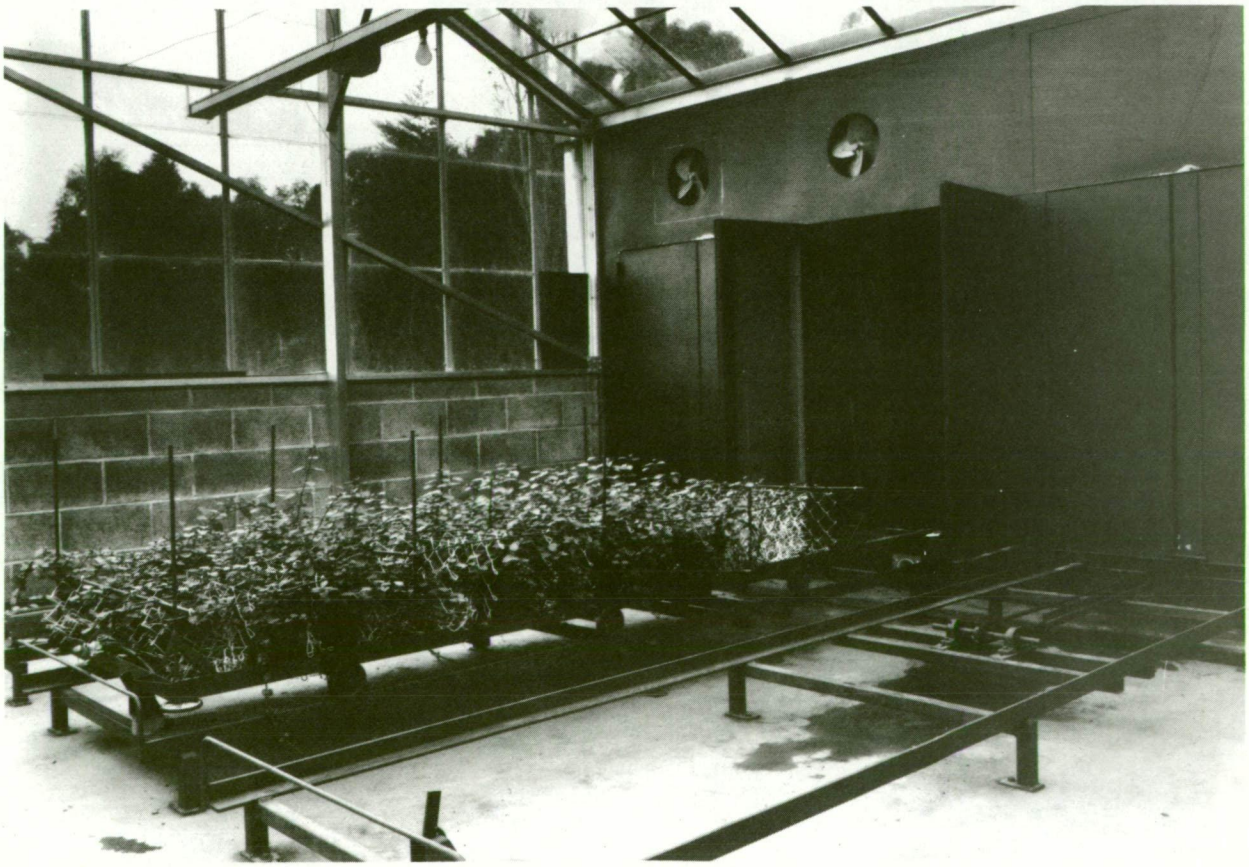
Growth Rooms

Growth rooms were each 1.5m x 4m in size, light proof, lined with aluminium foil and fitted with air conditioners. The air conditioners controlled day and night temperatures within the growth rooms and provided approximately the same air movement within these rooms as used in the main glasshouse. Temperature and relative humidity were monitored continuously using a thermohydrograph and relative humidity was consistently above 50 percent. Lighting was provided by 10 Osram MCFER 40W white fluorescent lamps, 2 Mazda 75W incandescent lamps and 2 Philips HLRG-N mercury vapour lamps in each room. The fluorescent and incandescent lamps were evenly distributed on the ceiling of the rooms, 2m above the plants, and the mercury vapour lamps were suspended 1.5m above the plants and 0.5m apart to provide uniform irradiance over all plant material. This provided $75\mu\Sigma\ m^{-2}s^{-1}$ at the bottom of the room and $150\mu\Sigma\ m^{-2}s^{-1}$ above the floor, as measured with the quantum flux sensor. Plate III 8.1 illustrates the design and layout of the growth rooms.

Plate III 8.1. Growth room facility, within which plants were grown either continuously or during part of the 24 hour cycle.

Plate III 8.2. Trolley system used to transfer plants between the glasshouse and growth rooms.





Combined Glasshouse-Growth Room Facility

By combining the previously described glasshouse and growth room systems it was possible to control daylength and night temperature without the necessity of maintaining plants constantly under the low light intensities characteristic of any growth room system.

Plants were placed in 15cm plastic pots on one of three trolley systems. Plants were separated from each other on these trollies by an interlocking system of galvanized chain wire (Titan, Hobart). The height of the chain wire system was adjustable and was increased as plants grew. This system allowed plants to be maintained as discrete units and facilitated removal for harvest and randomization.

The trollies on which plants were placed were capable of moving in and out of the growth rooms, from the glasshouse. This movement was automatically controlled by a system of time clocks, and each trolley was individually controlled. The doors to the growth rooms automatically closed when trollies moved into the rooms. Plate III 8.2 illustrates the design and layout of this trolley system.

Whether plants were grown in the glasshouse, growth room or combined system, they all received the same water regime, nutrients and basal fertilisers.

All plants were watered with tap water daily and nutrient solution at weekly intervals (Hoagland and Arnon, 1950). Both irrigation and nutrient solution were applied through to a permanent trickle irrigation system. Tap water and nutrient solutions were applied until pots were observed to drain freely.

The potting mixture for all experiments consisted of a mixture of equal volumes of coarse sand and Tasmanian peat moss. Equal amounts of both dolomite and limil were added to this potting mixture to bring the pH to approximately 6.5. The equivalent of 1g of Osmocote (3-4 month

formulation, 15% N:5.2% P:12.5% K) was added per 400cm^3 of mix.

9. Field Experiments

All field trials were located in commercial plantings of *Mentha piperita* L. var. Black Mitcham, in Southern Tasmania. The first of these areas was at "Rotherwood", Ouse, in the Derwent Valley area of Tasmania, and the second location was in the Huon Valley of Tasmania at Castle Forbes Bay.

With the exception of treatments imposed during the course of these trials, all areas were subjected to the normal cultural practices adopted by commercial producers. Therefore, a brief outline of these cultural practices will be provided.

Planting and Growing System

New areas are planted with peppermint during May to July with propagating material removed from established plantings. This material is planted in rows approximately 70cm apart and growth in the first season remains within these rows whilst spreading during the season to form an almost uniform canopy at the end of this season. In subsequent years a uniform stand of herb develops and no attempt is made to maintain the initial row system. First year plantings are referred to as "row mint" whilst growth in subsequent years is referred to as "meadow mint".

Rust Control

In most years and in all areas, peppermint rust (*Puccinia menthae* Pers.) becomes a severe problem during the latter part of the growing season. Severe infestations of rust result in the loss of many lower leaves. The recommended control of this disease involves winter ploughing to bury all leaves and stolons and propane gas burning in early spring.

Herbicide Programme

Weed control in peppermint is important because several weed species also produce essential oils which may cause 'off-flavours' in the final oil product. The current herbicide programme incorporates a spring application of the terbacil herbicide Sinbar (DuPont, Australia, Ltd.) followed by spot spraying during spring and early summer to control problem weeds.

Fertiliser and Irrigation Practices

The current fertiliser regime consists of 400kg of mixed fertiliser (8:4:10) per hectare, applied in early spring, followed by minimal amounts of ammonium sulphate later in the growing season. Irrigation is commenced in late November and the equivalent of 25mm is applied weekly throughout the growing season, no post-harvest irrigation being applied. In most areas, irrigation is applied by overhead sprinklers, using travelling irrigators.

Harvesting

On the appropriate harvest date, plant material is mown using a rotary mower, left in the field to dry for approximately 1 day, racked into windrows and transferred into distillation vats, using a forage harvester. As well as providing a means of collecting the partially dried plant material, the forage harvester chops the material which allows more material to be placed into each vat, avoiding uneven packing. The distillation vats (capacity of approximately 1 tonne of partially dried material) are transported to the distillation unit with minimum delay.

Distillation

Extraction of oil is achieved by water-steam distillation, using

fully saturated steam at low pressure, generated by an oil fired furnace.

Complete exhaustion of the herb requires about 45 minutes with the oil yield per vat being 4 to 5 litres. The distillation rate is maintained at 8ℓ/min and the condenser temperature at 45^{o}C. The separating system used is in accordance with that described by Hughes (1952).

IV MATERIALS AND METHODS, RESULTS AND DISCUSSION

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A. Glasshouse and Laboratory Experiments

1. A Preliminary Investigation of the Accumulation of Essential Oil in Peppermint Leaves

1.1 Introduction

The aim of this preliminary experiment was to investigate oil accumulation in peppermint, the effect of growing conditions on accumulation, changes in composition with leaf age and position on the plant, and the relationship between leaf age, gland development and oil accumulation. From the results of this experiment and results reported in the literature, it was anticipated that a basis for the interpretation of monoterpene metabolism and interconversions within the plant could be obtained. In addition, an attempt was made to explain the apparent disagreement between observations of gland development and oil accumulation.

1.2 Materials and Methods

a. Plant Material

Peppermint plants were propagated vegetatively from clonal material. Shoot cuttings were taken from plants growing under the same photoperiodic conditions that were to be used in the experiment. After cuttings had formed roots (5 to 7 days) they were transplanted into sand:peat mix (1:1), under the treatment growing conditions.

b. Growing Conditions

All experimental work was conducted in the combined glasshouse-growth room system previously described (Section III.8). The plants were subjected to glasshouse light intensities and day temperatures throughout the experimental period.

C. Treatments

On 1 August 1977 visually matched plants were transferred into two growing conditions:-

LD x LNT : long days (16 : 8) and low night temperatures ($10\pm2^{o}C$)

SD x HNT : short days (8 : 16) and high night temperatures ($18\pm2^{o}C$)

Glasshouse day temperatures were $20\pm3^{o}C$ and light intensities were 900-$1200\mu\Sigma m^{-2}s^{-1}$.

Initially, twenty visually matched rooted cuttings were transplanted into each of three blocks, in both growing conditions. After 7 days of growth under the experimental conditions, five visually matched plants were reselected within each block. At this time the lowest leaf pair on each plant was marked (white paint) and all subsequent leaf numbering was related to this leaf pair (lowest leaf pair = No. 1). After 4 weeks of growth it became obvious that the lowest leaf pair was senescing and therefore leaf pair 5 was marked and became the reference for subsequent leaf numbering. Although only five experimental plants were selected per block, a total of twenty plants were retained in each block, with the additional plants functioning as 'buffer plants'. All plants were re-randomised within each block at weekly intervals.

Three plants were harvested from each growing condition (one per block) on five harvest occasions - 17 August 1977, 24 August 1977, 1 September 1977, 18 September 1977 and 4 October 1977. Plants were selected at random from within each block.

d. Extraction

At harvest,leaf pairs were removed node by node from the main stem commencing with the basal leaf pair (No. 2). Following leaf area determinations using a Paton Electroplan (Paton Industries Pty. Ltd., Stepney, South Australia), leaf pairs were immediately solvent extracted. In addition to the solvent extraction procedure outlined in Section III 3,

a known amount of internal standard was added to all extraction solutions. The addition of internal standard (1ml of a 1 x 10^{-4} g/ml solution of β-methyl naphthalene) was considered necessary to allow a comparison of relative oil yield per leaf pair.

e. Determination of Yield and Composition

The extract solutions were concentrated, analysed by gas chromatography and the peak areas of all components eluting from the F.F.A.P., SCOT capillary column determined using a Pye Unicam DP88, computing integrator. Peak areas of the eleven components of interest were corrected for FID response in accordance with procedures outlined in Section III 4 and Appendix III 4.3, and weight percentages determined.

A measure of relative oil yield per leaf pair was obtained by comparing the total corrected peak areas of all peaks eluting from the capillary column to the peak area of the internal standard. [Unidentified peaks represented approximately 5 percent of the total peak area and the FID response to these compounds was assumed to be 1.00 and therefore no correction of peak area was required.] Since a constant amount of internal standard was added to all extraction solutions, an increase in the ratio of total corrected peak area to peak area of internal standard, reflected an increase in the oil yield per leaf pair.

The addition of internal standard was necessary to avoid variations arising from differences in the extent to which extract solutions were concentrated and differences in injection volumes. The method of expressing changes in oil yield was considered satisfactory for this experiment, since changes in oil yield rather than absolute oil yields were of interest. Corrected peak areas were used in calculations since small variations in FID response could have resulted in large errors in yield determinations when large compositional changes associated with different leaf pairs were considered.

f. Gland Development

Leaves on which microscopic examination was to be conducted were harvested from the same plants used above. Leaf pairs (odd numbered) were selected from plants within block 1, on all harvest dates and from both growing conditions. Although all leaf samples were prepared, not all prepared samples were finally examined due to the time consuming nature of these examinations. Sufficient leaf samples were examined from each growing condition and from each plant to establish general trends in gland development.

g. Analysis of Results

Due to several unavoidable limitations in the present experiment, the results should be considered to indicate general trends rather than specific differences between individual leaves, harvest dates or growing conditions. Although replication was included within each growing condition, it was not possible to replicate actual growing conditions. Secondly, it was never possible to select any specific leaf pair and say that it was exactly equivalent to a specific leaf pair on another plant. This latter consideration may partly explain the large standard errors often associated with mean values of oil yield, leaf area and oil composition.

Statistical significance of the results was based on a 't-test' between standard errors of each mean of three results. That is,

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{SE_1^2 + SE_2^2}} \quad (df = 2\,(n - 1) = 4;\ t\ (0.05) = 2.776)$$

1.3 Results

a. Changes in Oil Yield

The yield of oil increased from basal to midstem leaf pairs and decreased from midstem to apical leaf pairs (Table IV A 1.1).

Table IV A1.1. Relative Oil Yield. Mean Values[a] and Standard Errors[b].

Growing condition: LD x LNT.

Leaf Pair	Ratio of Total Peak Area : Peak Area of Internal Standard				
	Harvest No.				
	1 (17/8/77)	2 (24/8/77)	3 (1/9/77)	4 (18/9/77)	5 (4/10/77)
2	4.23[a](0.12)[b]	3.38 (0.60)	2.42 (0.60)	1.75 (0.18)	*
4	10.42 (0.88)	11.24 (0.59)	6.19 (1.92)	5.26 (1.18)	*
6	23.72 (2.39)	21.59 (1.69)	28.94 (3.58)	26.15 (6.03)	9.83 (0.47)
8	10.50 (4.23)	26.97 (2.12)	40.90 (7.54)	52.06 (6.54)	42.82 (2.36)
10		3.82 (0.52)	21.97 (4.30)	47.15 (4.37)	40.71 (1.04)
12			8.19 (1.55)	28.44 (1.42)	37.87 (1.26)
14			3.40 (0.74)	16.65 (2.50)	35.25 (2.31)
16				6.38 (1.91)	18.46 (3.79)

* At Harvest 5 (4/10/77) leaf pairs 2 and 4 had fallen from the plant.

——— Oil yield data underlined, represents the first harvest at which the monoterpene yield for a leaf pair was not significantly different from the maximum yield observed during the experimental period (t = 0.05).

------ Harvest date after which no further significant increase in leaf size was observed

Growing condition: SD x HNT

Leaf Pair	Ratio of Total Peak Area : Peak Area of Internal Standard				
	Harvest No.				
	1 (17/8/77)	2 (24/8/77)	3 (1/9/77)	4 (18/9/77)	5 (4/10/77)
2	1.66 (0.22)	1.53 (0.28)	1.60 (0.19)	2.04 (0.28)	*
4	2.65 (0.34)	2.43 (0.43)	2.69 (0.39)	3.13 (0.34)	*
6	5.28 (1.43)	6.05 (2.02)	5.74 (0.33)	7.95 (0.70)	9.88 (1.44)
8	3.99 (0.69)	9.15 (0.21)	9.96 (0.66)	14.82 (0.49)	16.28 (1.03)
10		5.76 (0.27)	12.90 (1.59)	22.35 (3.61)	21.07 (1.05)
12			3.32 (0.90)	24.23 (0.57)	25.52 (0.57)
14			1.86 (0.28)	9.12 (0.59)	21.12 (3.50)
16				3.60 (0.76)	8.53 (0.64)

* At Harvest 5 (4/10/77) leaf pairs 2 and 4 had fallen from the plant.

——— Oil yield data underlined, represents the first harvest at which the monoterpene yield for a leaf pair was not significantly different from the maximum yield observed during the experimental period (t = 0.05).

------ Harvest date after which no further significant increase in leaf size was observed

This change in oil yield with leaf position occurred in both growing conditions.

In the LD x LNT growing condition, basal leaves (2, 4 and 6) had accumulated their maximum amount of oil by harvest 1 and there was a significant decrease in oil yield at later harvests. Midstem leaves (8 and 10) continued to accumulate oil during initial harvests, after which no significant change occurred. Apical leaves (12, 14 and 16) continued to accumulate oil until the last harvest.

In the SD x HNT growing condition, oil yield did not change significantly from harvest 1 - 5, in basal leaves. Midstem leaves continued to accumulate oil during initial harvests, reached a maximum oil content at harvest 3 - 4, after which no significant change occurred. Apical leaves continued to accumulate oil throughout the experimental period.

Given that inflorescences on plants growing under LD x LNT conditions appeared between harvest date 3 and 5, it follows that maximum oil yield in basal, midstem and apical leaf pairs occurred prior to, at the time of, and following the appearance of inflorescences, respectively. The maximum quantity of oil accumulated by each leaf pair was significantly higher under LD x LNT conditions. In addition, the significant decrease in oil content observed in midstem and basal leaves from the LD x LNT conditions was not apparent under SD x HNT conditions.

Changes in leaf area with harvest date are included in Table IV A 1.2. In general, basal leaves on plants growing under both conditions were fully expanded and contained their maximum amount of oil at harvest 1. Midstem leaves expanded and accumulated oil until harvest 3-4. Generally the period of rapid oil accumulation corresponded to the period of rapid leaf expansion. In these basal and midstem leaves the maximum oil accumulation occurred at or before the fully expanded

Table IV A 1.2 . Leaf Area per Leaf Pair (cm^2). Mean Values[a] and Standard Errors[b].

Growing condition: LD x LNT.

Leaf Pair	Ratio of Total Peak Area : Peak Area of Internal Standard				
	Harvest No.				
	1 (17/8/77)	2 (24/8/77)	3 (1/9/77)	4 (18/9/77)	5 (4/10/77)
2	12.30[a](2.06)[b]	9.09 (0.98)	8.92 (0.83)	9.39 (1.08)	*
4	25.49 (1.50)	25.04 (3.24)	22.78 (2.28)	23.32 (4.33)	*
6	33.02 (0.67)	35.77 (2.10)	34.61 (2.02)	34.32 (1.31)	36.66 (2.33)
8	12.25 (0.33)	28.21 (3.13)	34.73 (2.74)	42.35 (0.62)	43.95 (0.86)
10		8.23 (0.66)	22.25 (3.73)	39.12 (0.51)	44.64 (0.78)
12			11.00 (3.34)	26.48 (1.77)	39.81 (1.50)
14			4.20 (0.54)	20.71 (4.27)	34.76 (3.38)
16				6.42 (1.75)	24.95 (2.55)

* At Harvest 5 (4/10/77) leaf pairs 2 and 4 had fallen from the plant.

Growing condition: SD x HNT.

Leaf Pair	Ratio of Total Peak Area : Peak Area of Internal Standard				
	Harvest No.				
	1 (17/8/77)	2 (24/8/77)	3 (1/9/77)	4 (18/9/77)	5 (4/10/77)
2	7.79 (0.28)	7.33 (1.51)	7.01 (0.72)	6.78 (0.40)	*
4	9.72 (1.71)	13.87 (0.86)	11.54 (1.25)	12.58 (0.48)	*
6	12.12 (1.03)	17.72 (1.92)	22.19 (1.62)	24.10 (0.96)	21.81 (1.44)
8	4.71 (1.14)	14.26 (1.44)	21.89 (1.46)	25.57 (1.27)	27.89 (0.85)
10		5.34 (0.51)	16.07 (0.98)	21.56 (4.14)	28.01 (2.49)
12			7.18 (1.46)	15.45 (1.88)	20.24 (1.11)
14			3.65 (0.79)	9.84 (1.28)	14.40 (1.54)
16				2.76 (0.78)	5.15 (0.57)

* At Harvest 5 (4/10/77) leaf pairs 2 and 4 had fallen from the plant.

Table IV A 1.3 Oil Composition (%) - LD x LNT Conditions. Mean values [a] and Standard Errors [b].

(i) Harvest 1. (17/8/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.13[a] (0.78)[b]	1.84 (0.22)	2.09 (0.11)	5.35 (0.48)	17.23 (4.50)	6.76 (1.01)	1.70 (0.34)	0.78 (0.33)	2.99 (0.13)	55.42 (4.34)	0.91 (0.37)
4	1.03 (0.02)	2.02 (0.13)	2.10 (0.11)	7.56 (0.09)	42.16 (8.19)	6.56 (0.29)	1.90 (0.23)	0.46 (0.23)	1.58 (0.16)	28.72 (7.60)	1.65 (0.08)
6	1.04 (0.13)	2.05 (0.34)	2.33 (0.12)	7.33 (0.66)	63.70 (3.74)	6.72 (0.89)	1.42 (0.21)	0.24 (0.02)	0.78 (0.24)	8.37 (3.48)	1.89 (0.49)
8	0.79 (0.05)	1.37 (0.09)	2.05 (0.04)	3.68 (0.21)	72.67 (0.14)	8.61 (0.15)	2.36 (0.48)	0.18 (0.01)	0.39 (0.21)	2.27 (0.37)	1.60 (0.08)

(ii) Harvest 2. (24/8/77)

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.83 (0.58)	2.05 (0.29)	2.24 (0.24)	5.71 (0.80)	7.67 (2.03)	7.15 (0.26)	2.10 (0.06)	2.04 (0.24)	4.27 (0.40)	58.20 (1.53)	2.12 (0.40)
4	0.89 (0.11)	1.81 (0.31)	2.47 (0.35)	7.30 (1.45)	24.14 (5.11)	5.67 (1.03)	2.40 (0.23)	1.00 (0.12)	2.47 (0.38)	46.09 (7.24)	2.15 (0.42)
6	1.15 (0.18)	2.34 (0.30)	2.72 (0.34)	8.66 (1.03)	36.22 (0.41)	6.01 (0.34)	2.04 (0.28)	0.68 (0.21)	2.10 (0.14)	31.57 (1.75)	2.76 (0.18)
8	1.02 (0.06)	1.80 (0.22)	2.75 (0.36)	6.57 (1.29)	64.84 (0.39)	5.30 (0.17)	2.09 (0.16)	0.17 (0.04)	1.87 (0.40)	6.56 (1.06)	2.90 (0.18)
10	1.03 (0.09)	1.81 (0.29)	2.92 (0.12)	3.83 (0.43)	70.99 (1.03)	6.44 (0.51)	2.98 (0.11)	0.27 (0.53)	1.23 (0.11)	1.34 (0.73)	2.97 (0.11)

(iii) Harvest 3. (1/9/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.34 (0.23)	1.51 (0.33)	1.35 (0.20)	5.75 (0.53)	8.65 (3.77)	2.11 (0.58)	1.33 (0.12)	9.93 (2.90)	1.82 (0.20)	62.52 (5.28)	0.85 (0.54)
4	1.14 (0.16)	1.33 (0.23)	1.37 (0.27)	7.21 (0.57)	9.09 (1.66)	3.15 (0.38)	1.09 (0.04)	3.86 (1.81)	1.53 (0.26)	65.19 (3.14)	1.48 (0.39)
6	1.15 (0.10)	2.20 (0.17)	1.78 (0.14)	5.96 (1.15)	23.12 (4.33)	3.49 (0.11)	1.76 (0.10)	0.67 (0.19)	2.30 (0.31)	51.75 (4.85)	2.33 (0.57)
8	1.19 (0.12)	1.87 (0.22)	1.62 (0.16)	8.55 (1.98)	35.26 (8.24)	4.61 (0.64)	1.42 (0.34)	0.46 (0.14)	1.49 (0.31)	36.84 (9.45)	3.40 (0.59)
10	1.15 (0.13)	2.06 (0.54)	1.70 (0.34)	6.85 (0.81)	58.29 (9.24)	5.31 (0.20)	1.41 (0.16)	0.36 (0.05)	1.47 (0.27)	14.75 (7.65)	3.60 (0.57)
12	0.92 (0.16)	1.41 (0.20)	1.40 (0.30)	3.91 (1.73)	70.23 (9.31)	7.03 (1.49)	1.54 (0.60)	0.34 (0.61)	1.27 (0.03)	6.32 (4.52)	3.08 (0.08)
14	1.00 (0.03)	1.16 (0.28)	1.12 (0.05)	2.57 (0.62)	74.46 (0.68)	7.36 (1.29)	1.60 (0.21)	0.40 (0.14)	1.02 (0.10)	3.10 (0.55)	2.62 (0.28)

(iv) Harvest 4. (18/9/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.93 (0.15)	1.86 (0.16)	2.46 (0.14)	9.40 (0.50)	6.94 (1.11)	1.47 (0.19)	2.62 (0.22)	17.21 (5.08)	5.77 (0.23)	44.60 (4.16)	1.62 (0.32)
4	1.45 (0.20)	2.20 (0.07)	2.20 (0.13)	9.06 (0.30)	6.37 (0.78)	1.79 (0.25)	2.47 (0.41)	2.91 (0.43)	5.62 (0.19)	59.60 (0.93)	1.83 (0.33)
6	1.96 (0.10)	2.43 (0.39)	2.78 (0.32)	9.90 (1.41)	11.19 (0.81)	2.82 (0.46)	2.17 (0.46)	1.36 (0.13)	5.18 (0.42)	54.74 (1.65)	2.04 (0.34)
8	1.90 (0.11)	2.31 (0.21)	2.70 (0.14)	8.95 (0.30)	18.41 (0.96)	2.52 (0.30)	2.46 (0.62)	0.69 (0.03)	4.83 (0.27)	48.48 (1.58)	2.13 (0.13)
10	1.62 (0.21)	2.11 (0.16)	2.49 (0.35)	10.17 (0.63)	24.92 (4.57)	1.91 (0.36)	2.09 (0.41)	0.37 (0.03)	4.90 (0.42)	41.81 (5.14)	2.93 (0.42)
12	1.77 (0.11)	2.38 (0.18)	2.98 (0.99)	10.02 (1.06)	42.35 (3.79)	3.03 (0.13)	2.36 (0.12)	0.30 (0.03)	4.72 (0.29)	23.35 (4.60)	2.91 (0.23)
14	1.50 (0.31)	1.93 (0.10)	2.35 (0.19)	9.24 (0.51)	54.30 (3.75)	3.96 (0.11)	2.19 (0.27)	0.15 (0.03)	4.69 (0.24)	12.99 (3.72)	3.29 (0.33)
16	1.30 (0.13)	1.92 (0.05)	2.10 (0.08)	6.41 (0.41)	67.18 (1.20)	3.33 (0.33)	2.96 (0.38)	0.26 (0.03)	4.39 (0.19)	2.99 (1.48)	3.46 (0.09)

(v) Harvest 5. (4/10/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
6	1.30 (0.15)	2.58 (0.28)	2.77 (0.34)	9.66 (1.09)	3.76 (1.10)	2.10 (0.17)	1.79 (0.26)	5.58 (0.87)	4.75 (0.26)	59.42 (1.49)	2.12 (0.10)
8	1.82 (0.10)	2.85 (0.35)	2.21 (0.26)	10.56 (1.20)	10.44 (2.07)	3.30 (0.46)	2.15 (0.12)	2.67 (0.32)	4.16 (0.50)	53.25 (1.53)	2.13 (0.07)
10	1.29 (0.06)	2.54 (0.26)	2.84 (0.23)	10.12 (0.48)	17.38 (1.59)	3.31 (0.16)	2.46 (0.26)	1.11 (0.03)	4.15 (0.59)	47.94 (0.32)	2.54 (0.33)
12	1.32 (0.08)	2.77 (0.15)	2.83 (0.06)	9.33 (0.69)	31.45 (0.33)	4.45 (0.42)	2.62 (0.28)	0.45 (0.04)	5.14 (0.02)	31.24 (1.11)	3.84 (0.15)
14	1.82 (0.29)	2.12 (0.12)	3.02 (0.14)	10.88 (0.21)	37.20 (1.41)	4.00 (0.31)	2.53 (0.23)	0.34 (0.08)	4.57 (0.25)	29.43 (4.89)	4.23 (0.41)
16	1.77 (0.18)	2.58 (0.07)	2.38 (0.17)	8.53 (0.40)	50.79 (0.47)	4.22 (0.28)	2.68 (0.31)	0.24 (0.04)	4.63 (0.25)	14.59 (0.53)	3.03 (0.28)

Table IV A 1.4 Oil Composition (%) - SD x HNT Conditions. Mean Values[a] and Standard Errors[b].

(i) Harvest 1. (17/8/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.18[a] (0.05)[b]	2.03 (0.32)	1.97 (0.07)	4.60 (0.42)	16.93 (3.41)	6.99 (0.89)	1.78 (0.18)	1.52 (0.42)	3.17 (0.29)	53.87 (4.33)	1.72 (0.13)
4	1.31 (0.13)	2.05 (0.05)	1.89 (0.08)	6.19 (0.21)	36.72 (4.93)	6.52 (0.69)	1.32 (0.15)	0.84 (0.04)	2.15 (0.28)	36.44 (5.65)	1.55 (0.44)
6	1.19 (0.17)	1.98 (0.06)	1.94 (0.03)	6.64 (1.13)	66.82 (0.28)	7.15 (0.11)	1.57 (0.30)	0.35 (0.09)	1.18 (0.09)	6.90 (0.94)	1.81 (0.30)
8	1.03 (0.13)	2.06 (0.03)	1.94 (0.10)	5.19 (0.67)	69.53 (0.54)	7.75 (0.12)	1.84 (0.06)	0.19 (0.01)	1.27 (0.15)	2.88 (0.65)	2.20 (0.07)

(ii) Harvest 2. (24/8/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.20 (0.20)	2.17 (0.13)	2.31 (0.16)	6.00 (0.93)	9.57 (0.28)	4.94 (1.21)	1.94 (0.11)	1.63 (0.15)	4.58 (0.36)	58.69 (1.53)	2.07 (0.43)
4	2.11 (0.08)	2.64 (0.12)	2.13 (0.29)	5.99 (1.15)	22.03 (2.49)	4.19 (0.62)	1.80 (0.16)	1.16 (0.10)	4.50 (0.61)	47.87 (3.10)	2.01 (0.47)
6	1.33 (0.36)	2.26 (0.53)	2.34 (0.29)	8.50 (1.51)	45.11 (1.27)	4.62 (0.34)	1.87 (0.19)	0.75 (0.06)	3.09 (0.52)	23.64 (1.43)	3.03 (0.12)
8	1.10 (0.09)	2.49 (0.25)	1.89 (0.35)	7.22 (1.16)	60.40 (0.52)	4.96 (0.19)	2.57 (0.43)	0.24 (0.06)	3.13 (0.55)	7.71 (0.30)	3.51 (0.41
10	1.00 (0.11)	1.78 (0.31)	2.61 (0.32)	4.08 (0.11)	72.43 (1.77)	5.19 (0.51)	1.92 (0.07)	0.21 (0.02)	2.13 (0.29)	1.68 (0.38)	2.86 (0.46)

(iii) Harvest 3. (1/9/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.74 (0.10)	1.68 (0.33)	1.73 (0.27)	10.41 (2.29)	2.02 (0.48)	0.50 (0.24)	2.11 (0.36)	18.01 (1.41)	3.88 (0.54)	52.68 (2.61)	0.59 (0.21)
4	1.92 (0.24)	2.21 (0.36)	1.92 (0.22)	9.28 (2.30)	1.97 (0.10)	0.32 (0.05)	2.49 (0.39)	7.07 (0.49)	3.89 (0.91)	63.55 (4.91)	0.72 (0.51)
6	2.04 (0.06)	2.62 (0.26)	2.34 (0.32)	11.26 (0.77)	10.87 (3.03)	3.02 (0.55)	2.69 (0.32)	0.93 (0.14)	5.78 (0.58)	52.85 (5.30)	1.09 (0.17)
8	1.97 (0.23)	2.52 (0.25)	2.80 (0.32)	15.89 (4.99)	22.19 (4.98)	3.74 (0.39)	3.15 (0.63)	0.44 (0.03)	6.30 (0.70)	33.65 (3.16)	2.08 (0.61)
10	1.15 (0.12)	2.06 (0.35)	1.98 (0.13)	8.14 (0.72)	47.01 (5.04)	4.00 (0.62)	2.21 (0.29)	0.37 (0.03)	3.02 (0.78)	22.61 (6.15)	2.89 (0.93)
12	1.22 (0.18)	1.55 (0.31)	1.65 (0.25)	5.58 (0.83)	69.00 (2.50)	4.43 (0.25)	1.90 (1.12)	0.31 (0.11)	1.18 (0.08)	6.47 (1.15)	3.31 (0.61)
14	1.05 (0.05)	1.56 (0.12)	1.23 (0.03)	3.03 (0.16)	74.58 (1.46)	5.49 (0.30)	1.80 (0.32)	0.53 (0.14)	1.25 (0.15)	3.74 (1.73)	2.09 (0.81)

(iv) Harvest 4. (18/9/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1.57 (0.06)	1.77 (0.13)	2.08 (0.06)	7.36 (0.96)	5.45 (1.07)	3.46 (0.89)	2.65 (0.27)	19.43 (4.05)	5.88 (0.29)	44.71 (2.53)	1.50 (0.13)
4	1.93 (0.26)	1.88 (0.67)	2.36 (0.48)	8.89 (1.81)	3.50 (0.31)	1.79 (0.36)	2.72 (0.31)	10.41 (3.09)	5.64 (0.38)	55.27 (4.61)	1.29 (0.34)
6	1.81 (0.32)	3.29 (0.45)	2.68 (0.13)	11.24 (0.83)	8.41 (1.49)	2.33 (0.22)	2.34 (0.10)	2.80 (0.20)	5.16 (0.36)	53.06 (3.50)	1.40 (0.15)
8	1.40 (0.10)	2.56 (0.58)	2.42 (0.34)	10.20 (1.34)	13.36 (2.10)	3.14 (0.33)	2.29 (0.16)	0.84 (0.09)	5.16 (0.18)	51.35 (3.08)	2.51 (0.67)
10	2.13 (0.14)	2.04 (0.07)	2.27 (0.45)	9.64 (1.14)	30.53 (2.54)	4.55 (0.27)	2.96 (0.20)	0.64 (0.05)	4.25 (0.36)	33.05 (3.87)	3.62 (0.41)
12	1.92 (0.35)	2.80 (0.20)	2.78 (0.17)	9.00 (1.01)	45.36 (3.67)	3.26 (0.31)	2.25 (0.34)	0.85 (0.50)	5.01 (0.15)	19.37 (4.08)	3.26 (0.19)
14	2.09 (0.05)	2.74 (0.47)	2.15 (0.16)	6.97 (1.07)	55.81 (1.60)	4.80 (0.26)	1.98 (0.40)	0.60 (0.07)	5.47 (0.38)	8.39 (1.63)	3.89 (0.09)
16	1.15 (0.08)	1.82 (0.06)	2.21 (0.15)	6.80 (0.93)	65.04 (0.86)	4.61 (0.28)	2.72 (0.35)	0.15 (0.01)	5.09 (0.25)	3.12 (0.63)	3.28 (0.40)

(v) Harvest 5. (4/10/77).

Leaf Pair No.	Component										
	β-Pinene	α-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
6	1.19 (0.04)	2.46 (0.37)	2.99 (0.11)	9.91 (0.61)	3.06 (0.16)	1.61 (0.25)	2.15 (0.10)	7.05 (1.08)	5.41 (0.75)	59.17 (0.48)	1.20 (0.13)
8	1.62 (0.20)	2.87 (0.63)	2.40 (0.22)	9.48 (0.77)	6.08 (0.31)	1.37 (0.32)	2.71 (0.27)	3.33 (0.42)	4.98 (0.35)	57.90 (1.76)	2.59 (0.25)
10	1.07 (0.04)	3.06 (0.50)	2.78 (0.40)	11.95 (0.33)	19.64 (4.21)	1.93 (0.12)	2.50 (0.42)	1.03 (0.09)	5.02 (0.10)	44.89 (4.58)	2.66 (0.37)
12	1.35 (0.21)	3.41 (0.36)	2.73 (0.25)	9.41 (0.46)	30.76 (1.80)	2.46 (0.25)	2.30 (0.33)	0.53 (0.12)	5.05 (0.47)	34.66 (1.69)	3.24 (0.60)
14	1.84 (0.19)	2.85 (0.23)	2.42 (0.26)	7.82 (0.75)	52.73 (1.58)	3.17 (0.89)	2.37 (0.22)	0.34 (0.07)	3.71 (0.23)	12.79 (2.58)	3.06 (0.08)
16	1.93 (0.11)	2.55 (0.24)	2.89 (0.13)	9.19 (0.60)	57.83 (1.83)	3.25 (0.26)	3.13 (0.64)	0.23 (0.58)	3.61 (0.33)	7.80 (1.53)	3.40 (0.48)

leaf stage. In LD x LNT conditions, apical leaves continued to expand and accumulate oil after the appearance of inflorescences. In SD x HNT conditions, apical leaves were not observed to expand from harvest 4-5, however, oil yield per leaf pair continued to increase until harvest 5 in leaf pairs 14 and 16.

b. Changes in Oil Composition

Several trends in oil composition are apparent from the data presented in Tables IV A 1.3 and 1.4. Generally there were no pronounced differences in composition between oils extracted from plants growing under LD x LNT and SD x HNT conditions. The effect of these growing conditions on oil composition is considered in Section IV A 3.

With respect to changes in oil composition with leaf position, menthol increased and menthone decreased in basal leaves relative to apical leaves, at all harvest dates. The decrease in menthol content of basal leaves at harvest 3 and 4 was associated with an increase in menthyl acetate. Where changes in other components were observed, menthofuran and pulegone tended to be highest in apical leaves, neomenthol tended to be highest in basal leaves, and cineole increased from basal to midstem leaves and decreased from midstem to apical leaves. These changes were less consistent and less pronounced than changes in menthol and menthone, and are only suggested as general trends.

From the results presented it is possible to follow the change in oil composition within equivalent leaves with time. Consistently menthol increased and menthone decreased with time in all leaves. These changes in oil composition occurred regardless of whether leaves were fully expanded and had reached their maximum oil content, or were rapidly expanding and accumulating oil. Menthyl acetate increased in fully expanded leaves with time and most leaves tended to have higher concentrations of cineole during latter harvests. However, no changes

were as pronounced or as consistent as the described changes in menthol, menthone and menthyl acetate with time.

c. Gland Development

From the observation of numerous leaf series it was apparent that changes in gland development from apical to basal leaves on any individual plant, regardless of growing condition, were similar to changes observed in equivalent leaves with time. The series of micrographs presented were selected to be representative of the above changes. The glandular structures considered are the ten-celled glandular trichomes and the three-celled glandular hairs.

Scanning Electron Micrographs. Glandular hairs were evident during very early leaf development and the appearance of these structures under the scanning electron microscope did not change significantly with leaf development. In contrast, the glandular trichomes appeared slightly later and a pronounced maturation of these glands occurred with time. Mature glandular trichomes will be considered to be those in which secretion of oil into the subcuticular space had taken place to the extent that the outer cuticle appeared fully distended.

Plates IV A 1(a)-(g) are micrographs taken at decreasing height on the plant, respectively. The first discernible leaf pair possessed numerous glandular hairs as well as many immature glandular structures [Plate IV A 1(a)]. At a stage when the leaf was approximately 2-5mm in length the formation of many glandular hairs as well as glandular trichomes had taken place. At this stage glandular hairs appeared to out-number glandular trichomes [Plate IV A 1(b)]. On these small leaves glandular trichomes at all degrees of maturity were evident. That is, both glandular trichomes in which significant filling of the subcuticular space had occurred and those in which cell division was still occurring, were present on leaves 2-5mm in length.

As leaf development continued (from 1-1.5cm to 2-2.5cm in length) both the formation and maturation of glandular trichomes was observed [Plates IV A 1(c) and (d)]. By the time leaves had reached 3-4cm in length, all glandular trichome formation was completed and the majority of these glands appeared mature [Plate IV A 1(e)]. When fully expanded leaves were examined, only mature glandular trichomes and glandular hairs (having the same appearance as those present on very young leaves) were observed [Plate IV A 1(f)]. The only noteworthy change in gland appearance with increasing age following the fully expanded leaf stage, was an increase in the number of seemingly 'broken' trichomes on senescing leaves [Plate IV A 1(g)]. These trichomes had lost their 'subcuticular gland cap' and only the eight secretory cells remained. No partially filled trichomes were evident on fully expanded leaves.

The variation in stage of maturity of both glandular trichomes and hairs during early leaf expansion are illustrated in Plates IV A 1(h) and (i). In contrast to the variation in maturity of trichomes on young expanding leaves, all trichomes appeared mature on fully expanded leaves [Plate IV A 1 (j)].

Light Micrographs. The light micrographs presented in Plates IV A 1 (k)-(o) were selected to represent changes in gland development observed on leaves of increasing age. Observations based on both scanning electron micrographs and light micrographs were in general agreement.

In the youngest discernible leaves, epidermal cells were observed to differentiate into immature glandular structures [Plate IV A 1(k)]. By the time leaves were 2-5mm in length, glandular trichomes at all degrees of maturity were evident [Plates IV A 1(l)-(n)]. Oil accumulation in both glandular trichomes and hairs appeared to be associated with increased age of these structures. Although difficulty

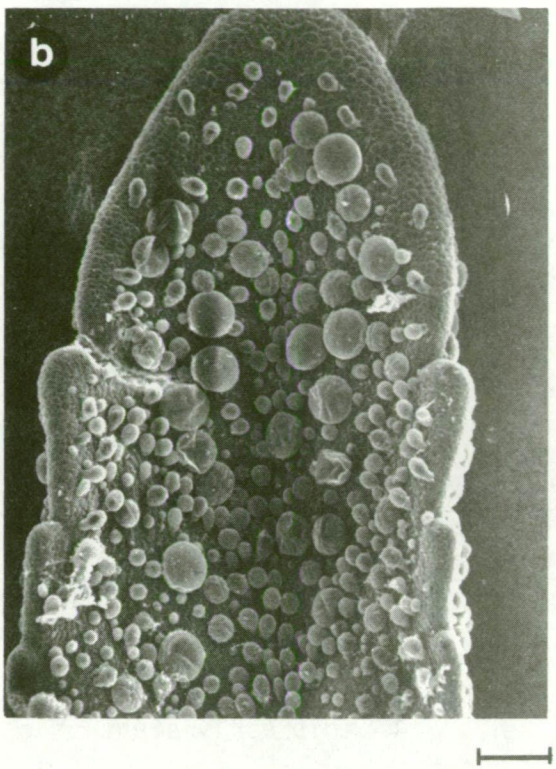
Plate IV A 1(a). Scanning electron micrograph (S.E.M.) of the growing tip of peppermint. Numerous well developed glandular hairs are evident at this early stage of development. The collapsed appearance of several glandular hairs was attributed to problems encountered during tissue preparation. (Fixation involved osmium tetroxide without prior fixation in glutaraldehyde.) Bar = 30μm.

Plate IV A 1(b). S.E.M. of leaf tissue, 2-5mm in length. Numerous well developed glandular trichomes are evident at this early stage of development. The smaller glandular structures appeared to be both immature trichomes as well as mature hairs. (Fixation in both glutaraldehyde and osmium tetroxide.) Bar = 100μm.

Plate IV A 1(c). SEM of leaf tissue, 1-1.5cm in length. At this stage of leaf development the formation of new glandular trichomes and the filling of existing trichomes with oil was observed. (Fixation in both glutaraldehyde and osmium tetroxide.) Bar = 100μm.

Plate IV A 1(d). SEM of leaf tissue, 2-2.5cm in length. The formation of new glandular trichomes appeared to have ceased, many mature and immature trichomes are evident. (Fixation in glutaraldehyde and osmium tetroxide.) Bar = 100μm.


a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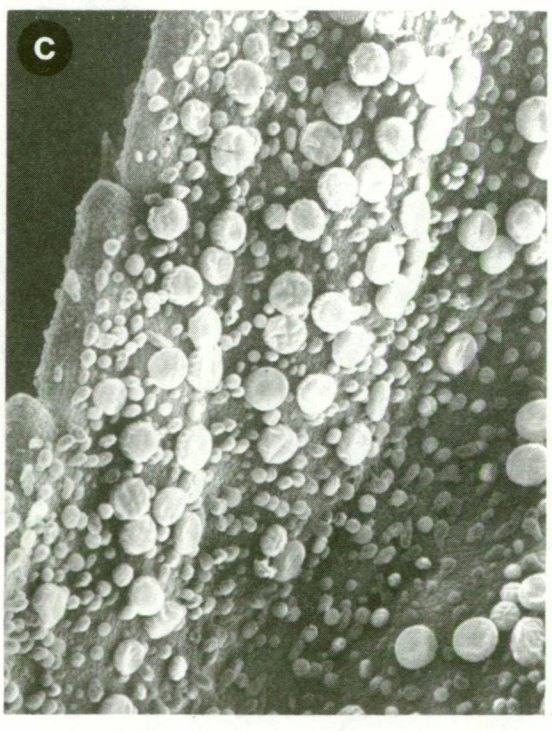
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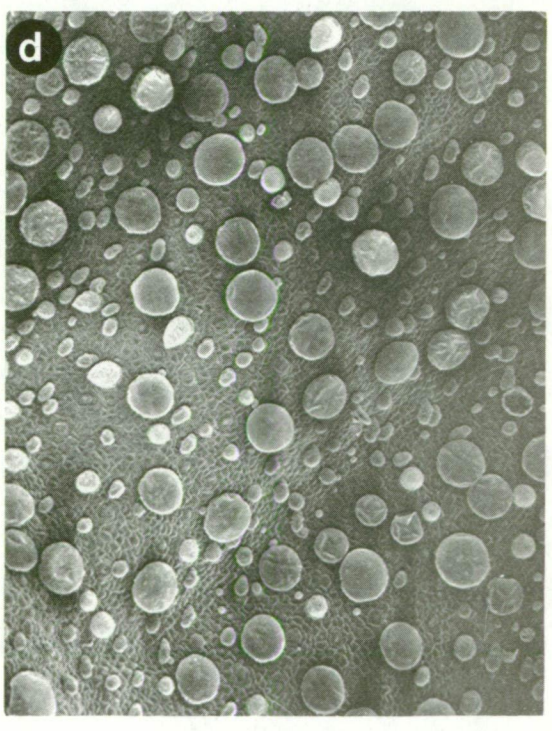
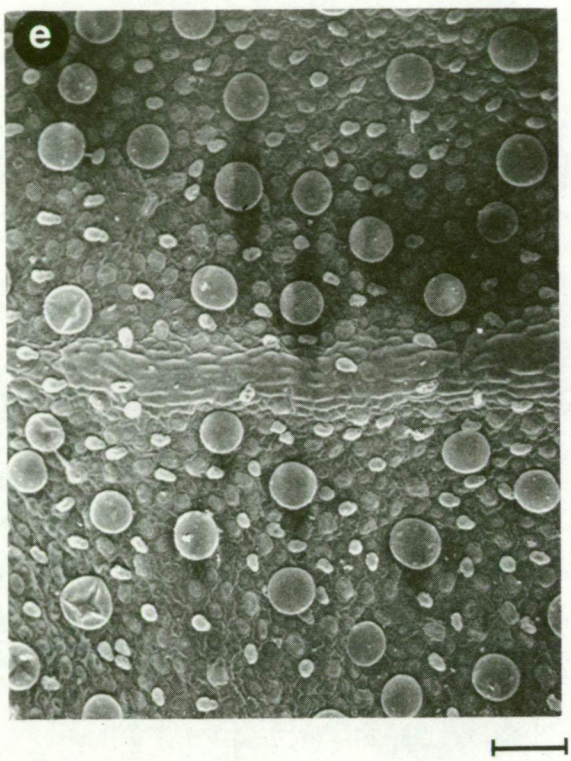

d

Plate IV A 1(e). SEM of leaf tissue, 3-4cm in length. Virtually all trichomes have filled with oil to the extent that the 'glandular caps' are fully distended. (Fixation in glutaraldehyde and osmium tetroxide.) Bar = 100μm.

Plate IV A 1(f). SEM of fully expanded leaf tissue, 4-5mm in length. Without exception, all glandular trichomes appeared to be mature and filled with oil. (Fixation in glutaraldehyde and osmium tetroxide.) Bar = 100μm.

Plate IV A 1(g). SEM of fully expanded leaf tissue (basal senescing leaves). Numerous glandular trichomes appeared to have lost their 'glandular cap'. This damage was only observed on leaves which had commenced to senesce and although the possibility of damage during tissue preparation cannot be discounted, the occurrence of such damaged glands was quite widespread and confined to this leaf tissue. Bar = 20μm.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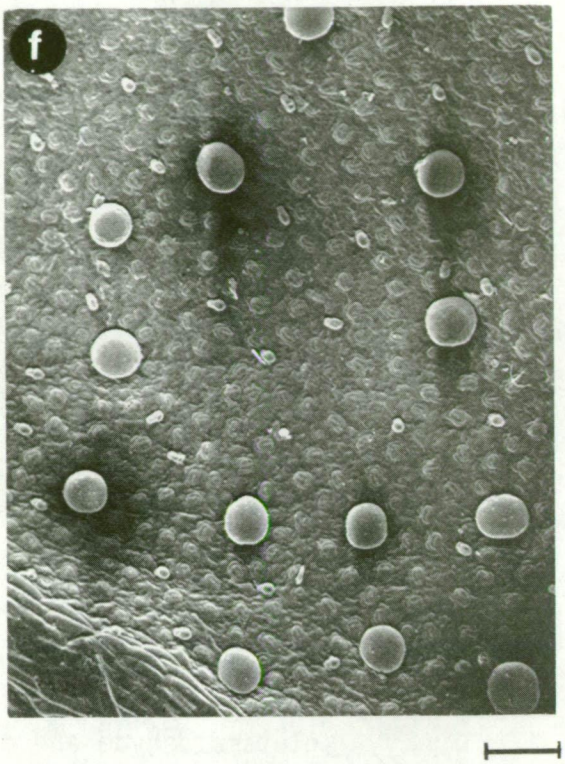

f

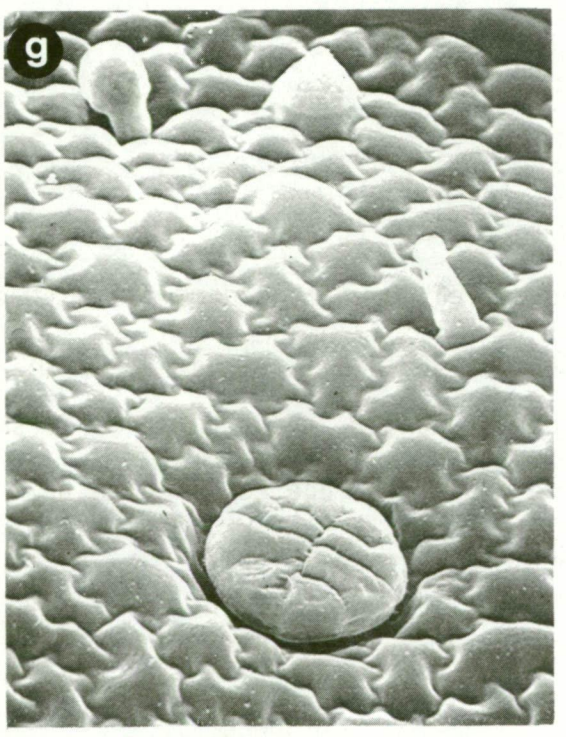

g

Plate IV A 1(h). SEM of leaf tissue, 1.0-1.5cm in length. Glandular trichomes at all stages of maturity are apparent. (Fixation in glutaraldehyde and osmium tetroxide.) Bar = 30μm.

Plate IV A 1(i). SEM of leaf tissue, 2-5mm in length. Glandular hairs appeared to be well developed at this early stage of leaf development. An immature glandular trichome is evident in the centre of the micrograph. (Fixation in glutaraldehyde.) Bar = 20μm.

Plate IV A 1(j). SEM of fully expanded leaf tissue. All glandular hairs and glandular trichomes appeared to be fully developed. (Fixation in glutaraldehyde and osmium tetroxide.) Bar = 10μm.


h


i

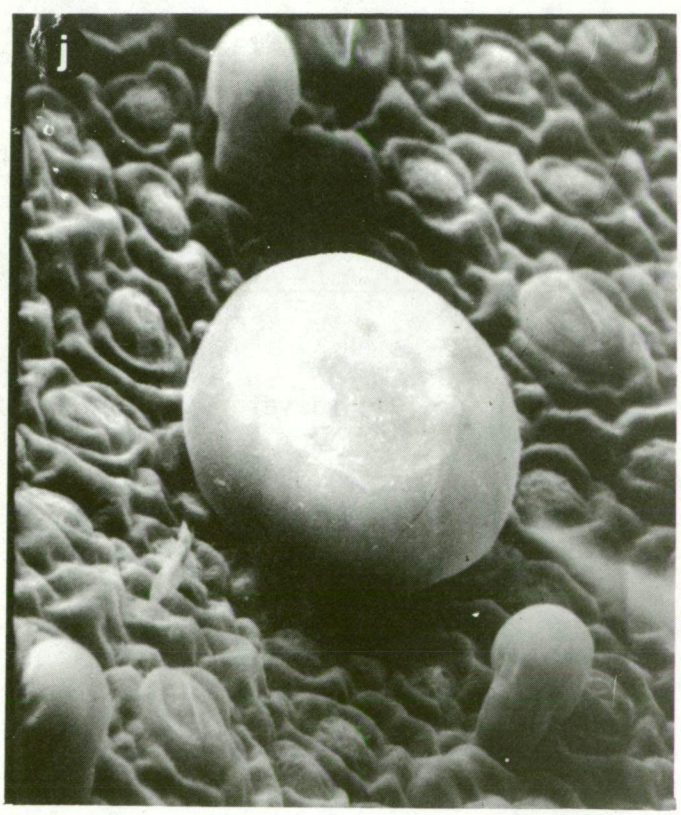
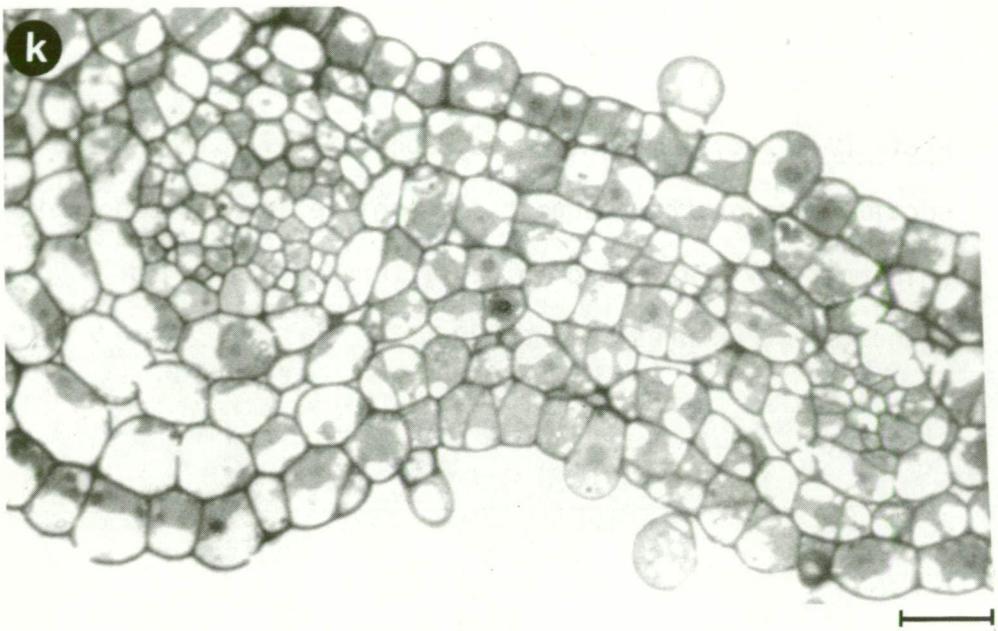

j

Plate IV A 1(k). Light micrograph (LM) of a transverse section through the youngest discernible leaf of peppermint. Epidermal cells appeared to be differentiating into glandular structures. Bar = 20μm.

Plate IV A 1(l). LM of leaf tissue, 2-5mm in length. Glandular trichome, prior to accumulation of significant amounts of oil (R.H.S.) and during early development of the glandular secretory space (L.H.S.). Bar = 20μm.

Plate IV A 1(m). LM of leaf tissue, 1.5-2.0cm in length. Glandular trichomes with well developed secretory spaces, as well as very immature trichomes (top left), were observed on these young leaves. Early stages of glandular hair development are evident at this stage (bottom right). Bar = 20μm.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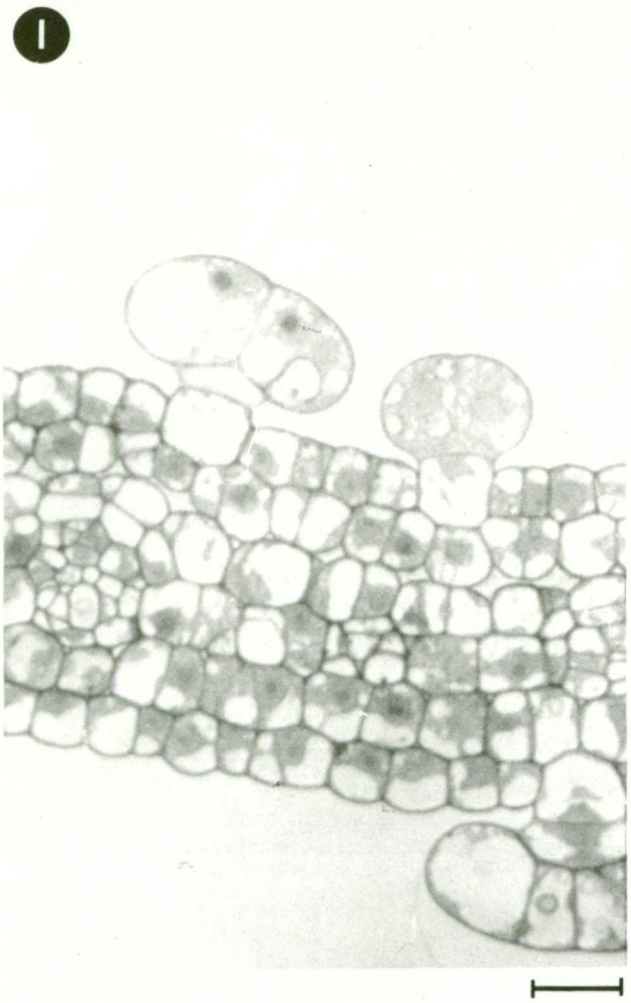
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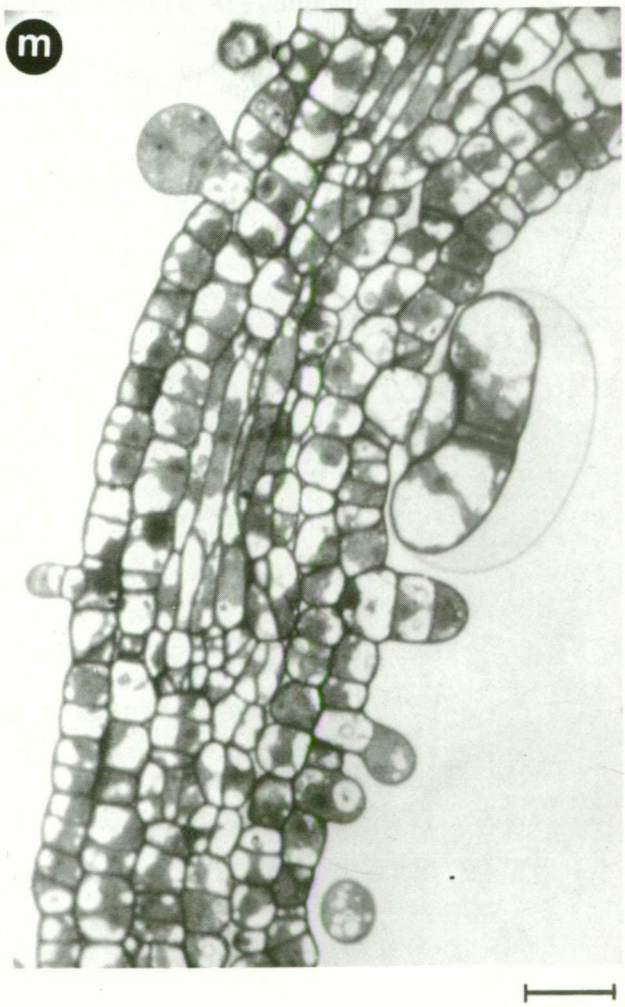
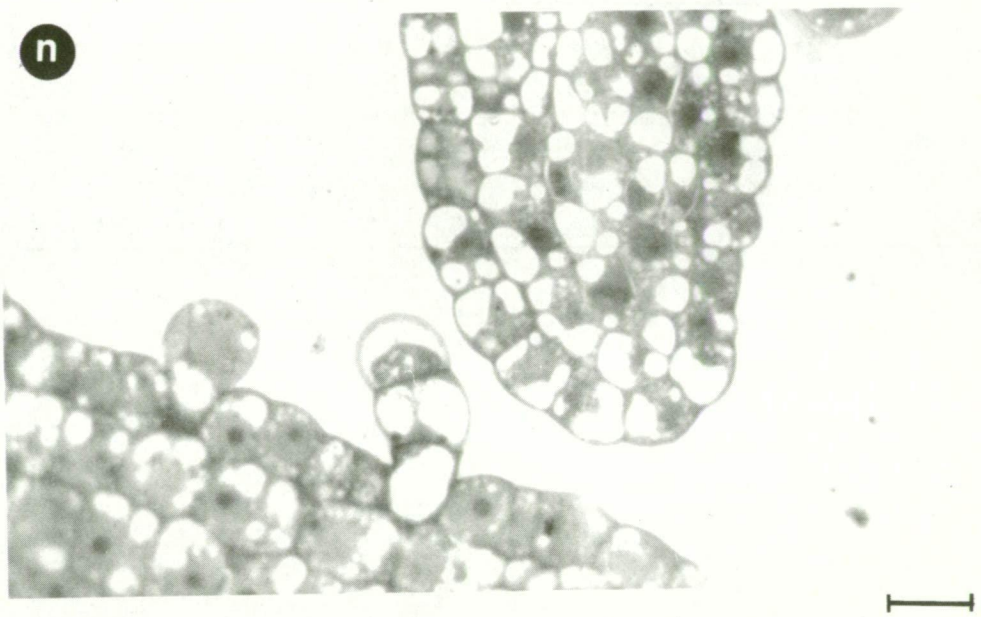
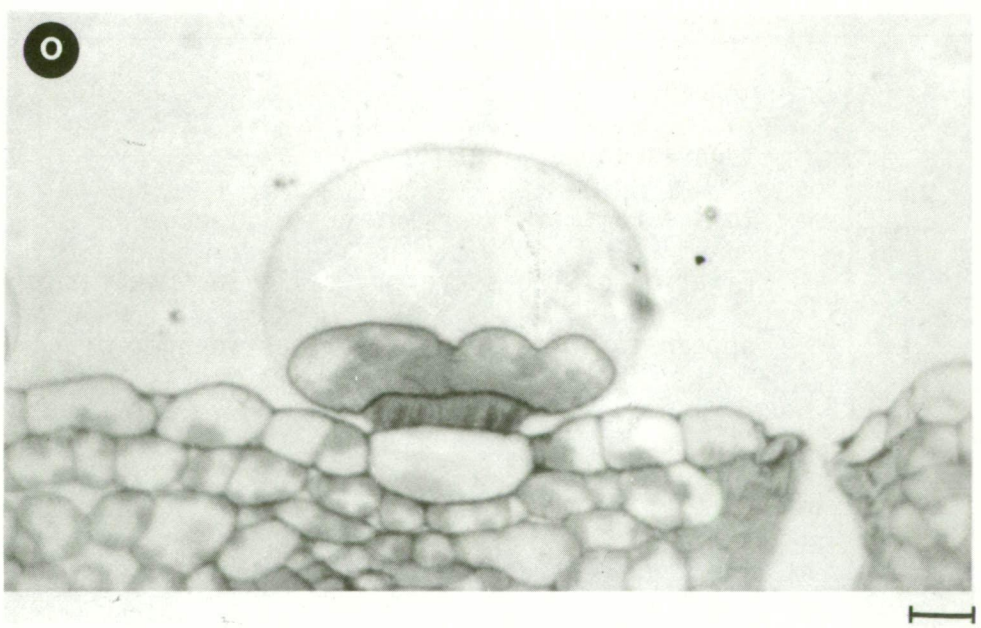

m

Plate IV A 1(n). LM of glandular hair (centre) on the same leave tissue as Plate IV A 1(m), showing increased development of the glandular secretory space. Bar = 20μm.

Plate IV A 1(o). LM of mature glandular trichome on a fully expanded leaf. Great difficulty was experienced in obtaining transverse sections through mature glands on these leaves due to the relatively low number of glands per unit area. Fixation of the secretory cells in such mature glands did not appear to be as satisfactory as younger glands. Bar = 10μm.


n


o

was experienced both in obtaining well preserved glandular trichomes on fully expanded leaves and in locating such glands during the sectioning procedure, the general appearance of all glands sectioned is illustrated in Plate IV A 1(o).

1.4 Discussion

At all stages of plant growth, basal and apical leaves contained less oil than midstem leaves, under both growing conditions. This observation may suggest that losses of oil occurred with time, below the midstem leaf position. For example, the relative oil yield decreased from approximately 40 to 2 from leaf pair 8 to 2 at harvest 3, from LD x LNT conditions. However, from the periodic analyses of equivalent leaves it was apparent that although a small decrease in oil yield occurred from basal leaves, such leaves did not at any stage accumulate oil to the same extent as midstem leaves. The lower yield of oil obtained from apical leaves relative to midstem leaves at any harvest date was due to the fact that these apical leaves were still accumulating oil, and the maximum amount of oil in these leaves had not been reached.

Burbott and Loomis (1969) reported that leaves expanding during the period in which cuttings were forming roots, synthesised but did not accumulate oil. This situation may have existed in leaf pairs 2 and 4. If these basal leaves had the same potential to synthesise oil as midstem and apical leaves (similar number of glands per leaf) and accumulation of oil did not occur because of the unfavourable conditions in the plant at the time these leaves were expanding, it would be expected that numerous partially filled glands should be evident on these leaves. In contrast, all glands observed on these leaves, with the exception of ruptured glands on senescing leaves,

appeared mature and 'full of oil'. This may suggest that the lower yield of oil obtained from basal leaves was due to fewer glands on these leaves. The smaller number of glands may have resulted from the conditions which existed in the plant during the period in which gland differentiation occurred. The other possibility is that the observation of glands by SEM may not have detected the decreased extent of fill, in these glands. That is, a large decrease in oil content may be necessary before the gland cuticle loses its fully distended appearance. Lemli (1963) reported that very young and old leaves had the smallest number of glands.

In agreement with the work of Burbott and Loomis (1969), the maximum accumulation of oil in midstem leaves from the LD x LNT condition did coincide with the approximate time inflorescences were observed macroscopically. Unlike the very rapid increase in oil yield from midstem leaves, observed to precede inflorescence appearance (Burbott and Loomis, 1969), under the present experimental conditions, the increase in oil yield tended to be more gradual and was associated with leaf expansion and gland filling. That is, the period of maximum oil yield in midstem leaves may have been associated with the fully expanded leaf stage, which in turn happened to occur at the time of inflorescence appearance. In addition, no rapid increase in gland size on midstem leaves was observed prior to the appearance of inflorescences. Although Burbott and Loomis (1969) did not mention such an increase, the storage capacity of glands in their study must have increased very rapidly to accommodate the observed peak in essential oil yield. Furthermore, the decrease in oil yield from basal leaves appeared to occur after the fully expanded leaf stage was reached, rather than following the appearance of inflorescences. This decrease in oil yield was much more gradual than observed by Burbott and Loomis (1969). As mentioned previously, no glands were observed on

basal leaves, that would suggest metabolic depletion of oil from these structures. Burbott and Loomis (1969) suggested that the metabolic turnover of oil in glands after inflorescence appearance may be associated with these long day, cool night temperature plants, losing their "energy-rich status" due to the increased demands placed on the plant during flowering. Given that the present experiment was conducted under higher light intensity conditions relative to the experiment of Burbott and Loomis (1969), it could be suggested that the increased availability of photosynthate arising under the higher light intensity conditions decreased the extent of oil metabolism necessary to supply the increased requirements for energy during flowering.

In the SD x HNT conditions, oil yield per leaf was generally lower than resulted from LD x LNT conditions. Croteau *et al.* (1972b) reported that storage or accumulation of oil was favoured by an abundance of photosynthate within the plant. Similarly, Burbott and Loomis (1967) suggested that an abundance of photosynthate favoured the reduction of pulegone to menthone as opposed to oxidation of pulegone to menthofuran. Therefore, if the availability of photosynthate was limiting in SD x HNT plants compared with LD x LNT plants, the rapid turnover of monoterpenes may have occurred at the expense of accumulation. Such a shortage of photosynthate was not reflected in the composition of oil extracted from these plants. The SD x HNT and LD x LNT plants both accumulated menthone, menthol and menthyl acetate rather than pulegone and menthofuran. Therefore, either the availability of photosynthate was not the important factor accounting for the lower accumulation of oil in the SD x HNT plants, or the accumulation and turnover of oil components was more sensitive to the availability of photosynthate than processes involved with the interconversion of oil components. Langston and Leopold (1954) reported that the number of oil glands per

unit leaf area was influenced by daylength. Long days resulted in increased numbers of glands. Therefore, in addition to the photosynthate effect on oil accumulation, a photoperiodic effect may operate by controlling the number of glands per unit leaf area.

With respect to the changes in gland development reported by Ameluxen (1964, 1965), it was suggested that the degeneration of structure in the gland cells and the filling of glands with oil occurred while the leaves were very young and had hardly started to expand. In the glandular hairs these changes were reported to be completed by the time the leaf was 1.0 to 1.5mm in length, and in the trichomes by the time the leaf was 4 to 5mm in length (cited by Loomis, 1967). From micrographs of glands obtained in the present study, it is apparent that the observations made by Ameluxen (1964, 1965) are somewhat misleading. That is, although trichomes do exist at a very early stage of leaf development (2-5mm), few are filled with oil and only a small proportion of the final number of trichomes are actually present at this stage. Therefore, although the observations of Ameluxen (1964, 1965) may well have been characteristic of a particular trichome on the 4-5mm leaf, such observations would certainly not appear to be representative of all glands on the leaf. The results obtained in the present work are in agreement with observations made by Lemli (1963). This worker observed that all glands required 2-3 weeks to fill with oil, after their formation. Furthermore, Lemli (1963) considered that the maximum capacity of glandular trichomes occurred 4-6 weeks after leaf formation, at a stage when leaf expansion had ceased. The observed non-uniformity in gland maturity on young leaves (1-2cm) suggested that synthesis of oil continued long after the stage at which Ameluxen (1964, 1965) observed individual trichomes to mature. This may explain the previous apparent disagreement in results obtained from $^{14}CO_2$ tracer studies, periodic

analyses of leaves, with results obtained by Ameluxen (1964, 1965).

If the overall changes in oil composition, oil yield and gland development are considered, several implications arise with respect to the metabolism and interconversion of oil components. Firstly, with respect to the observed decrease of oil yield in basal leaves (LD x LNT), several possible mechanisms may be proposed. Croteau and Martinkus (1979) observed rapid metabolism of menthone to glucosides (i.e. (+)-neo-menthyl glucoside) in midstem leaves of flowering peppermint. Although metabolism of menthone may have accounted for a portion of the yield reduction, it is unlikely that this mechanism alone accounted for the decrease. In basal leaves (e.g. leaf pair 2, LD x LNT), the percentage menthone in extracted oil was generally very low, even at harvest 1. In this leaf pair menthone decreased from 17 to 7 percent, during the period when an almost 50 percent reduction in oil yield was observed. Furthermore, the increase in menthol and menthyl acetate could have accounted for this 10 percent reduction in the percentage menthone. Alternatively, it could also be suggested that menthol (the major component of mature leaf oil) was converted to menthone which then metabolised to glucosides,or menthol may be envisaged as undergoing turnover in its own right. If the decrease in oil yield resulted from the metabolism of one or more of the major components, then it follows that unless a rapid dynamic equilibrium existed between all measured components, a large depletion of menthol or menthone would result not only in a decrease in oil yield but also a significant increase in the percentage composition of several other components (e.g. α- and β-pinene). Significant increases in these components was not associated with the decrease in oil yield.

Evaporation of oil from glands may also be suggested as the cause of the decrease in oil yield. If evaporation was the factor responsible for

the decreased yield from basal leaves with time (LD x LNT), then this would also be expected to have consequences with respect to oil composition. The highly volatile components such as α- and β-pinene would be expected to evaporate at a faster rate than the less volatile components such as menthol. As mentioned previously, there were no overall changes in the percentage α- and β-pinene in the oil extracted during the period of decreasing yield.

Since no metabolically depleted glands were observed and because of the above compositional considerations, it could be suggested that the decrease in oil yield resulted from the loss of complete units of oil (i.e. glands). Certainly, ruptured oil glands were evident on senescing leaves such as leaf pair 2 and 4 at harvest 4. However, if this mechanism is proposed, then it would be expected that leaves from SD x HNT plants would have behaved in a similar manner. This was not observed to be the case and no obvious explanation exists to account for this inconsistency. Therefore, it does not appear that any one of the individual avenues of oil loss are in agreement with the observed changes in oil composition, yield and gland development. It is possible that several of the above mechanisms were involved to varying extents in the observed decrease in oil yield in basal leaves.

Although oil accumulation in peppermint leaves was associated with leaf expansion and gland filling, interconversions of oil components (e.g. menthone → menthol → menthyl acetate) continued long after the leaf had reached the fully expanded stage. From light micrographs of glands on expanded leaves it appeared that at this stage of leaf development the major portion of oil was stored in the secretory space of glandular trichomes [Plate IV A 1(p)]. In agreement with the results of the present work, numerous workers have reported interconversions in oil from fully expanded leaves (Battaile and Loomis, 1961). From observations

of glands it would appear that the above interconversions need to take place between oil components existing within the secretory space, if such interconversions are to significantly affect oil composition. Therefore, either the enzyme systems and cofactors which are reported to be necessary for interconversions (e.g. $NADPH_2$), must operate within this secretory space, external to the secretory cells, or interconversions occur following re-absorption of oil into secretory cells. From transmission electron microscopic examination of hop glandular hairs (Menary, *pers. comm.**),it would appear that within the secretory space oil droplets are surrounded by an aqueous medium. If this system is common to peppermint glands, then the necessary enzyme systems and cofactors may operate within this aqueous medium in the secretory space. The release of the required enzymes and cofactors into the aqueous medium of the secretory space would need to be associated with loss of membrane integrity in secretory cells. Such changes in cell membranes may in turn be associated with the observed degeneration of the secretory cells which coincides with the formation of the secretory space. Within this system, the supply of reduced respiratory co-enzymes may present a formidable problem for glands and the provision of these requirements (e.g. $NADPH_2$ or $NADPH_2$-generating systems) from adjacent cells may be of utmost importance if interconversions are to proceed within the secretory space.

[*R.C. Menary, University of Tasmania]

2. The Effect of Photoperiod on the Yield and Composition of Peppermint Oil

2.1 Introduction

There are many indications in the literature that peppermint is affected by photoperiodic treatments. However, few workers have studied the effect of photoperiod on the monoterpene composition of peppermint oil. With respect to the existence of a true photoperiodic effect on the monoterpene composition of peppermint oil, there appears to be an apparent disagreement between the findings of Burbott and Loomis (1967) and Grahle and Holtzel (1963).

The aim of this study was to examine the influence of photoperiodic treatments on the yield and composition of the monoterpenes of peppermint oil.

2.2 Materials and Methods

Two experiments were designed to investigate the effect of photoperiod. The only difference in the two experiments was in the nature of the photoperiodic treatments imposed. In all other respects the two experiments were identical and will therefore be discussed together, in the following report.

a. Plant Material

Cuttings of *Mentha piperita* L. were propagated vegetatively from plants growing under a 14 hour photoperiod in the glasshouse. Cuttings consisted of short sections (5cm) of underground stem material that were rooted in sand and peat mix (50:50). Propagating material taken from the 14 hour photoperiod plants was rooted under the photoperiodic treatment conditions to be used in the experiments. These cuttings were transplanted into pots in the growth rooms when the plants were approximately 3cm tall and after they had produced three pairs of leaves.

b. Growing Conditions

All experiments were conducted in two identical growth rooms, each 1.5m x 4m in size, lined with aluminium foil and fitted with air conditioners. The specific details of these growth rooms has been described previously (Section III.8).

c. Sampling and Oil Extraction

Plants were harvested at ground level, dried in the glasshouse for 1 day, steam distilled and yield components and composition determined.

d. Treatments

This experimental work consisted of two photoperiodic treatments per experiment; a short photoperiodic and a long photoperiodic treatment.

Experiment 1

The short photoperiodic treatment involved 13 hours of light per day (13H) and the long photoperiodic treatment involved 12 hours of light per day followed by a 1 hour light break in the middle of the dark period (13I). Light intensity employed during the light break was identical to that used during either the 12 or 13 hour day.

Both treatments were harvested after 62 days in the growth rooms.

Experiment 2

The short photoperiodic treatment involved 12 hours of light per day (12H) and the long photoperiodic treatment involved 12 hours light per day with a 15 minute light flash in the middle of the night period (12I). The light intensity employed during the light flash was sufficient for photoperiodic effects but not sufficient for photosynthesis (i.e. 2 x 60 watt incandescent lamps). Both treatments were harvested after 79 days in the growth rooms.

In both experiments the temperature was maintained constant at 20°C (±1°C), and the relative humidity at approximately 50 percent during the day and night.

e. Experimental Design

Each photoperiodic treatment in both experiments consisted of three replications with ten plants in each replicate.

2.3 Results

Generally, the long photoperiodic treatment (13I) and the short photoperiodic treatment (13H) in experiment 1 behaved in a similar manner as the long photoperiodic treatment (12I) and the short photoperiodic treatment (12H) in experiment 2, respectively. Therefore, both experiments will be discussed together.

The growth habit of plants receiving 13I and 13H photoperiods is shown in Plate IV A 2.1. Plants grown under a 13I or 12I inductive photoperiod were erect and formed inflorescences during the course of the experiment. In contrast, growth under a 13H or 12H non-inductive photoperiod was poor, with plants being recumbent with many stolons and few erect stems.

The mean dry matter yield per plant, yield of oil per plant and percentage oil yield are listed in Table IV A 2.1 and 2.2 and from these results it appeared that plants grown under long photoperiodic (13I and 12I) conditions had significantly higher dry matter, oil and percentage oil yields, relative to that produced under short photoperiodic (13H and 12H) conditions. Both an increase in percentage oil yield and an increase in dry matter production per plant appeared to contribute to the increase in oil yield per plant.

Plate IV A 2.1. Peppermint (*Mentha piperita* L.) grown under two photoperiodic treatments:

12 + 1 (13I) indicates 12 hours light per day, plus 1 hour of light in the middle of the dark period;

13 (13H) indicates 13 hours light per day.

(Both plants were harvested after 62 days in the growth rooms.)

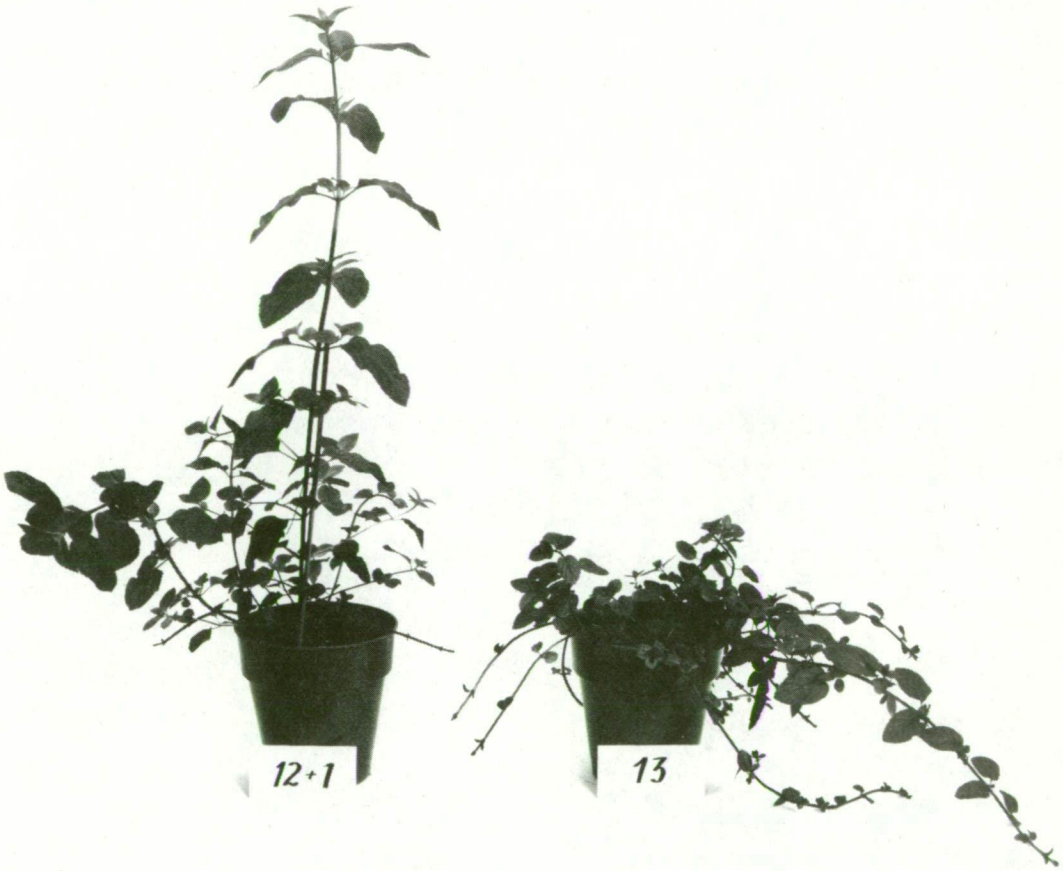

12+1
13

Table IV A 2.1. The effect of photoperiod on dry matter, oil and percentage oil yield; Experiment 1.

Mean values from 3 reps.	Photoperiodic Treatments		Variance ratio
	13I	13H	
Dry herb yield (g/plant)	4.32	2.16	27.87**
Oil Yield (mg/plant)	76.94	27.32	207.8***
% Yield (Dry Matter Basis)	1.78	1.26	11.12*

Table IV A 2.2. The effect of photoperiod on dry matter, oil and percentage oil yield; Experiment 2.

Mean values from 3 reps.	Photoperiodic Treatments		Variance ratio
	12I	12H	
Dry herb yield (g/plant)	3.94	2.08	166.29***
Oil yield (mg/plant)	72.54	24.16	689.21***
% Yield (Dry Matter Basis)	1.84	1.17	137.82***

Significance at 5% (*); 1% (**); or 0.1% (***) level.

The influence of photoperiod on oil composition is illustrated in Figures IV A 2.1 and 2.2. The mean value for percentage of total peak area represented by the major compounds is listed in Table IV A 2.3 and 2.4. The twelve compounds selected represent approximately 97% of the total peak area and no other compounds were observed to vary with photoperiod. From these results it appeared that the photoperiodic treatments imposed had several effects on oil composition. The most significant of these changes in oil composition was the increase in menthofuran, limonene, menthyl acetate and pulegone and decrease in the amount of cineole, menthone and menthol, in short photoperiodic treatments (13H and 12H), relative to long photoperiodic treatments (13I and 12I). Other changes in oil composition were decreases in β-pinene (and α-pinene in Experiment 2), trans-sabinene hydrate, neomenthol (+ unknown) and the unknown (peak 12) in treatments 13H and 12H, relative to 13I and 12I.

2.4 Discussion

Photoperiod clearly has an effect on vegetative growth and flowering in *Mentha piperita* L., both being promoted by long days or interrupted nights. This observation is in agreement with several other reports (Allard, 1941; Langston and Leopold, 1954; Reitsema, 1958; Burbott and Loomis, 1967).

The amount of essential oil accumulated in plants receiving a 13H or 12H non-inductive photoperiod was approximately one third that found in the plants exposed to a 13I or 12I inductive photoperiod.

Burbott and Loomis (1967) stated that photoperiod as such, does not directly influence the monoterpene composition of peppermint. These results were obtained using interrupted night and low light intensity

Figure IV A 2.1. Gas chromatogram of peppermint oil extracted from plants growing under long photoperiods provided by treatment 13I.

Figure IV A 2.2. Gas chromatogram of peppermint oil extracted from plants growing under short photoperiods provided by treatment 13H.

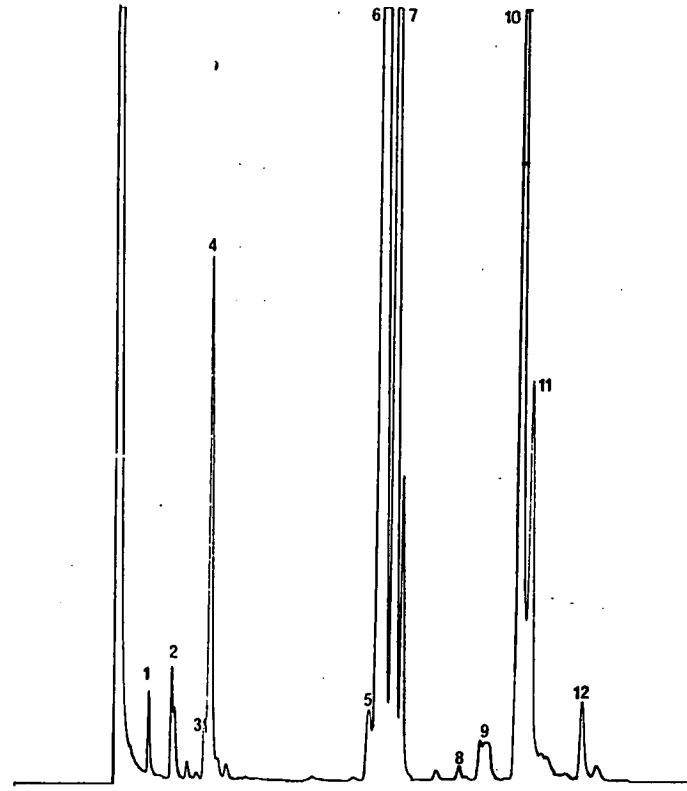
Key to Peaks on Gas Chromatograms

1. α-Pinene
2. β-Pinene
3. Limonene
4. Cineole
*5. Trans-sabinene Hydrate
6. Menthone
7. Menthofuran
8. Menthyl Acetate
**9. Neomenthol (+ unknown)
10. Menthol
11. Pulegone
12. Unknown

*The identity of this peak was not confirmed by GC-MS.

**On the basis of comparative retention times, this peak was first attributed to neoisomenthyl acetate but subsequent GC-MS results suggested that this peak was neomenthol or a closely related isomer of menthol.

2.1



2.2

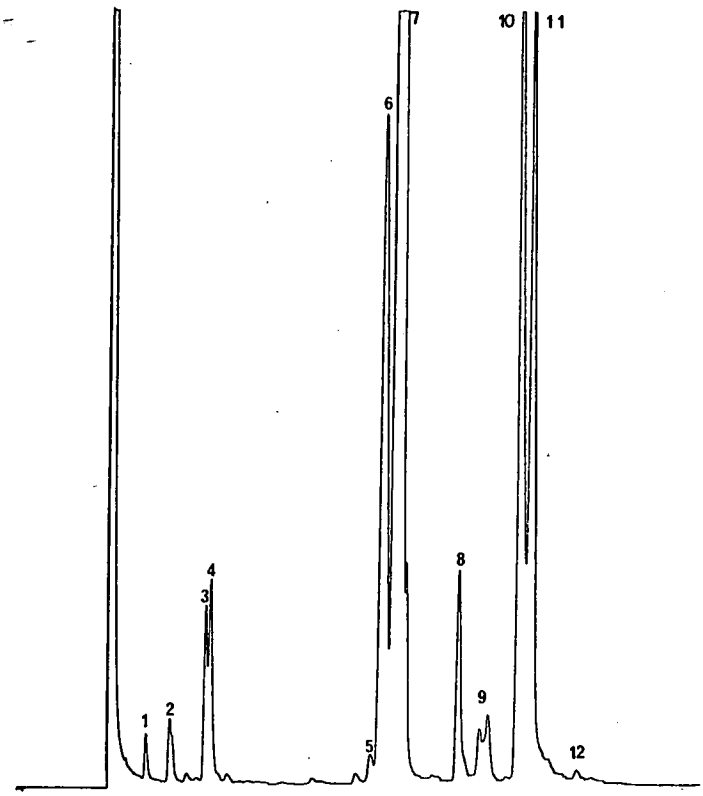


Table IV A 2.3. The effect of photoperiod on oil composition in peppermint; Experiment 1.

Peak No.	Compound	Photoperiodic Treatments (% total peak area)		Variance ratio
		13I	13H	
1	α-Pinene	0.703	0.404	4.788 ns
2	β-Pinene	1.568	0.770	15.061*
3	Limonene	0.541	1.612	183.844***
4	Cineole	6.371	0.877	228.500***
5	Trans-Sabinene Hydrate	1.325	0.487	24.877**
6	Menthone	43.77	8.135	631.55***
7	Menthofuran	21.098	64.340	884.60***
8	Menthyl Acetate	0.356	2.144	11752.05***
9	Neomenthol (+ Unknown)	2.077	1.360	9.059*
10	Menthol	13.869	9.545	86.86***
11	Pulegone	7.075	10.146	79.804***
12	Unknown	1.268	0.241	64.497**

Table IV A 2.4. The effect of photoperiod on oil composition in peppermint; Experiment 2.

Peak No.	Compound	Photoperiodic Treatments (% total peak area)		Variance ratio
		12I	12H	
1	α-Pinene	0.673	0.390	83.770***
2	β-Pinene	1.365	0.824	59.281**
3	Limonene	0.643	1.373	84.437***
4	Cineole	6.104	1.260	671.339***
5	Trans-Sabinene Hydrate	1.173	0.602	45.606**
6	Menthone	41.511	8.408	1469.886***
7	Menthofuran	23.871	64.907	1868.087***
8	Menthyl Acetate	0.413	2.030	323.689***
9	Neomenthol (+ Unknown)	1.673	0.933	11.707*
10	Menthol	13.092	8.759	106.763***
11	Pulegone	8.140	10.176	62.988**
12	Unknown	1.347	0.338	349.576***

Significance at 5% (*); 1% (**); and 0.1% (***); ns = not significant.

studies. The present study does not support the claim that photoperiod as such has no direct influence on monoterpene composition. In contrast, it would appear that photoperiodic treatments imposed in the present work had a profound influence on the monoterpene composition of peppermint.

The results obtained agree with the work of Grahle and Holtzel (1963) who reported that the proportions of individual monoterpenes in peppermint oil were strongly influenced by daylength. A possible criticism of the technique used by Grahle and Holtzel (1963) is that these workers did not completely separate the effects due to photoperiod from those due to photosynthesis. That is, the extra hour of light introduced into the middle of the dark period, increased the time available for photosynthesis by one hour. In experiment 1 an attempt was made to overcome this criticism and the total available time for photosynthesis was equal in both short and long photoperiodic treatments (13 hours). However, the photoperiodic effect on monoterpene composition was still evident. Secondly, in experiment 2, both the duration and distribution of the photosynthetic period within a 24 hour cycle were constant in both photoperiodic treatments. In experiment 1 it could be argued that the effect of treatments on oil composition resulted from the difference in distribution of the photosynthetic period within the 24 hour cycle. That is, if photosynthesis was subject to diurnal fluctuation, the 1 hour of light placed in the middle of the dark period may have been more effective, with respect to net CO_2 fixation, than adding 1 hour to the 12 hour light period. This possible criticism of experiment 1 was investigated by including experiment 2. The results of experiment 2 confirmed the existence of a true photoperiodic effect on monoterpene composition.

In addition to the reported change in proportions of compounds such as menthofuran, menthone and menthol (Grahle and Holtzel, 1963), the

present work indicated that several other compounds were significantly altered by the photoperiodic treatments. For example, the large change in the ratio of limonene to cineole with changes in photoperiod, is previously unreported. Smith and Levi (1961) considered a ratio of 0.2-0.7 characteristic of *Mentha piperita* L. From their observations, these workers suggested that this ratio was genetically controlled and could offer a means of identifying authentic oils. The wide variation in this ratio obtained in the present work, suggested a strong influence of environmental effects on the concentration of limonene and cineole.

The differences in oil composition which resulted from the imposed treatments, in general follows previously reported trends (Grahle and Holtzel,1963; Burbott and Loomis, 1967). Subjecting plants to long photoperiodic conditions had similar effects as were observed by Burbott and Loomis (1967) when cold nights and long days were employed. These treatments resulted in increased concentrations of menthone and menthol and decreased concentrations of menthofuran and pulegone. Such changes are in agreement with the scheme of reductive monoterpene interconversions proposed by Reitsema (1958). That is, interconversions proceed via pulegone either to menthofuran or to menthone and menthol. However, the biochemical relationships proposed by Reitsema (1958) do not explain how conditions which favoured the accumulation of menthofuran also favoured accumulation of menthyl acetate.

3. The Effect of Daylength, Light Intensity, Night Temperature and Day Temperature on the Yield and Composition of Peppermint Oil

3.1 Introduction

The aim of this work was to investigate the interacting effects of several environmental factors on the yield and composition of peppermint oil. The only other controlled study of the interaction of environmental factors on monoterpene composition was conducted by Burbott and Loomis (1967). This work resulted in a proposed model to explain the effect of various environmental factors on monoterpene composition. This model is the basis of the present understanding of factors affecting monoterpene composition.

3.2 Materials and Methods

a. Plant Material

Peppermint plants were propagated vegetatively from clonal material. Shoot cuttings were taken from plants growing under the same photoperiodic conditions that were to be used in the experiments. After these cuttings had formed roots (5-7 days), they were transplanted into sand:peat mix (1:1), under the treatment growing conditions.

b. Growing Conditions

All experiments were conducted in the combined glasshouse-growth room system previously described (Section III.8). The plants were subjected to glasshouse light intensities and day temperatures unless otherwise stated.

c. Treatments

Two experiments were conducted to investigate the effect of certain environmental factors on the yield and composition of peppermint oil.

Experiment 1. Analysis of Oil Components at Five Stages of Growth

On 1 August 1977 visually matched plants were transferred into two sets of growing conditions:

LD x LNT : long days (16 : 8) and low night temperature (10±2°C)

SD x HNT : short days (8 : 16) and high night temperature (18±2°C)

Glasshouse day temperatures were 20±3°C and light intensities were 900-1200$\mu\Sigma m^{-2}s^{-1}$, throughout the experimental period. At the time plants were transferred to the above growing conditions, the lowest leaf pair on each plant was marked (white paint) and all subsequent leaf numbering was related to this leaf pair. Three plants were harvested from each treatment at 10 day intervals, throughout the growing period. A total of five harvests were made and on each occasion leaf pairs were solvent extracted and the composition of oil determined. At the end of the experimental period (22 September 1977), plants from both treatments were harvested, steam distilled and oil composition determined.

[Note: This experiment was initially designed to investigate oil accumulation, gland morphology and the effect of growing conditions on these processes. The results reported in this section are presented in more detail in Section IV A 1, where oil accumulation and gland morphology, etc., were considered.]

Experiment 2. Interaction Between Environmental Factors

This consisted of a 2 x 2 x 3 factorial experiment with three replications and eight plants per replication. The experiment was conducted in the same glasshouse-growth room system as above and the treatments were as follows:

Daylength : long days (16 : 8), LD; short days (8 : 16), SD.

Night temperature : low night temperature (10±2°C), LNT;
high night temperature (20±2°C), HNT

Light intensity : 10% of glasshouse light intensity (≈150μΣm^{-2}s^{-1}), L1;
50% of glasshouse light intensity (≈600μΣm^{-2}s^{-1}), L2;
100% of glasshouse light intensity (≈1200μΣm^{-2}s^{-1}), L3.

Light intensities were reduced in treatments L1 and L2 with Sarlon shadecloth. Glasshouse day temperatures were 26±3°C throughout the experimental period. The experiment was commenced on 28 November 1977 and harvesting of all treatments was conducted on 3 January 1978.

In experiment 1 individual leaf pairs were solvent extracted and whole plants were steam distilled at the end of the experiment. In experiment 2 all plant material was steam distilled. The determination of yield components and oil composition were in accordance with techniques described in Section III.

3.3 Results

Experiment 1

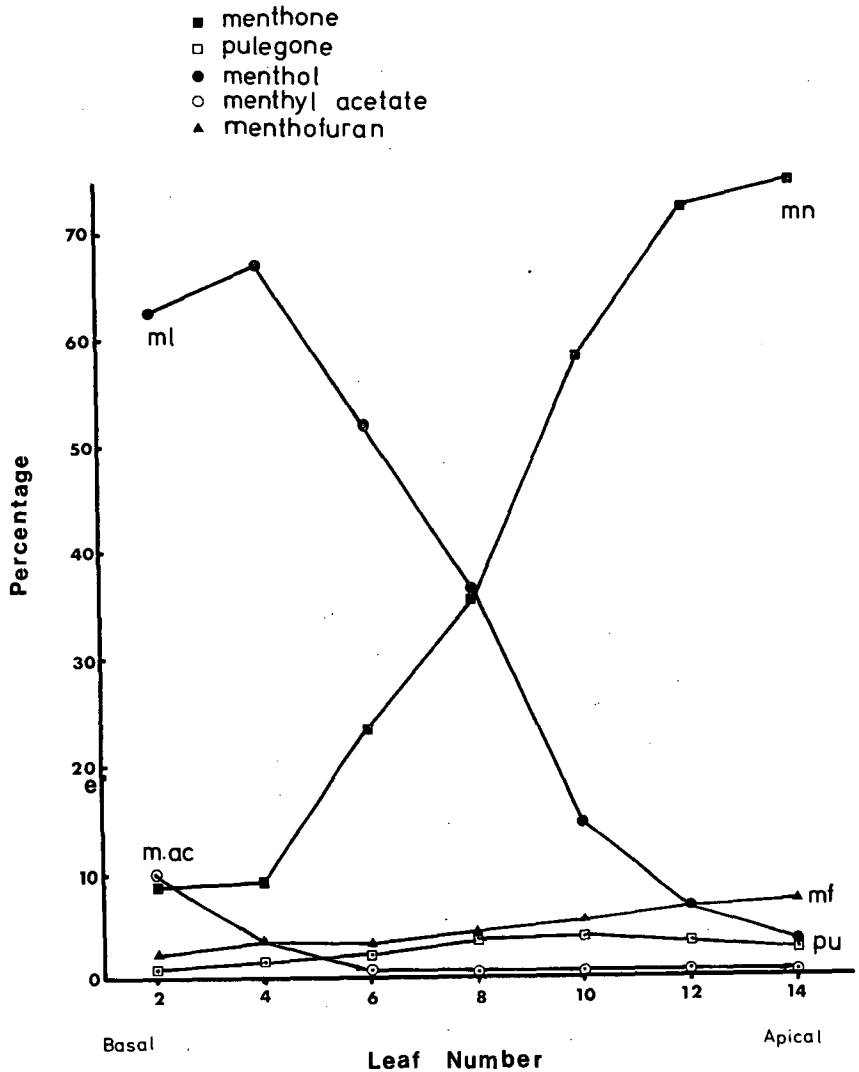
Oil Composition. The results presented are those obtained at harvest 3 (1 September 1977) and are in general agreement with results obtained at harvests 1, 2, 4 and 5 listed in Section IV A 1.

In both LD x LNT and SD x HNT treatments, menthone decreased from the apical to the basal leaf pairs (Figures IV A 3.1 and 3.2). Changes in menthofuran and pulegone were less pronounced with low concentrations occurring in all leaves. Menthone and menthol were the predominant monoterpenes in both LD x LNT and SD x HNT treatments. Subsequent analysis of steam distilled oil from whole plants supported the above finding that oil composition was not affected by the treatment growing conditions (Table IV A 3.1).

Figure IV A 3.1. The percentage of menthone, menthofuran, menthyl acetate, menthol and pulegone in oil extracted from individual pairs of leaves, from plants growing under LD x LNT conditions. (Mean results from 3 plants.)

Figure IV A 3.2. The percentage of menthone, menthofuran, menthyl acetate, menthol and pulegone in oil extracted from individual pairs of leaves, from plants growing under SD x HNT conditions. (Mean results from 3 plants.)

Footnote: The results presented in Figures IV A 3.1 and 3.2 were subjected to statistical analyses and because of the residual correlation between leaves from the one plant, multivariate analysis of variance was used to test for significant leaf treatment interactions. Statistically significant differences in leaf treatment interactions were found for both menthone and menthofuran. Both menthone and menthofuran were significantly lower under SD x HNT conditions but such differences were not considered biologically significant.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mf
pu

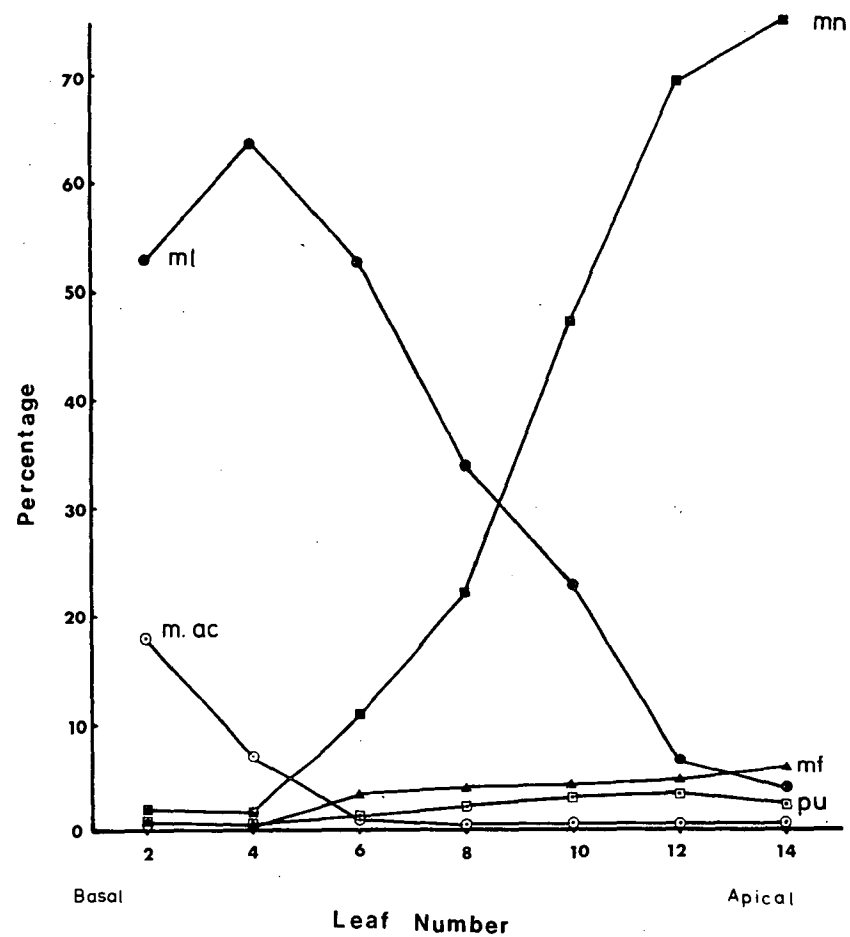

Percentage
Leaf Number
Basal
Apical
ml
mn
m. ac
mf
pu

Table IV A 3.1. Effect of night temperature and daylength on peppermint oil composition.

Compound (%)	Growing condition*		Variance ratio
	LD x LNT	SD x HNT	
Menthone	29.96	29.73	0.081 ns
Menthofuran	4.85	5.06	0.410 ns
Menthyl acetate	1.20	1.23	0.022 ns
Menthol	51.09	50.45	1.854 ns
Pulegone	1.92	1.90	0.038 ns

*Mean values; 3 replications, 5 plants/replication

ns; not significant at 5% level.

Experiment 2

Dry Matter, Oil and Percentage Oil Yield

Dry matter and oil yield increased as light intensity increased from L1 to L3 in all daylength and night temperature treatments (Table IV A 3.2). These increases were most pronounced in LD treatments, resulting in significantly higher dry matter and oil yields from LD treatments at high light intensity (L3). Within daylength treatments, high night temperatures (HNT) favoured highest dry matter and oil yields. The percentage oil yield was highest in plants growing in LD treatments. Night temperature and light intensity had no significant effect on percentage oil yield.

Oil Composition

α- and β-Pinene. Increasing daylength, increased α- and β-pinene at all light intensities, except the lowest light intensity (L1) (Table IV A 3.3 and 3.4). α- and β-pinene were lower under low light intensity (L1) and a significant increase in both components occurred when light intensity was increased from L2 to L3, under long days (LD). Increasing night temperature resulted in an increase in α-pinene under short day (SD) conditions. Night temperature did not significantly affect β-pinene concentrations. Generally, increased daylength and light intensity favoured highest concentrations of α- and β-pinene.

Limonene. High concentrations of limonene were favoured by short day (SD), high light intensity (L3) and low night temperature (LNT) treatments relative to long day (LD), low light intensity (L1) and high night temperature (HNT) treatments (Table IV A 3.5). No significant interaction between light intensity, daylength and night temperature occurred with respect to the concentration of limonene.

Table IV A 3.2. Dry matter, oil and percentage oil yield.

Growing conditions	Dry Matter yield (g)*	Oil yield (g)*	% Oil yield (Dry matter basis)
LD x LNT x L1	3.27	0.0828	2.54
LD x LNT x L2	12.85	0.3182	2.48
LD x LNT x L3	19.49	0.4856	2.49
LD x HNT x L1	4.44	0.1099	2.48
LD x HNT x L2	15.04	0.3662	2.44
LD x HNT x L3	24.03	0.5770	2.40
SD x LNT x L1	1.11	trace only+ (-0.0335)	- (2.04)
SD x LNT x L2	4.22	0.0792	1.88
SD x LNT x L3	8.74	0.1634	1.87
SD x HNT x L1	1.25	trace only+ (-0.0363)	- (2.01)
SD x HNT x L2	7.14	0.1307	1.83
SD x HNT x L3	10.39	0.1886	1.82
lsd (5%) (3 factor interaction)	1.44	0.034	0.12

*g/8 plants.

+Missing values. (Sufficient oil was obtained to allow determination of oil composition but not oil yield.)

- missing values were calculated using a Genstat package (Genstat Mark 4.01 (c) 1977, Lawes Agric. Trust, Rothamsted Exp. Sta.).

Cineole. At low light intensity (L1) there was no significant difference in the concentration of cineole between SD and LD treatments (Table IV A 3.6). The increase in cineole with increased light intensity was most pronounced under LD conditions and as a result cineole was significantly higher in LD treatments at high light intensity (L3). No significant increase in cineole occurred when light intensity was increased from L2 to L3 under SD or HNT conditions. The only other significant effect on cineole concentration was the increased cineole in HNT relative to LNT conditions at L2.

Menthone. The concentration of menthone increased as light intensity was increased from L1 to L2, and remained constant from L2 to L3, irrespective of daylength and night temperature conditions (Table IV A 3.7). Increased daylength and decreased night temperature favoured higher levels of menthone. The decrease in menthone with decreased daylength was most pronounced in high night temperature treatments.

Menthofuran. At low light intensity (L1) there was no significant difference in the concentration of menthofuran between SD and LD treatments or LNT and HNT treatments (Table IV A 3.8). As light intensity was increased, menthofuran decreased. This decrease was most pronounced under LD and LNT conditions, resulting in significantly lower concentrations of menthofuran in LD and LNT treatments relative to SD and HNT conditions, at high light intensity (L3). The increase in menthofuran with increased night temperature was most pronounced under SD conditions.

Overall, the conditions favouring low concentrations of menthofuran were low night temperature, long days and high light intensity.

Isomenthone. Isomenthone increased as light intensity was increased from L1 to L3 (Table IV A 3.9). Although night temperature

had no significant effect, increased daylength resulted in higher concentrations of isomenthone.

Menthyl Acetate. At low light intensity(L1), SD and HNT favoured higher concentrations of menthyl acetate than LD and LNT (Table IV A 3.10). Increasing light intensity from L1 to L2 resulted in a decrease in menthyl acetate under SD and both night temperature treatments and no significant change occurred in these treatments when light intensity was increased to L3. In the case of LD conditions, light intensity had no significant effect. Overall, night temperature had no effect on the concentration of menthyl acetate and SD conditions yielded higher concentrations than LD conditions.

Neomenthol (+ Menthol Isomers). At low light intensity (L1), SD and HNT conditions resulted in significantly higher levels of neomenthol (Table IV A 3.11). As light intensity was increased to L2 a rapid decrease occurred in SD and HNT treatments, after which the concentration remained constant. Under LD conditions, increasing light intensity, increased neomenthol. Under LNT conditions, light intensity had no significant effect. As a result of the above trends, at high light intensity (L3) neomenthol was significantly higher under LD conditions, whilst no difference existed between night temperature treatments. Whereas increased night temperature had no effect under LD conditions, a significant increase was observed under SD conditions.

Menthol. At low light intensity (L1), SD and HNT conditions resulted in significantly higher levels of menthol (Table IV A 3.12). As light intensity was increased to L2 a decrease in menthol concentration occurred in SD and both night temperature treatments. Under LD conditions the only significant change in menthol levels was an increase from L2 to L3. At high light intensity (L3), LNT conditions

Tables IV A 3. 3-3.13. The interacting effect of environmental conditions on the composition of peppermint oil.

Table IV A 3.3. α-Pinene (%)

	LNT	HNT	LSD (5%)
LD	1.26	1.15	0.21
SD	0.64	0.90	

	L1	L2	L3	
LD	0.65	1.38	1.59	0.26
SD	0.50	0.84	0.98	

	L1	L2	L3	
LNT	0.55	1.02	1.28	0.26
HNT	0.60	1.19	1.29	

3.4. β-Pinene (%)

	LNT	HNT	LSD (5%)
LD	1.48	1.44	0.21
SD	0.93	1.12	

	L1	L2	L3	
LD	0.85	1.64	1.88	0.26
SD	0.80	1.09	1.19	

	L1	L2	L3	
LNT	0.81	1.31	1.49	0.26
HNT	0.84	1.42	1.58	

3.5. Limonene (%)

	LNT	HNT	LSD (5%)
LD	2.55	1.69	0.32
SD	2.98	2.52	

	L1	L2	L3	
LD	1.16	2.32	2.88	0.39
SD	1.89	2.54	3.27	

	L1	L2	L3	
LNT	1.98	2.74	3.57	0.39
HNT	1.07	2.12	2.57	

3.6. Cineole (%)

	LNT	HNT	LSD (5%)
LD	4.49	5.01	0.61
SD	2.86	3.30	

	L1	L2	L3	
LD	2.39	5.31	6.54	0.75
SD	2.26	3.38	3.59	

	L1	L2	L3	
LNT	2.19	3.91	4.92	0.75
HNT	2.46	4.78	5.22	

3.7. Menthone (%)

	LNT	HNT	LSD (5%)
LD	51.41	49.33	2.99
SD	48.31	36.01	

	L1	L2	L3	
LD	45.71	52.50	52.91	3.66
SD	33.60	46.69	46.19	

	L1	L2	L3	
LNT	42.83	53.56	53.20	3.66
HNT	36.48	45.63	45.90	

3.8. Menthofuran (%)

	LNT	HNT	LSD (5%)
LD	17.00	18.49	2.72
SD	19.18	24.66	

	L1	L2	L3	
LD	25.56	16.19	11.48	3.33
SD	25.68	19.09	20.99	

	L1	L2	L3	
LNT	26.45	14.88	12.94	3.33
HNT	24.80	20.40	19.52	

3.9. Isomenthone (%)

	LNT	HNT	LSD (5%)
LD	4.43	4.19	0.82
SD	3.01	3.40	

	L1	L2	L3	
LD	3.18	4.43	5.32	1.00
SD	1.74	3.50	4.38	

	L1	L2	L3	
LNT	2.33	3.94	4.89	1.00
HNT	2.59	3.99	4.81	

3.10. Menthyl Acetate (%)

	LNT	HNT	LSD (5%)
LD	0.29	0.25	0.17
SD	0.81	0.99	

	L1	L2	L3	
LD	0.40	0.18	0.22	0.21
SD	2.13	0.36	0.21	

	L1	L2	L3	
LNT	1.16	0.28	0.21	0.21
HNT	1.38	0.25	0.22	

3.11. Neomenthol (%)

	LNT	HNT	LSD (5%)
LD	0.86	1.01	0.15
SD	0.63	1.01	

	L1	L2	L3	
LD	0.76	0.87	1.19	0.19
SD	1.34	0.59	0.53	

	L1	L2	L3	
LNT	0.67	0.72	0.85	0.19
HNT	1.43	0.73	0.87	

3.12. Menthol (%)

	LNT	HNT	LSD (5%)
LD	7.63	6.30	1.47
SD	12.83	12.26	

	L1	L2	L3	
LD	6.26	6.26	8.37	1.80
SD	18.13	10.10	9.40	

	L1	L2	L3	
LNT	11.61	9.51	9.56	1.80
HNT	12.77	6.84	8.21	

3.13. Pulegone (%)

	LNT	HNT	LSD (5%)
LD	4.90	7.71	1.70
SD	5.34	12.77	

	L1	L2	L3	
LD	9.81	5.32	3.80	2.09
SD	10.30	8.44	8.43	

	L1	L2	L3	
LNT	6.90	4.55	3.92	2.09
HNT	13.21	9.21	8.31	

resulted in higher concentrations of menthol, while no significant difference existed between LD and SD conditions.

Pulegone. At low light intensity (L1) there was no significant effect of daylength on the level of pulegone,high levels being obtained from both SD and LD treatments (Table IV A 3.13). The decrease in pulegone with increased light intensity was most pronounced under LD conditions, resulting in significantly higher levels under SD conditions at L3. High night temperatures (HNT) favoured high levels of pulegone and this increase in pulegone with increased night temperature was greatest under SD conditions.

3.4 Discussion

Long days, high light intensity and high night temperatures favoured highest oil yields. The increase in oil yield with increased daylength was associated with an increase in both dry matter per plant and percentage oil yield. This is in agreement with the effect of photoperiod on oil yield, dry matter and percentage oil yield reported in Section IV A 2. Percentage oil yield was not affected by light intensity or night temperature.

Monoterpene composition of peppermint was not affected by daylength or night temperature when plants were grown at glasshouse light intensity and 20^{o}C day temperature (experiment 1). In contrast, the monoterpene composition was affected by the above changes in daylength and night temperature when plants were grown at glasshouse light intensity and 26^{o}C day temperatures. Therefore, day temperature is an important interacting factor determining oil composition. For day temperature to operate within the limits of the photosynthate model, increasing the day temperature from 20^{o}C to 26^{o}C must favour the depletion of respiratory

substrates by increasing utilization and/or decreasing fixation of photosynthate.

The effect of light intensity, night temperature and daylength on oil composition is in general agreement with the model proposed by Burbott and Loomis (1967). Within this model, the balance between day time accumulation of photosynthate and night time utilization of photosynthate is seen as the determinant of monoterpene composition. Factors favouring the maintenance of high levels of photosynthate (i.e. long days, high light intensity, low night temperatures) favoured high concentrations of cineole and menthone and low concentrations of menthofuran and pulegone.

The proposed model is also supported by the nature of treatment interactions. For example, at the lowest light intensity (L1), menthofuran was high irrespective of daylength and night temperatures. Neither increased photosynthetic production (long days) nor decreased utilization of photosynthate (low night temperatures), could compensate for the low level of photosynthesis which would be expected in such low light intensity treatments. As light intensity decreased, night temperature and daylength became important determinants of oil composition.

In the case of limonene, the photosynthate model did not account for the observed results; short days, high light intensity and low night temperatures favoured high limonene concentrations. Within the photosynthate model, decreased daylength has the opposite effect to increased light intensity and decreased night temperature. In Section IV A 2 a true photoperiodic effect on limonene was described; short days resulted in high limonene concentrations. It could be proposed that short days had an effect via the photoperiodic mechanism and that light intensity and night temperature affected the photosynthetic mechanism.

In the model of Burbott and Loomis (1967), the subsequent reduction of menthone to menthol and menthol to menthyl acetate would be favoured by high levels of photosynthate. It has been demonstrated that such reductions require $NADPH_2$ as a cofactor, as does the reduction of pulegone to menthone (Battaile *et al.*, 1968). Therefore, conditions favouring accumulation of menthofuran would not be expected to favour accumulation of menthol and menthyl acetate. Croteau and Hooper (1978) reported that all leaves of peppermint as well as flowers contain menthyl acetate. Flowers are known to contain oil high in menthofuran, large amounts of non-photosynthetic tissue and have a high requirement for respiratory substrates. As a consequence, a shortage of respiratory substrates could account for the high menthofuran concentrations associated with this tissue. In Section IV A 2 it was demonstrated that photoperiodic conditions favouring the accumulation of high concentrations of menthofuran also favoured menthyl acetate accumulation. In the present experiments, treatments favouring low levels of photosynthate (SD, L1, HNT) resulted in the highest concentrations of menthol and menthyl acetate. Neither photoperiodic nor photosynthetic effects adequately account for such changes. There are many reports that the conversion of menthone to menthol and menthol to menthyl acetate are associated with plant maturation. Unlike the rapid conversion of menthone to menthol in *M. arvensis* (Murray *et al.*, 1972), such conversions are quantitatively slower in *M. piperita* (Croteau and Hooper, 1978).

4. The Effect of Temperature on Photosynthesis, Photorespiration and Dark Respiration in Peppermint

4.1 Introduction

The scheme of monoterpene interconversions in peppermint proposed by Reitsema (1958) assigned a central role to pulegone as the precursor of menthofuran and menthone. The direction in which monoterpene interconversions proceed from pulegone is of utmost importance in determining oil quality. Oxidation of pulegone to menthofuran gives rise to an oil of low quality, whilst reduction of pulegone to menthones, precursors of menthols, favours high oil quality. High day temperatures, high night temperatures, low light intensity and short days have been found to favour high menthofuran and low menthone. Burbott and Loomis (1967) suggested that these environmental factors influenced the metabolism of monoterpenes through an effect on the photosynthate balance within the plant.

The aim of the present experiment was to investigate the effect of day temperature, night temperature and light intensity on photosynthesis, photorespiration and dark respiration in peppermint. An attempt was also made to relate the net CO_2 exchange characteristics of peppermint at different temperatures to the observed effects of temperature on monoterpene composition.

4.2 Materials and Methods

The youngest fully expanded leaves of peppermint were used in all experiments. Plants possessed ten fully expanded pairs of leaves when subjected to experimental treatments. Plant material, propagation and growing system are discussed in detail in Section III.

a. Growing Conditions

Plants were grown under both glasshouse and growth room conditions. Glasshouse conditions were 25±3°C days, 20±2°C nights (16:8) photoperiod at a light intensity of 1000-1200$\mu\Sigma m^{-2}s^{-1}$. Growth room conditions were 25±2°C days, 20±2°C nights (16:8) photoperiod at a light intensity of 150$\mu\Sigma m^{-2}s^{-1}$.

b. Net CO_2 Exchange Measurements

Rates of net CO_2 exchange were measured on attached leaves in a leaf chamber placed inside a light cabinet. An open circuit CO_2 monitoring system incorporating an infra-red gas analyser(I.R.G.A.) was used to monitor net CO_2 exchange in the leaf chamber. Details of the open circuit CO_2 monitoring system and the leaf chamber are provided in Section III.5.

Plants on which measurements were to be made were removed from the glasshouse or growth room at the commencement of the light period and preconditioned in the light cabinet for 1 hour. Leaf diffusive resistance measurements (using a Lambda L1-65 autopormeter) were conducted on plants during the preconditioning period. Only leaves with low leaf diffusive resistances (abaxial resistance less than 2.0 s cm^{-1}) were used in subsequent experiments.

Net CO_2 exchange was measured on leaves from plants grown under the above growing conditions at several temperatures and light intensities. At each temperature or light intensity the net CO_2 exchange was allowed to stabilize for 15 minutes before measurements were taken; during this time rates were stable, indicating a constant plant response to the experimental leaf environment. Light intensity was controlled by inserting varying thicknesses of Sarlon shadecloth between the light source and the leaf chamber, and was measured using a Lambda L1-185 meter fitted with a quantum flux sensor. The temperature

of the leaf chamber was controlled by adjusting the temperature of the surrounding water jacket and was continuously monitored using a thermocouple placed inside the leaf chamber. Likewise, a water bath was used to control the temperature of the leaf chamber air supply before and during humidification. The leaf chamber air supply was maintained at the same temperature as the leaf chamber throughout all experiments.

Rates of "apparent" photosynthesis and dark respiration were determined by measuring net CO_2 exchange in air (21% O_2), in the light and dark, respectively. Photorespiration rate was estimated as the enhancement of net CO_2 exchange in 2% O_2 as compared with 21% O_2. An estimate of "true" photosynthesis was obtained by adjusting the net CO_2 exchange rate in 2% O_2 for the contribution due to dark respiration. The infra-red gas analyser was calibrated using gas mixtures of known CO_2 concentration. The instruments response to a known CO_2 differential was checked before and after each days operations.

c. Light Response and Net CO_2 Exchange

Leaves from plants grown in the glasshouse and growth room were exposed to varying levels of light intensity, in the leaf chamber, and net CO_2 exchange measured at 20°C. All subsequent experiments were conducted at saturating light intensities.

d. Temperature Response and Net CO_2 Exchange

Leaves from plants grown in the glasshouse were used to determine the influence of temperature on net CO_2 exchange. Net CO_2 exchange was monitored in 21% O_2 and 2% O_2, saturating light intensities and in the dark; whilst temperature was increased from 5°C to 35°C.

The net CO_2 exchange curves for the temperature range 5-35°C were completely reproducible irrespective of whether the measurements commenced at the lower or upper limits of the temperature range.

However, additional equilibration time was required when measurements commenced at the upper limit, due to the hysteresis effect.

4.3 Results

a. Light Response and Net CO_2 Exchange

Increasing light intensity from 100 to 300$\mu\Sigma m^{-2}s^{-1}$ resulted in an increased rate of net CO_2 fixation (Figure IV A 4.1). Light saturation occurred between 400 and 500$\mu\Sigma m^{-2}s^{-1}$. At light intensities above saturation, the net CO_2 fixation was highest in plants grown at high light intensities.

b. Temperature Response and Net CO_2 Exchange

Net CO_2 fixation in 21% O_2 and 1000$\mu\Sigma m^{-2}s^{-1}$ ('apparent' photosynthesis) reached a maximum at 20°C and decreased with increasing temperature (Figure IV A 4.2 , curve 1). Efflux of CO_2 in the dark (dark respiration) increased with increasing temperature (Figure IV A 4.2, curve 2), and had a Q_{10} value of approximately 2. The enhancement of net CO_2 fixation in 2% O_2 as compared with 21% O_2 was most pronounced at high temperatures (Figure IV A 4.2, curve 4). Enhancement of net CO_2 fixation in 2% O_2 was an estimate of the contribution of photorespiration to the overall net CO_2 exchange, and represented an efflux of CO_2 from the leaf (Figure IV A 4.2, curve 4). By eliminating the contribution of both dark respiration (this assumes that dark respiration continues in the light) and photorespiration from the overall net CO_2 exchange, it was possible to obtain an estimate of 'true' photosynthesis (Figure IV A 4.2, curve 5). 'True' photosynthesis reached a maximum at 25°C and decreased when temperature was increased to 35°C.

4.4 Discussion

In the photosynthate model proposed by Burbott and Loomis (1967), the balance between production and utilization of photosynthate was

Figure IV A 4.1.

Light saturation curves for peppermint grown under high and low light intensity.
(High light intensity, LSD = 0.812;
Low light intensity, LSD = 0.970.)

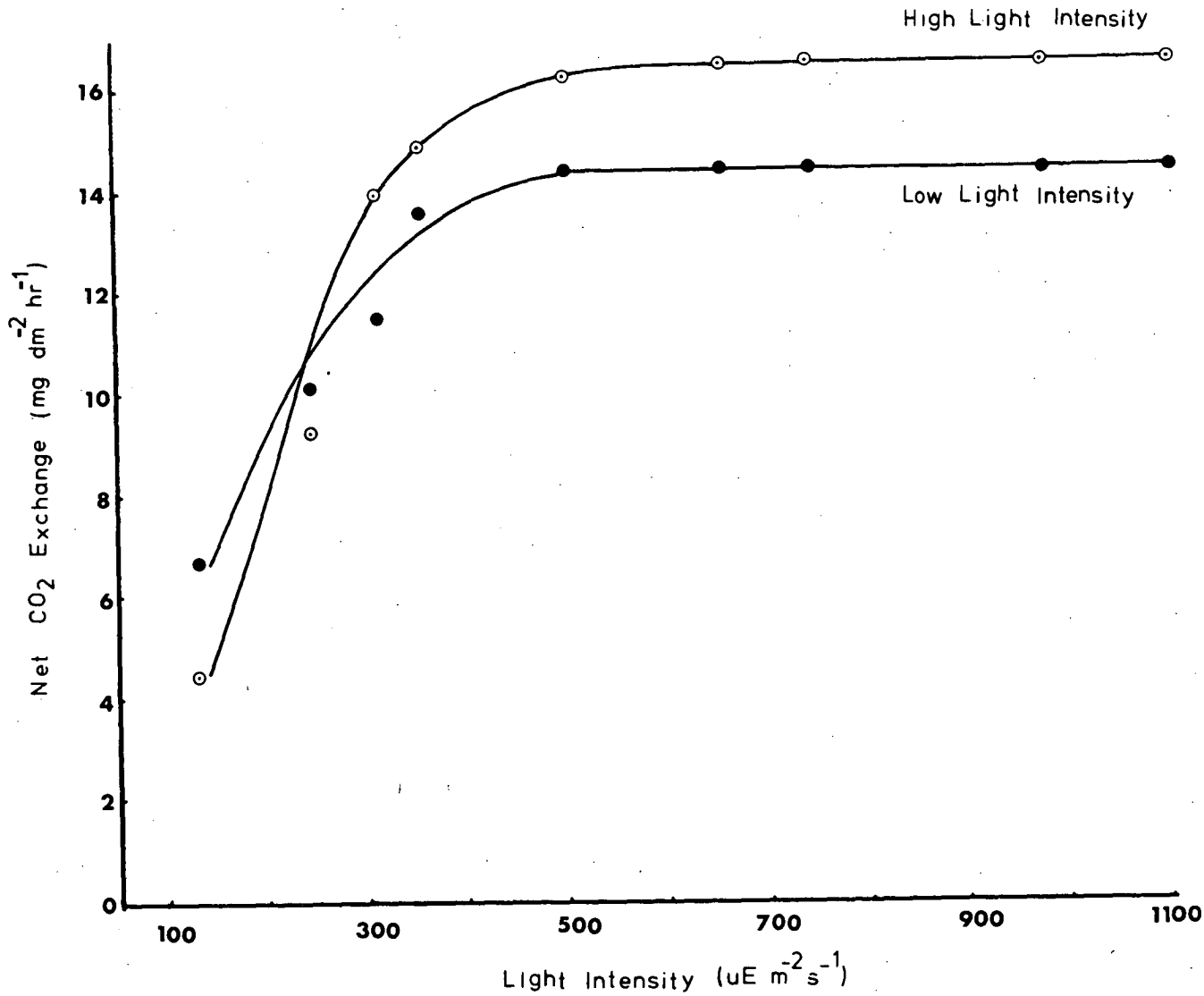
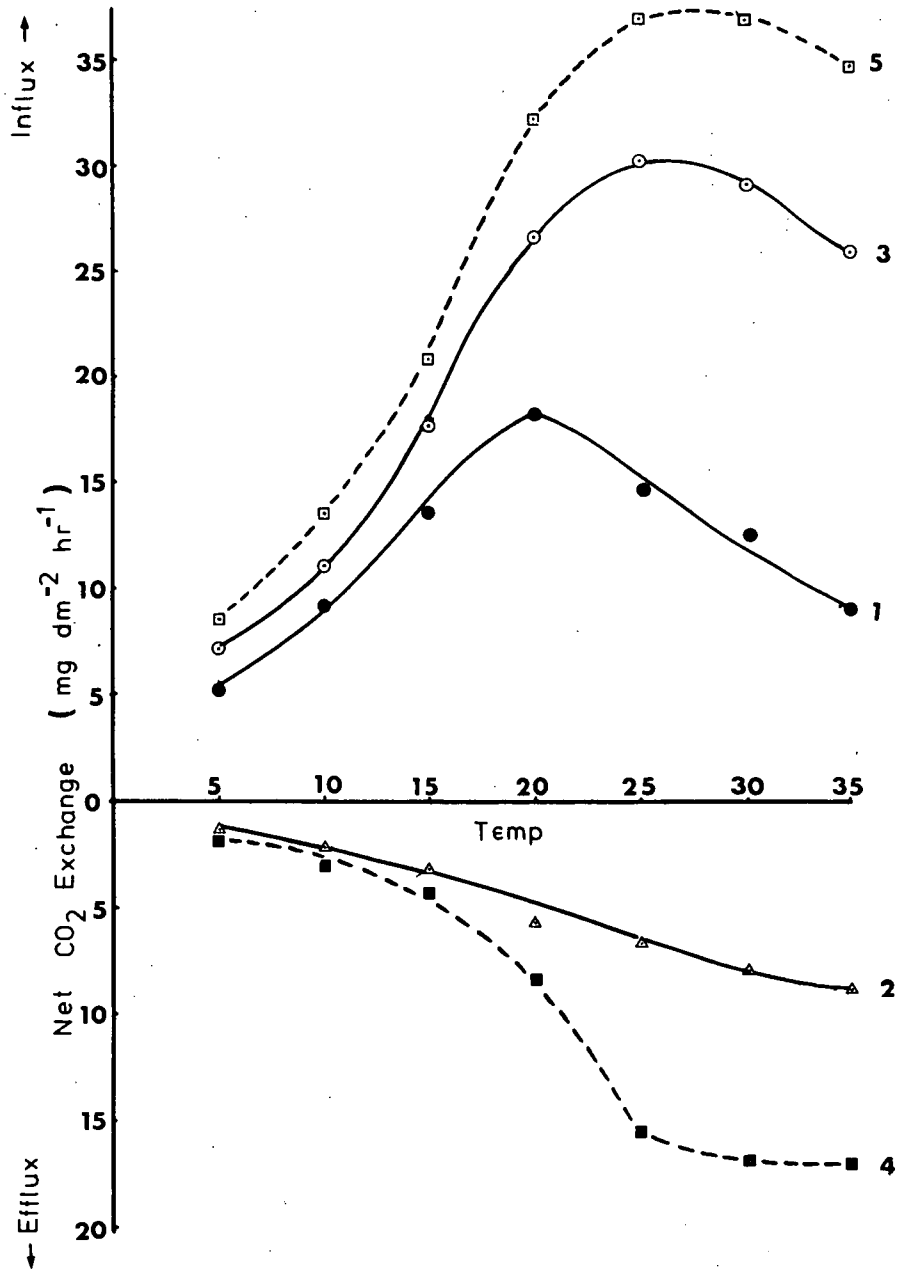

High Light Intensity
Low Light Intensity
Net CO_2 Exchange (mg dm^{-2} hr^{-1})
0
2
4
6
8
10
12
14
16
100
300
500
700
900
1100
Light Intensity (uE m^{-2} s^{-1})

Figure IV A 4.2.

Net CO_2 exchange characteristics of peppermint.

1. 'Apparent' photosynthesis (21% O_2, 310 ppm CO_2, $1000 \mu \Sigma m^{-2} s^{-1}$)(LSD = 0.63).
2. Dark respiration (21% O_2, 310 ppm CO_2, in the dark)(LSD = 0.35).
3. Enhancement of net CO_2 exchange (2% O_2, 310 ppm CO_2, $1000 \mu \Sigma m^{-2} s^{-1}$)(LSD = 0.68).
4. Photorespiration (1-3)(LSD = 0.83).
5. 'True' photosynthesis (3-2)(LSD = 0.81).


Influx →
35
30
25
20
15
10
5
0
5
10
15
20
Net CO2 Exchange (mg dm-2 hr-1)
← Efflux
5
10
15
20
25
30
35
Temp
1
2
3
4
5

seen as an important determinant of oil composition. Assuming that increased CO_2 fixation and increased CO_2 evolution by the plant reflected increased production and increased utilization of photosynthate, respectively, then factors contributing to changes in 'apparent' photosynthesis are important determinants of oil composition.

'Apparent' photosynthesis can be considered to have three components; 'true' photosynthesis, photorespiration and dark respiration. The increase in 'apparent' photosynthesis in the range 5 to 20°C was associated with an increase in 'true' photosynthesis and an increase in both dark respiration and photorespiration. 'Apparent' photosynthesis decreased in the range 20°C to 35°C as a result of the rapid increase in photorespiration between 15°C and 30°C and a continuous, more gradual, increase in dark respiration with no associated increase in 'true' photosynthesis above 25°C.

Burbott and Loomis (1967) reported that increasing night temperature from 8°C to 25°C increased dark respiration, shifting the photosynthate balance towards utilization; resulting in increased menthofuran. From the present work it is apparent that an increase in night temperature would increase dark respiration. The results presented in Section IV A 3 suggested that day temperature was also an important determinant of oil composition. At day temperatures above 25°C, oil composition was more sensitive to changes in daylength and night temperature relative to 20°C day temperatures. In the present work it is apparent that day temperatures of 20°C resulted in maximum rates of 'apparent' photosynthesis. The decrease in 'apparent' photosynthesis when day temperature was increased above 20°C resulted in maximum rates of 'apparent' photosynthesis. The decrease in 'apparent' photosynthesis when day temperature was increased above 20°C resulted from a steady increase in dark respiration and to a greater extent from the rapid increase in photorespiration.

Nelson *et al.* (1971a) reported that evaporative cooling of peppermint by sprinkler irrigation, when the ambient temperature exceeded 30°C, resulted in lower concentrations of menthofuran. These workers suggested that the evaporative cooling had the same effect as cool nights, reported by Burbott and Loomis (1967). Evaporative cooling would increase net CO_2 fixation by decreasing both photorespiration and dark respiration, whereas cool nights would only decrease dark respiration.

Therefore, it would appear that the effect of temperature on the net CO_2 exchange characteristics of peppermint supported the photosynthate model proposed. That is, light intensities in excess of $500 \mu\Sigma m^{-2} s^{-1}$, cool nights and 20°C day temperatures are most conducive to the maintenance of high levels of photosynthate, which favours the reduction of pulegone to menthone.

5. The Effect of Pre-Treatment Growing Conditions on the Monoterpene Composition of Peppermint Oil produced under Long Day Conditions

5.1 Introduction

Langston and Leopold (1954) described the effect of pre-treatment growing conditions on the photoperiodic response of peppermint. These workers reported that peppermint became photoperiodically receptive during early stages of growth. Plants subjected to long days (18:6) prior to the commencement of long day treatments, initiated inflorescences earlier, had higher oil yields and possessed a larger number of glands per unit area of the lower epidermis, than plants exposed to short days (10:14) for 30 days prior to the commencement of the treatment photoperiod (18:6).

In Section IV A 3, the possible confounding effect of pre-treatment growing conditions on the results presented by Burbott and Loomis (1967) was discussed. The aim of the present experiment was to investigate the effect of pre-treatment growing conditions on the monoterpene composition of peppermint oil.

5.2 Materials and Methods

a. Pre-Treatment Growing Conditions

Two pre-treatment growing conditions were used: Short days (8:16) and long days (16:8). Within both short day and long day growing conditions, the temperature was constant at 20^{o}C during the day and night, and the light intensity within the growth rooms was 150$\mu\Sigma m^{-2}s^{-1}$. All plant material was maintained within the above conditions for at least 60 days, prior to commencement of the experiment. Shoot cuttings were taken from plants growing under both short day and long day pre-treatment conditions. After cuttings had formed roots (5-7 days) under

the two pre-treatment conditions, they were transferred to the treatment growing conditions. At the time of transfer the youngest leaf pair (not including the tuft of very young leaves at the growing tip) which was approximately 2cm in length, was marked (end cut off one leaf). This leaf pair was numbered 1 and all subsequent numbering was related to this pair. Twenty visually matched plants (same number of expanded leaf pairs) were initially selected from each of the above pre-treatment growing conditions, and all were transferred to the treatment growing condition. At the time of transfer, visually matched plants were dissected under a stereo microscope and the number of leaves above leaf pair 1 was determined. Under both long days and short days, approximately 5-7 leaves had been differentiated above leaf pair 1.

b. Treatment Growing Conditions

Twenty visually matched, rooted cuttings from both short day (8:16) and long day (16:8) plant material were transferred to long day conditions (16:8). Temperature was maintained at $15^{o}C$ during both the day and night and the light intensity was $150\mu\Sigma m^{-2}s^{-1}$. After 30 days growth under the treatment conditions, visually matched plants were harvested and leaves were removed node by node from each plant for extraction and monoterpene analysis. Three plants were harvested from each pre-treatment growing condition.

5.3 Results

a. Plant Growth Habit

The growth habit of plants taken from long day conditions (16:8) was typical of long day plants. That is, plants were erect with large leaves and few stolons. In contrast, plants taken from short day conditions (8:16) retained a growth habit typical of short day plants, after being placed in the long day treatment conditions. Only after a

considerable period of time in long day conditions did the short day plants take on the characteristic long day growth habit. Plate IV A 5.1 illustrates the growth habit of plants taken from long day (16:8) conditions. Plate IV A 5.2 illustrates the short day appearance of plants taken from short day (8:16) conditions. This short day growth habit persisted under long day conditions for a considerable period; the plant shown in Plate IV A 5.2 had been growing under long day conditions for 25 days. At harvest (30 days after being transferred to long day conditions) many of the short day plants were beginning to adopt a growth habit similar to long day plants (Plate IV A 5.3). Although the plants shown in Plates IV A 5.1 to 5.3 were not those harvested (they were taken from a preliminary experiment), they are representative of the extremes of growth habit observed during the experimental period.

b. Oil Composition

From the results presented in Figures IV A 5.1 and 5.2 and Tables IV A 5.1 and 5.2, it is apparent that the pre-treatment growing conditions had pronounced effects on the monoterpene composition.

Plants subjected to a long day pre-treatment growing condition (Figure IV A 5.2) were observed to have an oil composition typical of the long day plants discussed in Section A 1. That is, menthol decreased and menthone increased with increasing height on the plant (basal → apical). In all leaves, menthone and menthol were the predominant monoterpenes. Changes in the percentage limonene, cineole, menthofuran, pulegone and menthyl acetate, with position on the plant, were less pronounced.

In plants subjected to a short day pre-treatment, menthone decreased from apical to basal leaves (Figure IV A 5.1). Menthol increased from leaf pair 9 to 5, after which it decreased in basal leaves. This

Plate IV A 5.1

Growth habit observed to be typical of long day plants, throughout the experimental period. Pre-treatment growing conditions; LD (16:8); Treatment growing conditions; LD (16:8).

Plate IV A 5.2

Growth habit observed to be typical of short day plants, during the initial part of the experimental period. [The plant shown actually maintained a short day appearance for approximately 25 days after being placed under long days (16:8).] Pre-treatment growing conditions; SD(8:16); Treatment growing conditions; LD(16:8).

Plate IV A 5.3

After a considerable period under long day conditions (16:8), short day plants adopted a long day growth habit. (The plant shown in Plate IV A 5.3 was typical of the short day plants harvested after 30 days in the long day conditions, of the present experiment.)

5.1

5.2

5.3

Key to Figures IV A 5.1 and 5.2

●- - - - - - ● Limonene

■- - - - - - - ■ Cineole

▲- - - - - - - ▲ Menthone

✱- - - - - - ✱ Menthofuran

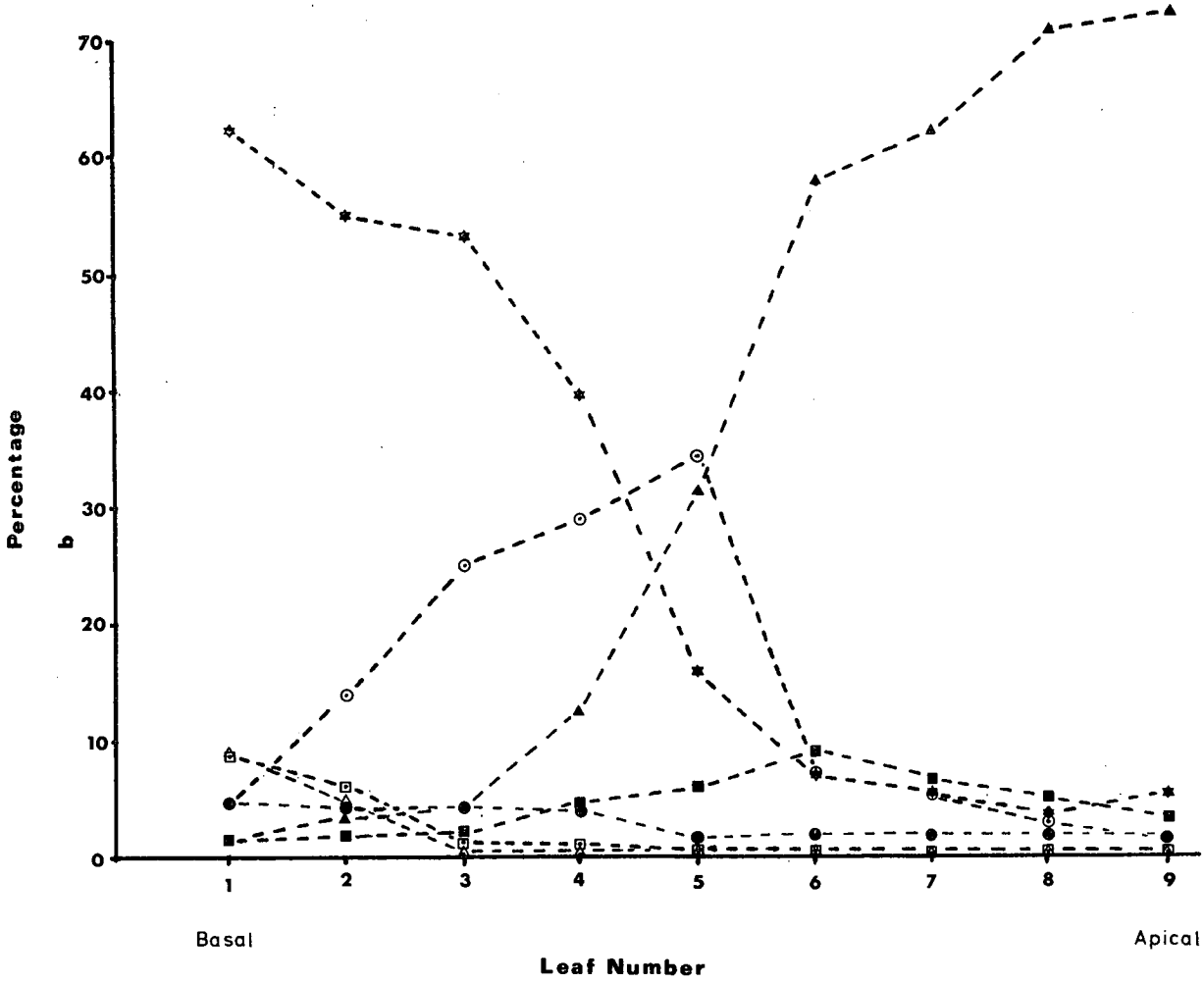
□- - - - - - □ Menthyl Acetate

○- - - - - - ○ Menthol

△- - - - - - △ Pulegone

Figure IV A 5.1. Plant grown for 30 days, long days (16:8), $15^{o}C/15^{o}C$, $150\mu\Sigma m^{-2}s^{-1}$. Pretreatment = Planting material taken from plants growing under short days (8:16), $20^{o}C/20^{o}C$, $150\mu\Sigma m^{-2}s^{-1}$.

Figure IV A 5.2. Plant grown for 30 days, long days (16:8), $15^{o}C/15^{o}C$, $150\mu\Sigma m^{-2}s^{-1}$. Pretreatment = Planting material taken from plants growing under long days (16:8), $20^{o}C/20^{o}C$, $150\mu\Sigma m^{-2}s^{-1}$.


Percentage
b
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60
50
40
30
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10
0
1
2
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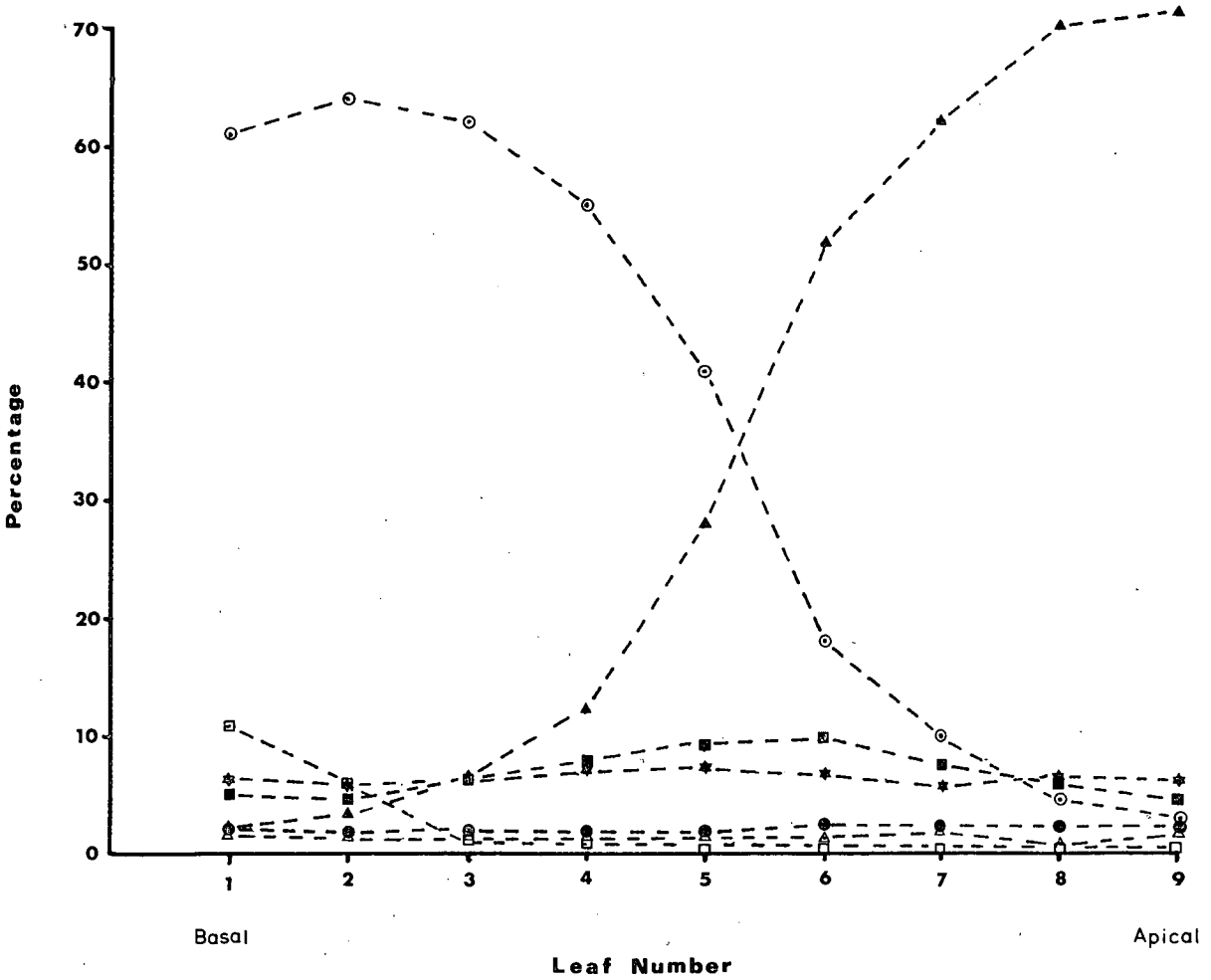
4
5
6
7
8
9
Basal
Apical
Leaf Number


Percentage
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Basal
Apical
Leaf Number

Table IV A 5.1. Oil composition (%) - Short day pre-treatment. Mean values[a] and Standard errors[b].

Compound (%)	Leaf Number*								
	1	2	3	4	5	6	7	8	9
Limonene	4.84[a] (0.06)[b]	4.41 (0.10)	4.19 (0.14)	3.89 (0.10)	1.94 (0.15)	2.32 (0.20)	2.11 (0.08)	2.20 (0.06)	2.06 (0.05)
Cineole	1.64 (0.05)	1.91 (0.03)	2.34 (0.12)	4.81 (0.15)	6.10 (0.06)	9.37 (0.21)	6.90 (0.21)	5.47 (0.25)	3.67 (0.22)
Menthone	1.33 (0.02)	3.14 (0.09)	4.86 (0.09)	12.51 (0.53)	30.17 (2.29)	58.02 (1.47)	62.34 (0.70)	70.91 (0.60)	72.40 (0.70)
Menthofuran	62.08 (1.10)	54.91 (0.48)	53.54 (0.84)	39.65 (1.79)	16.30 (1.17)	7.20 (0.17)	5.81 (0.46)	4.27 (0.46)	5.33 (0.59)
Menthyl Acetate	8.79 (0.51)	6.18 (0.09)	1.53 (0.33)	1.23 (0.15)	0.53 (0.10)	0.38 (0.07)	0.26 (0.04)	0.17 (0.03)	0.12 (0.04)
Menthol	4.80 (0.21)	14.50 (0.38)	25.07 (0.61)	28.94 (0.92)	34.40 (0.72)	7.13 (0.51)	5.50 (0.16)	3.63 (0.74)	2.05 (0.03)
Pulegone	8.28 (0.58)	4.97 (0.38)	1.05 (0.14)	0.86 (0.04)	0.62 (0.17)	0.62 (0.06)	0.56 (0.16)	0.62 (0.32)	0.61 (0.03)

*Leaf pair no. 9 = youngest apical leaf pair.

Table IV A 5.2. Oil composition (%) - Long day pre-treatment. Mean values[a] and Standard errors[b].

Compound (%)	Leaf Number*								
	1	2	3	4	5	6	7	8	9
Limonene	2.36[a] (0.27)[b]	2.02 (0.08)	2.17 (0.19)	2.19 (0.16)	2.16 (0.19)	2.58 (0.24)	2.52 (0.10)	2.42 (0.30)	2.44 (0.31)
Cineole	5.07 (0.10)	4.85 (0.34)	6.45 (0.35)	8.17 (0.56)	9.30 (0.64)	9.86 (0.23)	7.70 (0.30)	5.97 (0.14)	4.61 (0.39)
Menthone	2.20 (0.53)	3.67 (0.66)	6.86 (1.35)	12.16 (1.02)	27.78 (4.81)	51.82 (1.17)	62.13 (1.47)	69.99 (0.68)	71.29 (0.50)
Menthofuran	6.53 (0.87)	5.92 (0.85)	6.73 (0.69)	7.20 (0.85)	6.99 (0.23)	6.43 (0.99)	5.76 (0.96)	6.50 (0.87)	6.18 (0.94)
Menthyl Acetate	10.81 (0.69)	5.90 (0.71)	1.18 (0.29)	0.94 (0.07)	0.44 (0.05)	0.26 (0.02)	0.17 (0.06)	0.24 (0.05)	0.10 (0.02)
Menthol	60.90 (1.93)	64.01 (0.79)	62.20 (1.21)	55.20 (1.51)	40.96 (5.34)	17.92 (1.37)	9.99 (2.28)	4.39 (1.83)	2.94 (1.16)
Pulegone	2.63 (0.07)	1.68 (0.12)	1.53 (0.38)	1.85 (0.19)	1.88 (0.23)	1.41 (0.22)	2.04 (0.18)	1.09 (0.33)	1.83 (0.27)

*Leaf pair no. 9 = youngest apical leaf pair.

decrease in menthol in basal leaves coincided with a rapid increase in menthofuran. Menthyl acetate increased to a lesser extent, in basal leaves. In leaf pair 9 to 5, cineole was higher than limonene. However, the opposite was the case in basal leaves.

5.4 Discussion

In addition to the reported effect of pre-treatment growing conditions on the initiation of inflorescences, oil yield and number of glands (Langston and Leopold, 1954), the present experiment suggested a significant effect on growth habit and monoterpene composition of peppermint.

Leaves that were observed to form prior to the transfer of plants into treatment growing conditions, contained oil with a composition typical of the pre-treatment conditions. That is, plants from short day conditions (8:16) contained predominantly menthofuran in basal leaves. The higher concentration of limonene and lower concentration of cineole observed in basal leaves has been reported to be characteristic of short day plants (see Section IV A 2). Although basal leaves had formed before plants were subjected to the final treatment conditions (16:8), all leaves expanded under these conditions. From the results presented in Section IV A 1, it was concluded that oil accumulation continued during leaf expansion. Therefore, at the time of transfer, leaf pair 1 would be expected to have accumulated a significant proportion of its final oil content. With respect to leaf pair 1, the conditions under which accumulation occurred were characteristic of the pre-treatment conditions, therefore it was not unexpected that menthofuran should represent a significant proportion of oil formed under short days (and low light intensity). In contrast, leaf pairs 6 and 7,although discernible at the time of transfer, possessed very few glands and accumulation had hardly

commenced. Therefore, the bulk of oil finally accumulated in these leaves would have been synthesised under the treatment conditions, hence the long day characteristics of these oils. With respect to the high concentration of menthofuran in basal leaves of short day plants, it could be suggested that conditions which initially favoured accumulation of menthofuran (short days), persisted in these leaves and hence menthofuran was not reduced to menthol when the plant was transferred to long day conditions. Alternatively, it could be suggested that the pathway leading to the biosynthesis of menthofuran from pulegone was not readily reversed by altering the growing conditions.

In light of the reported effects of pre-treatment growing conditions on monoterpene composition, plant growth habit, oil yield, inflorescence initiation and number of glands, caution should be exercised in the selection of planting material to be used in experiments designed to investigate the effect of environmental effects on peppermint. In particular, the leaves selected for compositional studies should be those initiated under treatment conditions.

B. Field Experiments

1. A Study of Variations in Composition of Peppermint Oil in Relation to Production Areas

1.1 Introduction

The objective of the present work was to study the composition of peppermint oil produced in the commercial production areas of Tasmania, investigate the variations in oil composition within this State, and compare the composition of Tasmanian-produced oils with oils from other major production areas. From the results of this survey, it was anticipated that an assessment of the suitability of Tasmania for the production of high quality peppermint oil could be obtained. In addition, the variation in oil composition from different locations was related to several cultural and environmental factors.

1.2 Materials and Methods

a. Tasmanian Peppermint Oil Samples

Essential oil was obtained from *M. piperita* L. grown at various locations within Tasmania (Figure IV B 1.1). Random samples of plant material were harvested from trial plantings (S.E. Tasmania, W. Tasmania, N.E. Tasmania, N.W. Tasmania, N. Tasmania and King Island) as well as from commercial production areas (S. Tasmania and King Island), throughout the growing season.

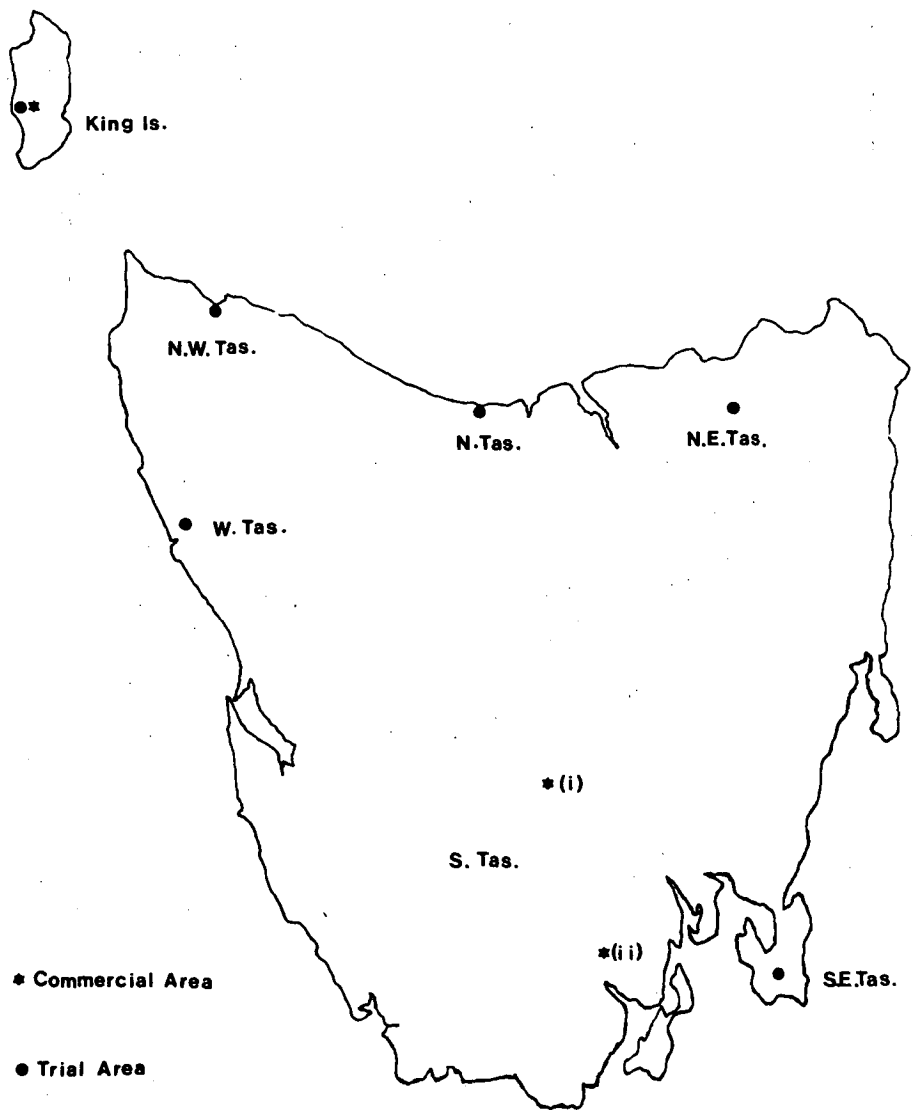
Samples of plant material were air dried and steam distilled in accordance with techniques previously described. Additional samples of 'bulk' oil were obtained from commercial steam distillation units at commercial harvest.

b. Peppermint Oil Samples from Other Production Areas

Data on the composition of peppermint oil from various

Figure IV B 1.1

Peppermint oil production areas within Tasmania.



production areas were obtained from the literature; New Zealand (Lammerink and Manning, 1971), India (Chandra *et al.*, 1968), and Alberta (Embong *et al.*, 1977). Reference data for oils produced in other areas were obtained from Smith and Levi (1961). Since the data available for production areas other than Tasmania were based on that reported in the literature, neither the authenticity nor the extent to which such data were representative of the given area, is known. The assumption that data were authentic and representative has been made by numerous workers (Hartmann and Hawkes, 1970; Elliot *et al.*, 1971), and has been made in the present study. The data presented should not be regarded as offering a final, unequivocal scheme of characterisation of production areas.

c. Principal Co-ordinates Analysis

This technique, due to Gower (1966), requires the user to define a similarity matrix between sampling units, which in this case were the eighty two oils from various locations. The variates were six compounds of peppermint oil, limonene, cineole, menthone, menthofuran, menthyl acetate and menthol. An 82 x 82 matrix of similarities between each pair of oils was defined using the so-called 'Canberra Metric', given in Lance and Williams (1967). The principal co-ordinates analysis ordinated this matrix so as to display the variation in as small a dimensionality as possible. Numerous examples of principal co-ordinates analysis, application and interpretation are cited in Blackith and Reyment (1971).

1.3 Results and Discussion

a. Ordination Diagrams

An officially adopted criteria for quality appraisal of peppermint oil involves quantitative determination of only two of the

many oil compounds, menthol and menthyl esters (British Pharmacoepia, 1968). In the present work several other important compounds are included to obtain a comparison of oil composition from different geographical areas, including Tasmania. Compositional data obtained in the present study, as well as that available from the literature, are included in Table IV B 1.1. Subsequent treatment of these data using principal co-ordinates analysis resulted in the ordination diagrams presented. Three dimensions were found to represent the variation adequately without there being any apparent important variation in higher dimensions. Figures IV B 1.2 and 1.3 display the variation accounted for by the combination of the first and second, and first and third principal co-ordinates, respectively.

The compounds involved in determining the first three principal co-ordinates and their relative importance, expressed as the correlation between the compound and the axis, are included in captions to Figures IV B 1.2 and 1.3. For example, large positive values for principal co-ordinate 1 are indicative of oils having low limonene, low menthofuran, low menthyl acetate and to a somewhat lesser extent high menthone, low cineole and to a much lesser extent high menthol.

It is apparent from Figure IV B 1.2 that sufficient variation in oil composition is explained by principal co-ordinates 1 and 2, as to allow separation of the oils into groups according to geographic origin. However, due to the number of compounds which together determine the principal co-ordinates and the differing importance of each compound, caution is required when these diagrams are interpreted. Interpretation should be made only in conjunction with the data included in Table IV B 1.1. In addition to the 'Canberra Metric' the data were also analysed using a coefficient of similarity based on Euclidean distance. The use

Table IV B 1.1.

Compositional data for peppermint oil from various production areas.

Sample		Composition of *M. piperita* L. oils (% compound)					
Sample Origin	Ref. No.	Limonene	Cineole	Menthone	Menthofuran	Menthyl Acetate	Menthol
U.S.A., Mid-West	1	3.00	8.30	29.90	1.90	4.20	39.30
	2	3.40	7.10	30.70	1.70	4.50	38.80
	3	3.60	8.70	31.60	.70	5.10	37.00
U.S.A., Oregon	4	4.30	8.30	19.50	3.20	6.10	43.10
	5	3.60	8.00	23.80	3.50	4.40	41.80
	6	3.20	7.50	21.30	2.60	4.50	46.20
U.S.A., Washington (Yakima)	7	4.30	8.10	16.70	8.80	6.90	43.20
	8	3.70	7.80	17.10	8.10	7.40	42.20
	9	3.50	13.50	17.90	6.20	5.30	40.20
	10	3.70	6.40	8.90	9.40	11.60	48.70
Italian	11	6.80	8.90	18.10	6.40	4.80	38.70
	12	6.20	9.90	19.60	5.50	5.60	35.30
	13	3.90	7.50	19.80	6.10	5.50	40.00
English (E)	14	6.80	8.80	20.30	1.70	4.60	43.90
	15	4.00	9.40	21.00	1.60	3.80	46.70
	16	4.30	8.70	16.90	1.80	6.90	47.60
	17	3.90	6.10	15.90	5.80	9.60	44.40
	18	5.50	7.10	15.60	5.80	8.50	39.70
	19	4.00	12.40	18.20	4.50	4.60	38.60
Bulgarian (B)	20	3.50	7.50	16.90	6.20	7.20	43.90
South African	21	5.80	7.40	19.10	8.80	6.90	36.10
	22	6.30	7.40	17.90	9.20	10.50	33.20
Argentina (Arg.)	23	3.80	6.70	12.80	8.40	7.50	46.90
Netherlands (N)	24	1.00	4.40	17.80	.30	6.50	54.50
Polish (P)	25	3.90	7.90	24.80	3.10	4.50	40.10
Spanish (S)	26	3.10	6.70	30.60	2.90	3.80	36.00
New Zealand	27	3.01	6.52	16.30	8.90	6.40	39.00
	28	2.73	5.93	14.30	8.10	6.00	43.00
	29	2.42	6.42	9.90	7.90	5.00	48.90
Indian (I)	30	3.50	4.00	7.70	.70	7.50	60.60
Alberta (A)	31	3.00	5.90	27.30	1.90	3.40	42.00
	32	2.70	6.30	20.70	6.30	4.20	43.20
	33	2.60	5.10	15.10	6.00	9.60	47.00
	34	1.60	5.20	29.50	1.10	3.40	36.30
	35	1.50	5.70	26.00	1.20	3.70	40.30
	36	2.00	5.10	21.20	2.90	3.80	44.20
	37	.80	4.20	11.90	.30	1.90	58.90
S. Tasmania (I)	38	1.38	6.06	31.50	1.73	1.48	39.07
	39	1.72	5.98	26.27	1.65	1.94	41.17
	40	2.03	7.07	23.63	1.53	2.80	45.66
	41	1.57	6.27	25.57	1.33	2.83	48.11
'Bulk'	42	1.83	5.04	24.91	2.01	3.10	45.05
S. Tasmania (II)	43	1.40	6.14	30.51	1.37	1.40	40.00
	44	1.57	5.79	27.75	1.60	2.00	42.17
	45	1.39	5.38	28.92	1.60	2.09	43.23
	46	1.95	6.23	25.64	1.69	2.62	44.87
'Bulk'	47	1.68	5.52	23.19	2.00	3.01	46.57
N.E. Tasmania	48	1.27	5.38	24.01	.78	2.35	45.58
	49	1.29	6.11	18.84	1.07	1.96	45.65
	50	.26	3.44	19.97	1.04	2.12	55.30
	51	.63	3.87	23.52	1.44	2.88	51.29
	52	.41	3.97	17.17	.99	2.80	50.25
N. Tasmania (+)	53	1.50	5.22	30.57	3.45	4.44	36.19
(+)	54	1.43	5.42	31.24	3.84	3.69	35.98
N.W. Tasmania	55	.28	1.74	31.63	.43	1.52	48.94
(+)	56	1.23	5.64	43.90	.27	.94	30.67
(+)	57	.99	3.86	44.49	.54	.77	34.87
W. Tasmania	58	.17	4.23	33.10	.95	2.11	48.78
	59	.16	3.88	27.62	1.01	1.93	55.32
	60	1.47	4.28	26.64	1.31	1.93	49.53
	61	.34	4.51	27.87	1.01	2.31	53.09
King Island	62	1.12	3.62	21.79	4.38	6.51	46.21
(+)	63	1.46	3.57	31.85	2.59	2.17	40.67
(+)	64	1.86	2.48	34.57	3.70	4.35	36.87
(+)	65	1.84	4.40	37.57	5.17	1.65	29.11
(+)	66	2.23	2.83	43.38	4.63	2.16	27.13
S.E. Tasmania	67	0.68	6.95	30.52	1.54	1.30	46.03
	68	1.67	5.25	21.83	2.97	1.49	47.40
	69	1.89	5.85	30.34	1.57	1.90	43.95
	70	1.46	6.40	31.29	1.73	1.75	42.99
King Island*	71	1.98	3.27	39.71	7.20	2.17	40.21
*	72	1.36	4.34	31.47	2.60	1.39	40.27
*	73	1.06	3.26	31.03	4.70	2.63	40.68
*	74	1.18	3.48	17.49	4.72	4.24	50.86
*	75	1.03	3.85	22.27	1.99	3.14	52.93
(+)	76	.95	1.03	38.51	8.34	1.57	35.17
*	77	1.61	3.31	20.65	10.60	5.17	40.87
*	78	1.51	3.30	13.29	13.65	4.90	48.20
*	79	1.45	4.05	35.87	1.72	2.78	41.11
S. Tasmania(*)	80	1.62	2.71	19.39	7.58	4.42	50.23
	81	1.50	2.77	9.71	6.55	8.48	59.25
	82	2.01	2.13	2.01	7.41	23.59	49.35

*Regrowth.

+Rust affected crop.

N.B. Rectified or partially dementholized oils reported by Smith and Levi (1961) are not included in Table 1 or any subsequent analysis.

Figure IV B 1.2

Ordination diagram of peppermint oil composition from various production areas. Correlation coefficients between the principal co-ordinate axis and the six variates are as follows:

Principal co-ordinate 1 (P1); limonene -0.8021, menthofuran -0.7286, menthyl acetate -0.6869, menthone +0.6869, cineole -0.5156, menthol +0.1394.

Principal co-ordinate 2 (P2); cineole +0.6881; menthofuran -0.4442, limonene +0.3827, menthol -0.2674, menthyl acetate -0.2586, menthone +0.1787.

Figure IV B 1.2.

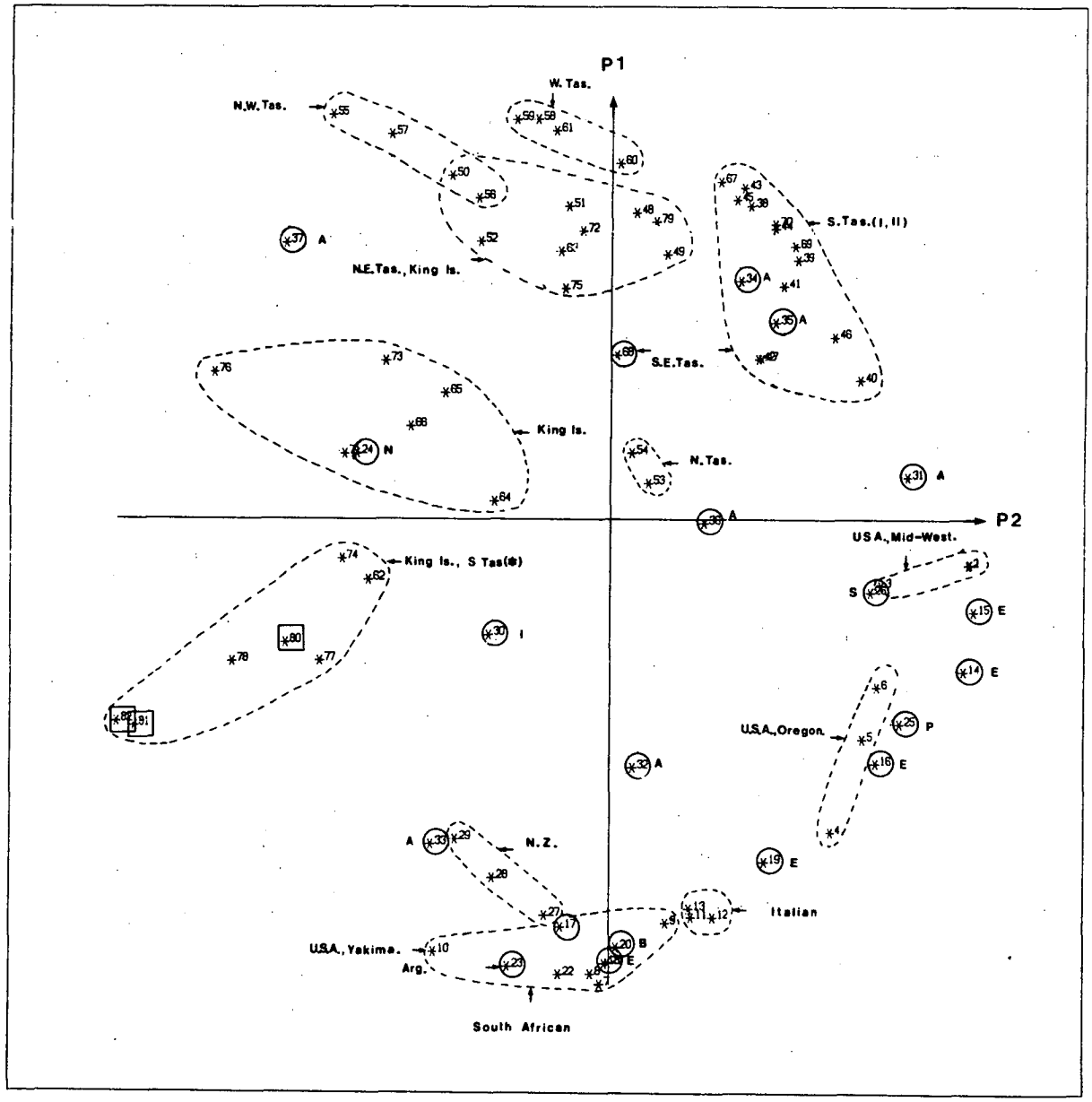
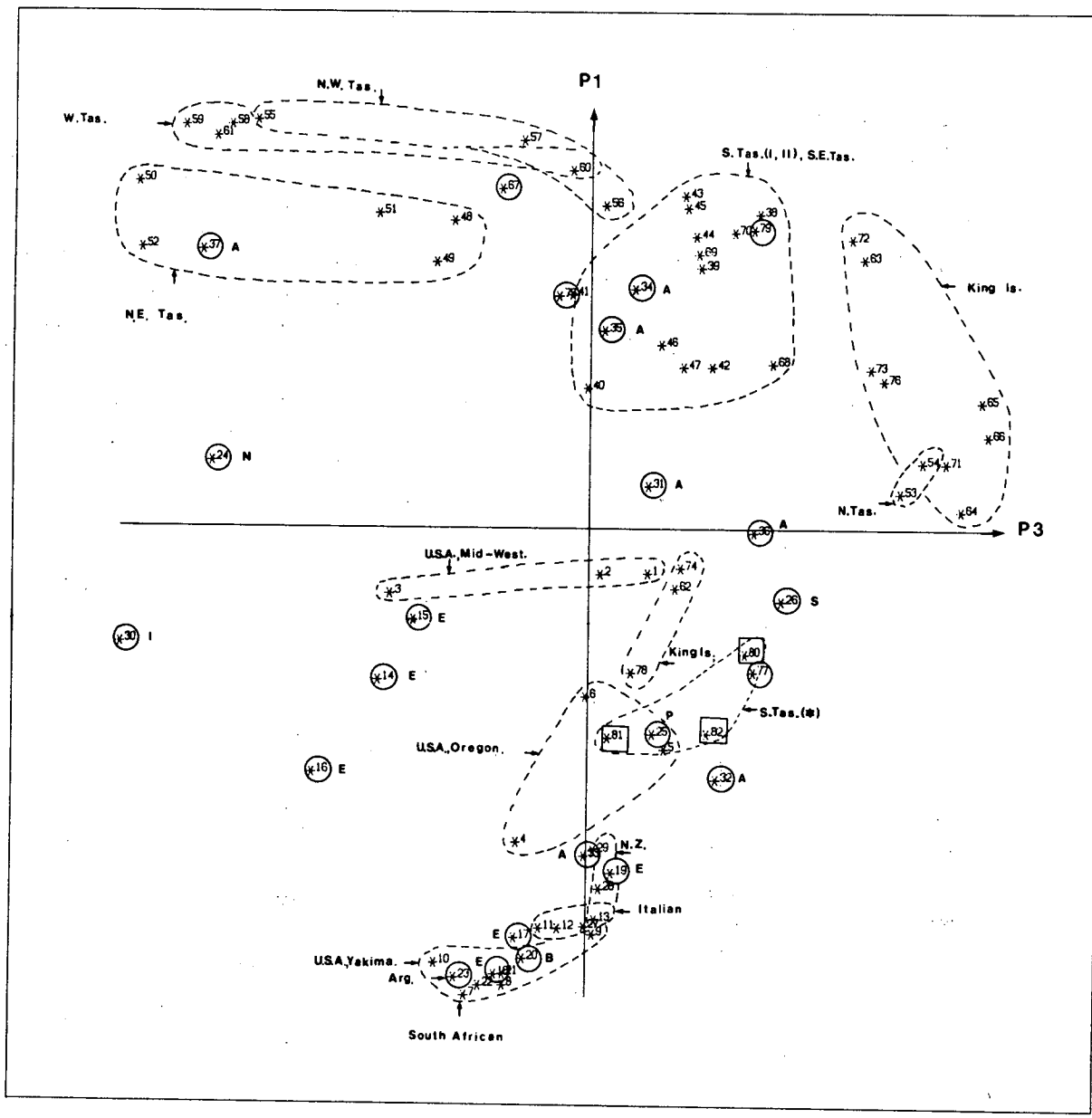


Figure IV B 1.3.

Ordination diagram of peppermint oil composition from various production areas. Correlation coefficients between the principal co-ordinate axes and the six variates are as follows:
Principal co-ordinate 1 (P1); limonene -0.8021, menthofuran -0.7286, menthyl acetate -0.6869, menthone +0.6869, cineole -0.5156, menthol +0.1394.
Principal co-ordinate 3 (P3); menthol 0.6156, menthone +0.4282, menthofuran +0.2693, cineole -0.1471, menthyl acetate -0.1119, limonene -0.0586.

Figure IV B 1.3.



of the latter coefficient of similarity did not result in any major differences in either the ordination diagrams obtained or their interpretation.

b. Menthofuran

The major commercial growing regions of peppermint are in the U.S.A., mostly in the Midwest States of Michigan,and Wisconsin and in Oregon and Washington. Oils produced in the Midwest States are lower in menthofuran than oils produced in Oregon and Washington. It was reported that oils from the latter areas often require fractionation to remove the ill-smelling compound menthofuran (Ellis, 1960). Loomis (1977a)associated the higher menthofuran content of oils obtained from the Oregon and Washington areas, with the high proportion of flowers present in these areas. The difference in flowering and plant growth habit was considered to be determined by daily moisture stress patterns, which in turn were determined by atmospheric moisture conditions and by irrigation practice. Moisture stress was observed to increase flowering in peppermint (Loomis, 1967).

Peppermint oil from South Africa, Argentina, Italy, New Zealand, Bulgaria, as well as several oils from England and Alberta, have menthofuran concentrations similar to those reported for Washington. Such oils are grouped together in Figure IV B 1.2 and have large negative values for principal co-ordinate 1. With respect to peppermint oils produced in Alberta, Embong *et al.* (1977) suggested that climatic conditions were important determinants of menthofuran content. Increased daylength and hours of sunshine were associated with lower concentrations of menthofuran. Climatic data presented by Embong *et al.* (1977) are included as follows:-

Alberta

	Southern	Central	Northern
Ref. no. of oil	31, 32, 33	34, 35, 36	37
Latitude	$50^{o}33'$	$53^{o}42'$	56^{o}
Hours of bright sunshine			
M	143	137	
J	312	260	
J	350	280	
A	328	282	
S	118	93	

With the exception of several King Island oils and southern Tasmanian oils extracted from regrowth herb, Tasmanian oils were generally low in menthofuran and in this regard were similar to oil produced in the Midwest States of the U.S.A.. Spanish, Netherlands, Polish, Indian and several English and Alberta oils were also relatively low in menthofuran. Although menthofuran is known to vary with stage of plant maturity (high menthofuran being associated with inflorescences), it is well established that environmental conditions such as night temperature, day temperature, daylength and light intensity are important determinants of menthofuran (Grahle and Holtzel, 1963; Burbott and Loomis, 1967). Since environmental factors vary with geographical area, it was not unexpected that menthofuran was of utmost importance in separating oils according to geographic origin. For example, menthofuran was an important determinant of both principal co-ordinates 1 and 2. Within Tasmania, oils with higher menthofuran were associated with herb produced late in the growing season from regrowth herb (shorter days) or from plants

severely affected by rust (a high proportion of leaves were lost, leaving a small number of upper leaves and an inflorescence).

c. Limonene and Cineole

Smith and Levi (1961) and Embong *et al.* (1977) reported that the ratio of limonene to cineole was genetically controlled, ranging from 0.2 to 0.7 for genuine oil of *M. piperita* L. In Section IV A 2, values approaching 2.0 were found characteristic of plants grown under short day conditions. The value of this ratio as a specific criterion for the recognition of genuine *M. piperita* L. oils would appear questionable. The importance of both limonene and cineole in accounting for the variation in oil samples displayed by principal co-ordinates 1 and 2, respectively, is further evidence that the concentration of these compounds is strongly influenced by environmental-geographic effects.

A characteristic of Tasmanian oils was their low concentration of limonene. Similar low concentrations were reported for oils produced in some areas of Alberta, Netherlands and to a lesser extent New Zealand. Cineole was also low in Tasmanian oils, as it was in oils from Netherlands, New Zealand, India and Alberta. Within the production areas of Tasmania, cineole was generally highest in Southern Tasmanian oils, during the commercial growing season. Lower concentrations of cineole were associated with Northern Tasmanian oils (including King Island) and Southern Tasmanian oils extracted from regrowth arising after commercial harvest.

d. Menthone, Menthol and Menthyl Acetate

In the scheme of interconversions proposed by Reitsema (1958) pulegone was either oxidised to menthofuran or reduced to menthones. The menthones were further reduced to menthols and menthols to menthyl acetate. The conversion of pulegone to menthofuran has been associated with environmental or plant conditions which favour the depletion of respiratory

substrates. The conversion of menthones to menthols and menthols to menthyl esters has been associated with increased plant maturity as well as environmental conditions (Ellis, 1945; Burbott and Loomis, 1967).

For peppermint oils to satisfy the requirements outlined by the British Pharmacoepia (1968), menthol must exceed 45 percent and menthyl acetate must range from 4 to 9 percent. A characteristic of many U.S.A., Italian, English, Bulgarian, South African, Polish, New Zealand and Alberta peppermint oils was their low menthol concentrations. Although menthol concentrations were often below the required 45 percent, menthyl acetate levels were generally satisfactory. Satisfactory concentrations of menthyl acetate would suggest the oil was quite mature whereas the low concentration of menthol would suggest the oil was quite immature. The large variation in menthol concentrations within some production areas (e.g. South Africa, 31.1 to 46.9%) would indicate that these areas were capable of producing oils of acceptable menthol concentration, and that within these areas there was a confounding effect due to plant maturity. With respect to menthol and menthone concentrations, a plant maturity effect was evident in the commercial production areas of Tasmania; satisfactory menthol concentrations were obtained at commercial harvest (late February, Ref. No. 42, 47). Low menthyl acetate concentrations were characteristic of these Tasmanian oils. High menthol and low menthyl acetate concentrations were also characteristic of other production areas within Tasmania, with the exception of oil extracted from plants which were severely infested with rust; these plants had low menthol concentrations. Rust affected plants were observed to lose up to 80 percent of their lower leaves (e.g. Ref. Nos. 53, 54, 56, 57, 63, 64, 65, 66). Therefore, an assessment of rust damage would appear necessary if compositional data are used as a means of assessing the

potential of an area for the production of high quality oil. Oil extracted from regrowth following commercial harvest was high in menthyl acetate and menthol and low in menthone. High menthyl acetate and menthol concentrations are normally associated with the onset of flowering. In regrowth plants flowering did not occur and the observed maturation of oil may have resulted from the cooler nights prevailing during the regrowth period or the cessation of growth due to the onset of dormancy.

2. The Effect of Harvest Date on the Yield and Composition of Peppermint Oil

2.1 Introduction

The aim of the present experiment was to monitor changes in oil yield per unit area and oil composition, throughout the growing season in the major commercial production areas of Tasmania. This work was a continuation of an investigation reported by Clark (1976). By monitoring the above changes during several growing seasons an attempt has been made to obtain results on which future predictions of harvest time, may be based.

2.2 Materials and Methods

a. Location

In 1978, two experimental areas were established to investigate the effect of harvest date on oil yield and oil composition. The first of these areas was at "Rotherwood", Ouse, in the Derwent Valley area of Tasmania (Site 1), and the second location was in the Huon Valley area of Tasmania at Castle Forbes Bay (Site 2).

Both locations were within commercial plantings of *Mentha piperita* L. which had been established for 3 years. Plant densities at site 1 and site 2 were 30 to 60 plants/m^2 and 10 to 20 plants/m^2, respectively. With the exception of harvest date, all plots received the same treatments as the larger commercial area.

b. Layout and Treatments

Both trials consisted of three randomised complete blocks with nine plots within each block. Treatments (harvest dates) were allocated to plots within blocks according to tables of random numbers (Fisher and Yates, 1948). All plots were 1.5 x 1.5m in size. On the appropriate harvest date the central $1m^2$ of each plot was harvested. In this way, a

0.5m border was maintained between treatments. Samples were harvested at weekly intervals throughout the growing season commencing on 2 January 1978. The harvested samples were dried, subsampled, distilled and analysed in the normal way.

2.3 Results

a. Dry Matter Yield

Dry matter yield of plant material increased with time at both sites (Figure IV B 2.1). At site 1, a decrease occurred at the end of the experimental period (27 February 1978). With the exception of the last harvest date, dry matter yield from site 1 was significantly higher than from site 2.

b. Oil Yield

Oil yield per unit area increased initially at both sites (Figure IV B 2.2). At site 1 oil yield did not change significantly from 9 January to 20 February 1978, after which a decrease occurred. Oil yield continued to increase throughout the growing season at site 2. Site 1 yielded more oil per unit area than site 2, from 2 January to 13 February 1978.

c. Percentage Oil Yield

Generally, percentage oil yield (dry matter basis) decreased with time. There was no significant difference between sites (Figure IV B 2.3).

d. Oil Composition

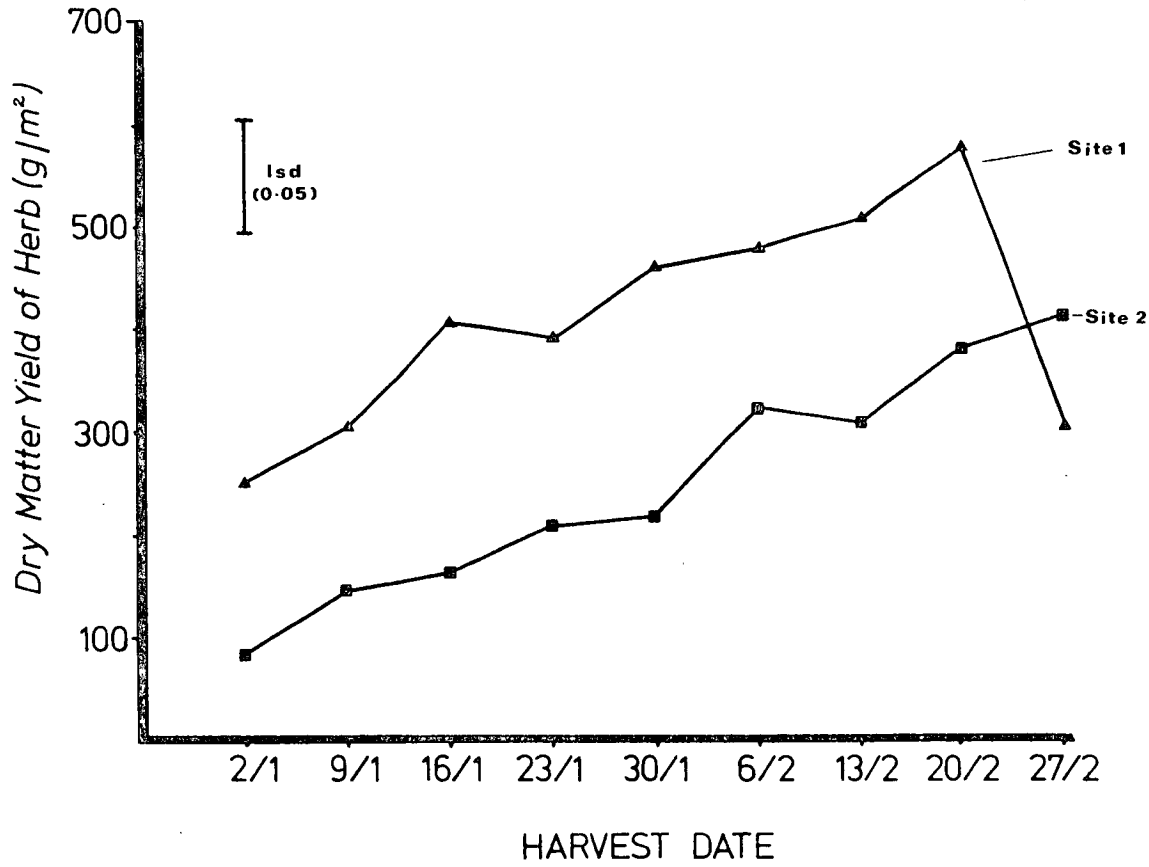
With respect to oil composition, menthone decreased from 2 January to 27 February 1978 at both sites (Figure IV B 2.4). At the beginning of the experimental period the concentration of menthone at site 2 was higher than at site 1, but these differences became less pronounced as the growing season progressed, resulting in no significant difference in menthone concentration between sites at the end of the

Figure IV B 2.1.

Dry matter yield of peppermint in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).

Figure IV B 2.2

Yield of peppermint oil in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).


700
500
300
100
Dry Matter Yield of Herb (g/m²)
lsd (0·05)
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

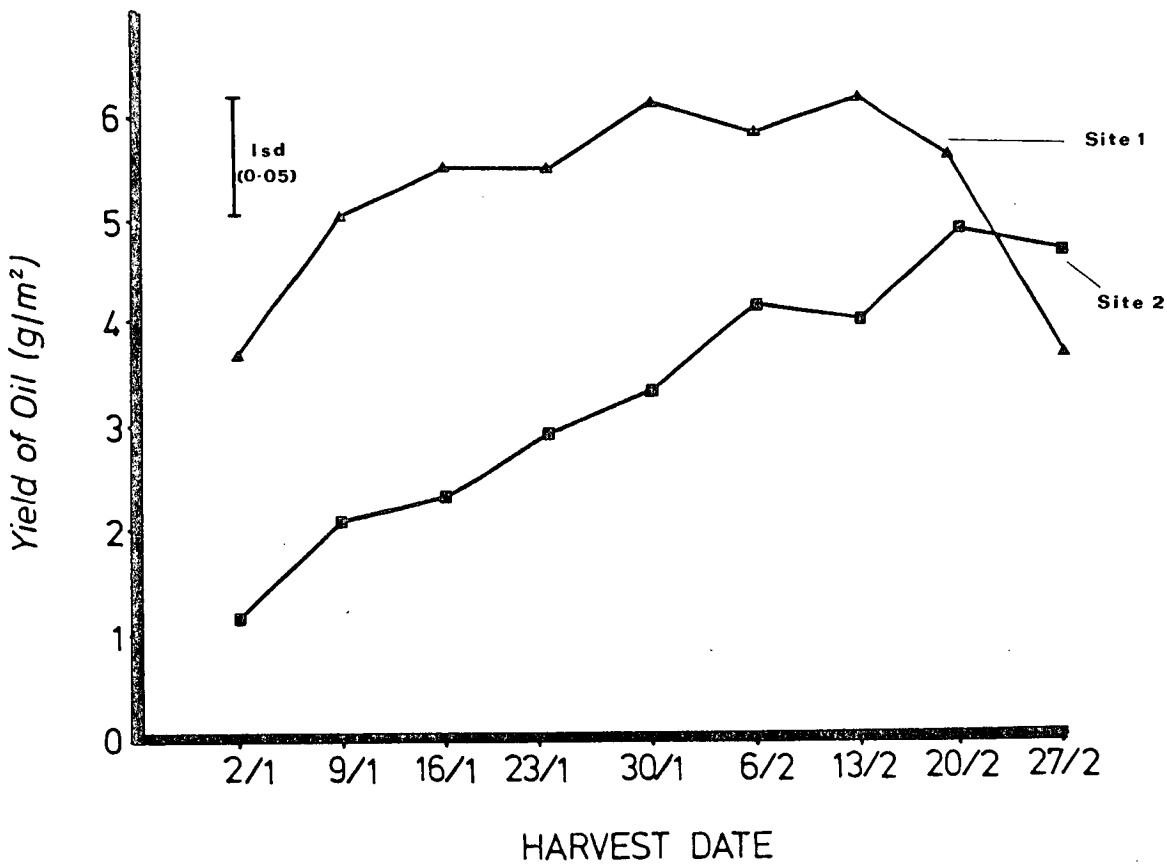
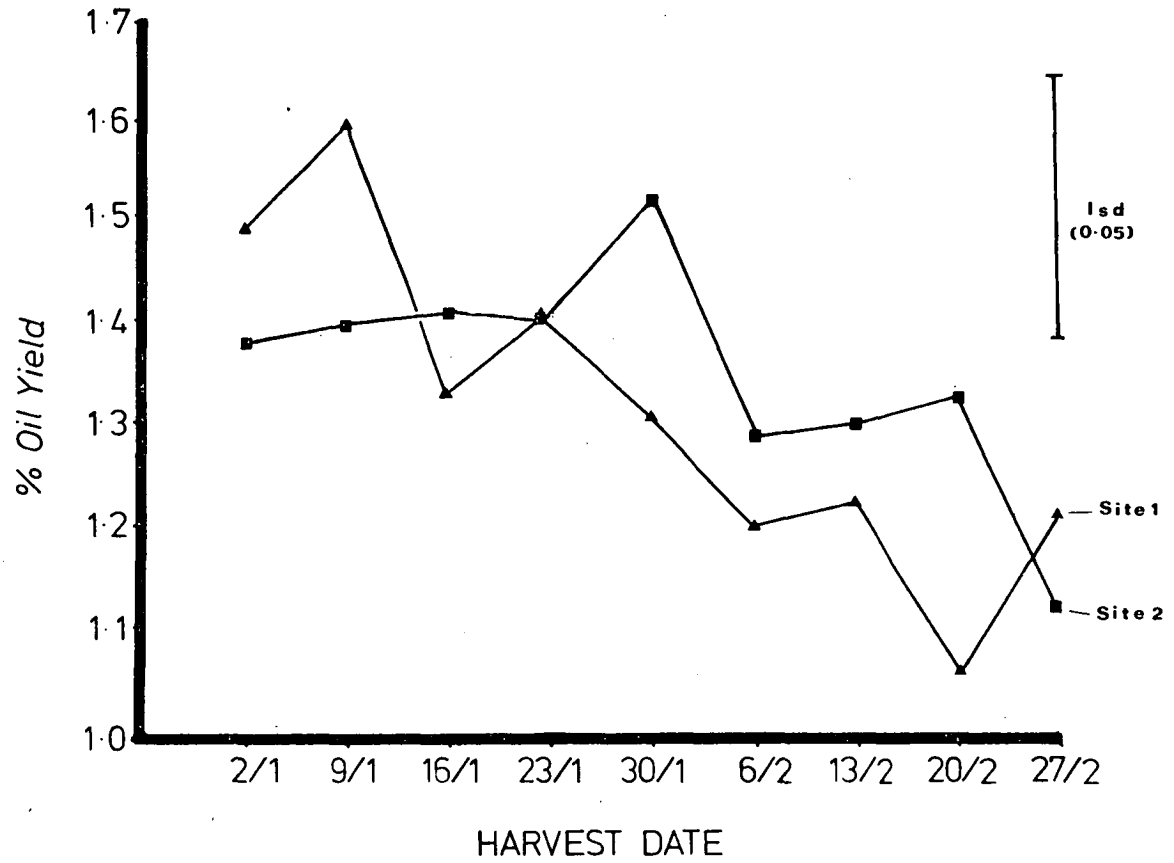

6
5
4
3
2
1
0
Yield of Oil (g/m²)
lsd (0·05)
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

Figure IV B 2.3

Percentage oil yield (dry matter basis) in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).

Figure IV B 2.4

Percentage menthone in peppermint oil in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).


1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
% Oil Yield
lsd (0·05)
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

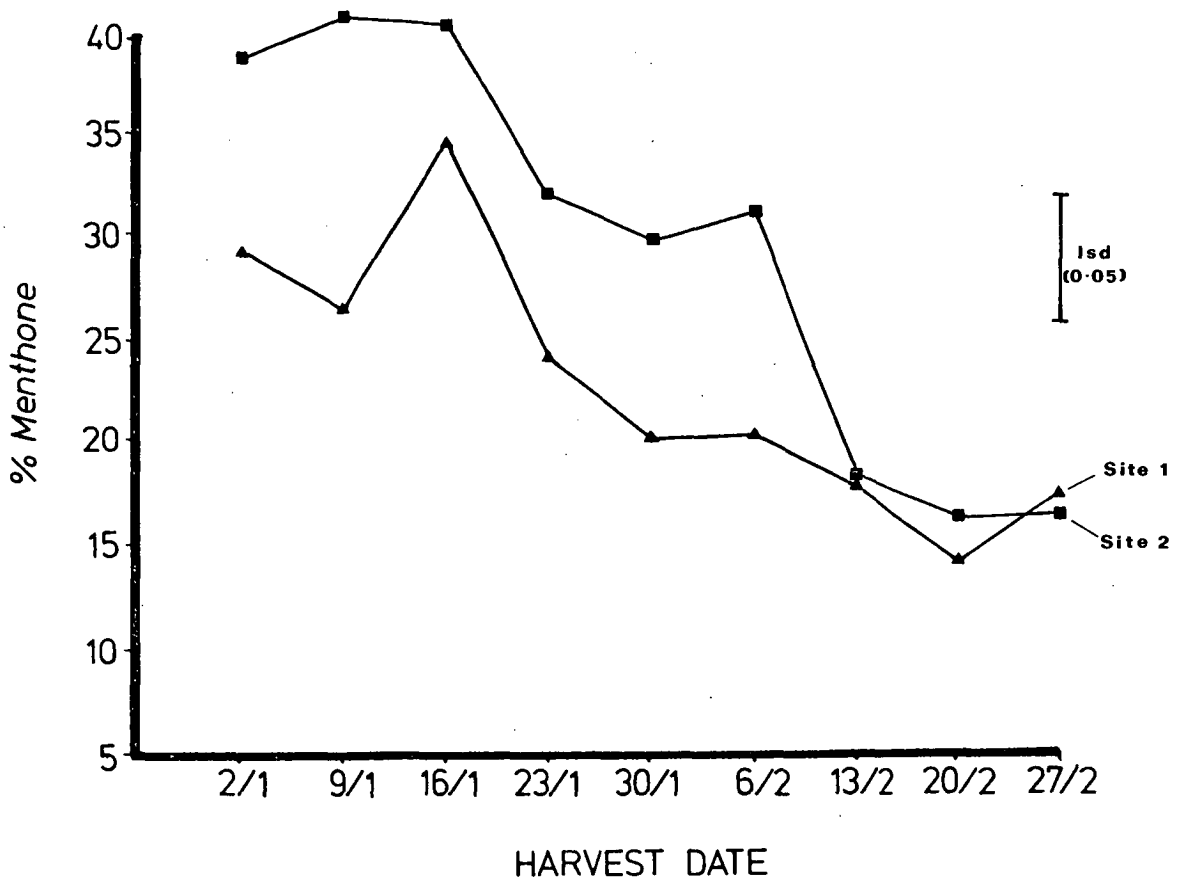
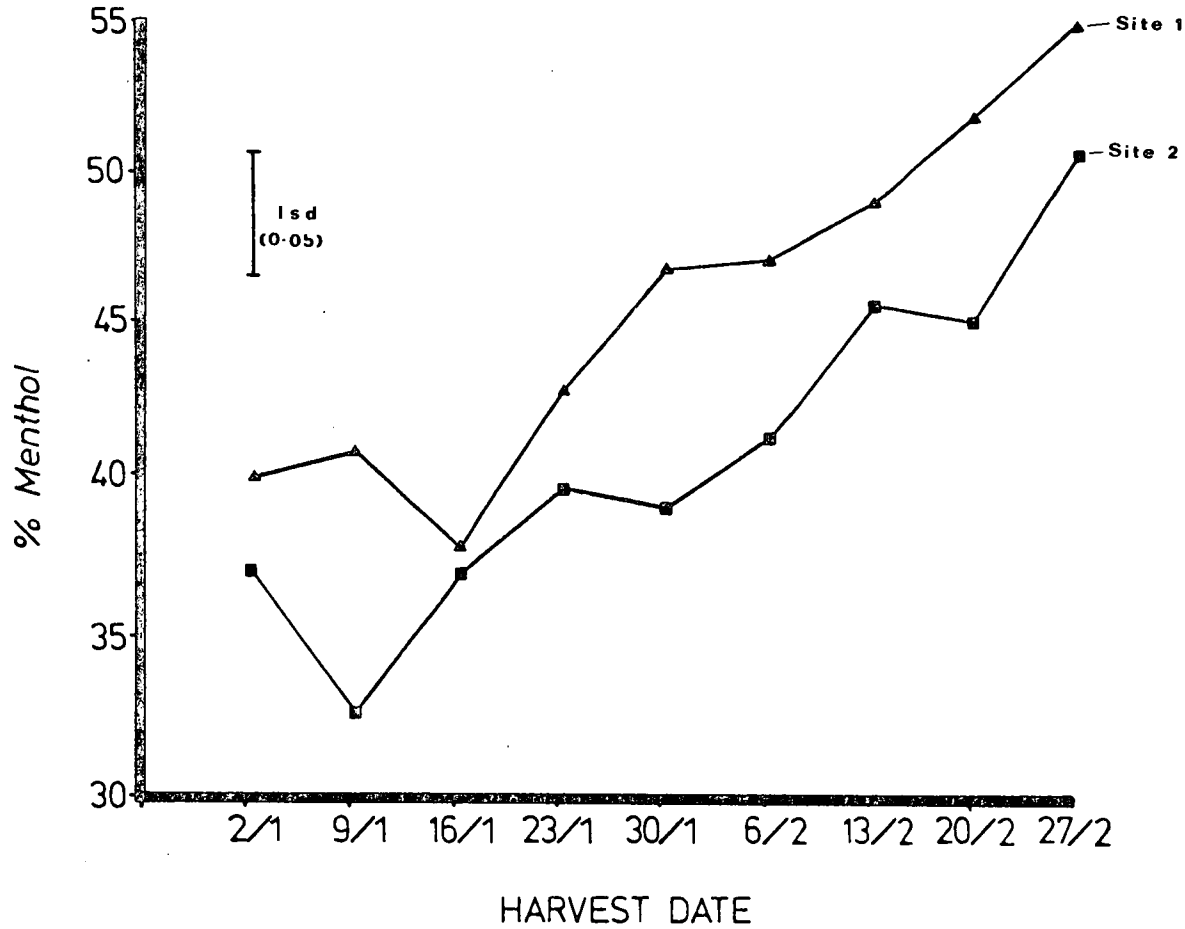

40
35
30
25
20
15
10
5
% Menthone
lsd (0·05)
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

Figure IV B 2.5

Percentage menthol in peppermint oil in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).

Figure IV B 2.6

Percentage menthyl acetate in peppermint oil in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).


55
50
45
40
35
30
% Menthol
lsd
(0·05)
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

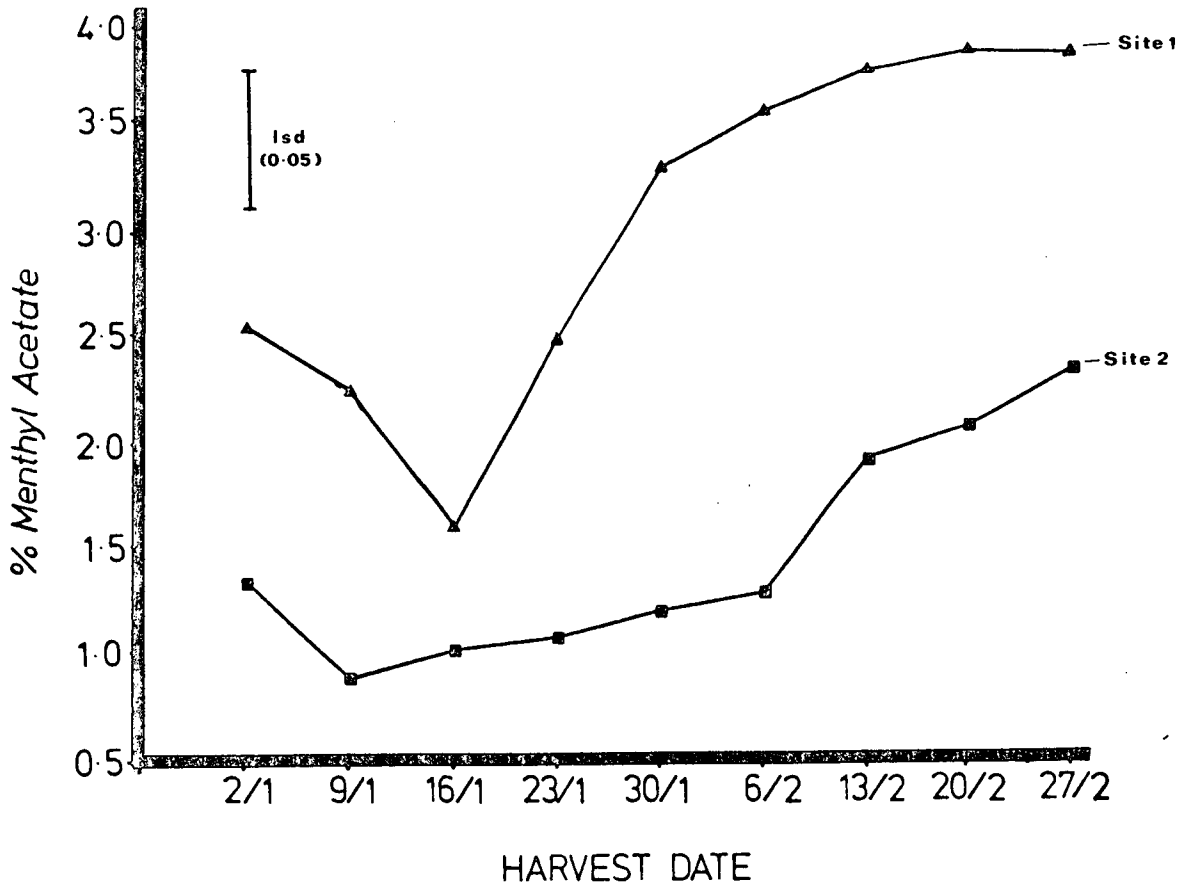
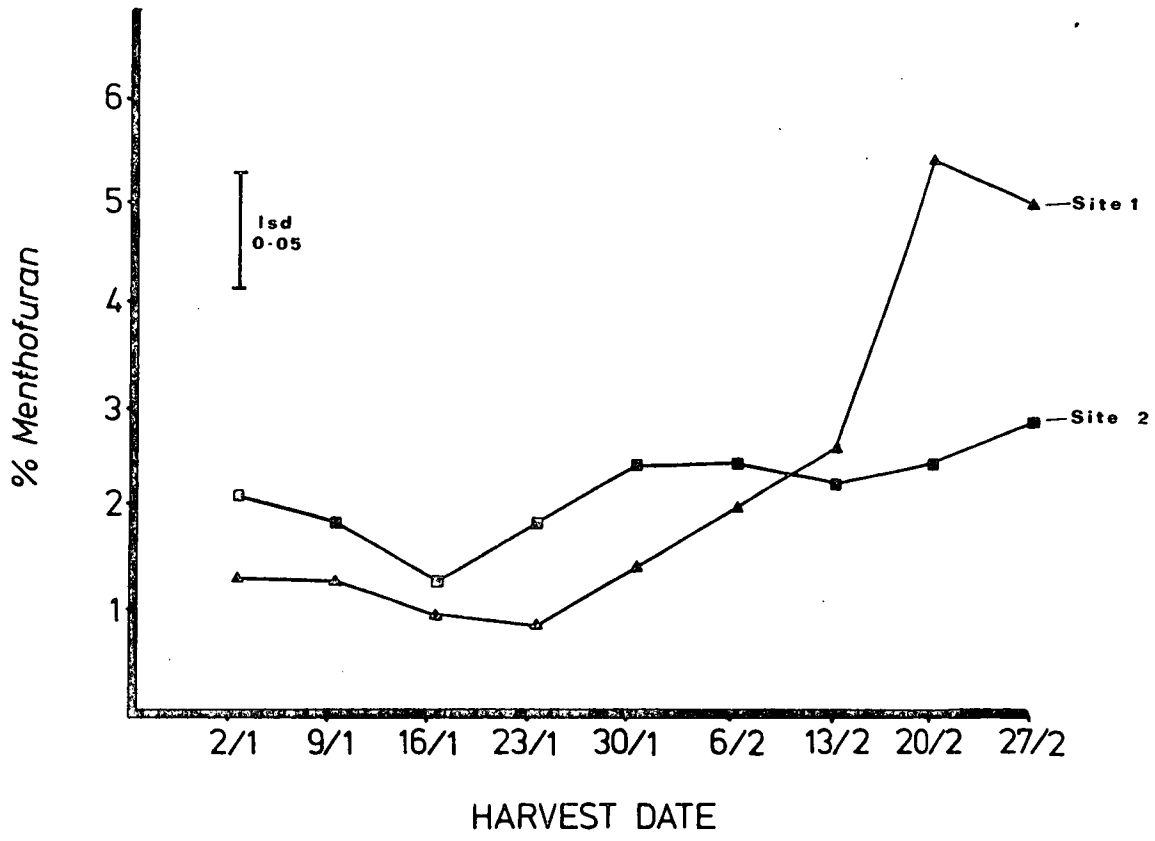

4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
% Menthyl Acetate
lsd
(0·05)
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

Figure IV B 2.7

Percentage of menthofuran in peppermint oil in relation to harvest date at "Rotherwood", Ouse (site 1) and Castle Forbes Bay (site 2).


% Menthofuran
6
5
4
3
2
1
lsd
0·05
Site 1
Site 2
2/1
9/1
16/1
23/1
30/1
6/2
13/2
20/2
27/2
HARVEST DATE

experiment.

Menthol concentration increased significantly from 2 January to 27 February 1978 at both sites (Figure IV B 2.5). At all harvest dates where a significant difference in the concentration of menthol existed between sites, oil from site 1 was higher in menthol.

Menthyl acetate increased overall from site 2 to site 1, and increased significantly during the experimental period (Figure IV B 2.6). Whereas harvest date had no significant effect on the concentration of menthofuran at site 2, an increase in menthofuran occurred at site 1 on 20 February and 27 February 1978 (Figure IV B 2.7). This increase in menthofuran concentration at site 1 resulted in a significant difference between sites on the last harvest date.

2.4 Discussion

Clark and Menary (1979) reported that at high plant densities (30 to 40 plants/m^2) oil yield per unit area increased initially during early January after which it remained constant for several weeks, under Southern Tasmanian conditions. During this period of constant oil yield per unit area, menthol increased to 45 percent. At low plant densities (10 plants/m^2), oil yield per unit area increased throughout the growing season. However, at these low plant densities an increase in oil yield per plant was not able to compensate for the very low number of plants present, within acceptable limits of oil quality. Therefore, oil yields per unit area were significantly lower when the low plant densities were considered.

As mentioned previously, plant densities at site 1 and site 2 were 30 to 60 plants/m^2 and 10 to 20 plants/m^2, respectively. In many respects the changes in oil yield per unit area at site 1 and site 2 were similar to changes in oil yield per unit area at high and low plant densities, respectively (Clark and Menary, 1979). At site 1 the yield of oil per

unit area reached a maximum early in the growing season, after which it remained unchanged for approximately 6 weeks. Thus harvesting should take place during the period of maximum oil yield per unit area and before any decrease occurs, provided the quality of the oil falls within acceptable levels.

Peppermint oil of high quality should contain no less than 45 percent menthol, have low levels of menthofuran as well as balanced amounts of the many other compounds (Guenther, 1949b). Provided that high plant densities were employed (site 1 or 30 to 40 plants/m^2), a menthol content of 45 percent was achieved during the period of maximum oil yield per unit area. In addition, the results indicated that if harvesting was delayed once the menthol levels were considered satisfactory, menthol did continue to increase but at the expense of increased levels of menthofuran.

3. The Effect of Irrigation and Nitrogen on the Yield and Composition of Peppermint Oil

3.1 Introduction

The intention of this work was to determine whether, by manipulating factors such as irrigation and nitrogen, the commercial yield of peppermint oil could be increased above that presently obtained (35 to 40 kg/ha). That is, are factors such as daylength, light intensity and temperature exerting a limiting effect on peppermint oil yields per unit area or are the low yields a reflection of the inadequacies associated with current cultural practices such as irrigation and nitrogen regimes. It was not intended to determine the specific irrigation and fertiliser requirements of peppermint. Manipulation of oil yield and quality by correct timing of irrigation to alleviate moisture stress late in the growing season, as suggested by Loomis (1977a),was attempted. Finally the possibility of obtaining two harvests of peppermint per season and the effect of irrigation and nitrogen on this possibility were examined.

3.2 Materials and Methods

a. Site and Layout

This experimental work was conducted in a commercial planting of *Mentha piperita* L. at "Rotherwood", Ouse, in the Derwent Valley area of Tasmania. The experiment was located in a 12 hectare field of row mint. The soil at this site had the following chemical properties:

Determination	Mean Value*
pH	6.2
Total Soluble Salts (%)	0.2
Nitrogen (N) aqueous extract (ppm)	20
Phosphorus (P) exchangeable (ppm)	21
Potassium (K) exchangeable (ppm)	28

*Mean value from three soil samples; one collected from each of the three blocks within the experiment. Each soil sample consisted of twenty cores taken at random, to a depth of 10cm. Sampling date was 1 December 1978 and analyses were conducted by the Government Analyst Laboratory, Hobart.

A split plot design with three randomised complete blocks was used. The main plots (irrigation treatments) were 8m x 12m in size. Each main plot was divided into four subplots (nitrogen fertiliser treatments) which were 4m x 6m in size.

b. Treatments

Irrigation. Irrigation commenced on 1 December 1978 and four irrigation treatments were included. These treatments were: 25mm of irrigation weekly, $I_{(L)}$; 25mm of irrigation twice weekly, $I_{(H)}$; 25mm of irrigation weekly during the first half of the growing season and twice weekly during the last half of the season, $I_{(L\rightarrow H)}$; 25mm of irrigation twice weekly during the first half of the growing season and 25mm weekly during the last half of the season, $I_{(H\rightarrow L)}$.

All plots received 25mm of irrigation weekly by overhead sprinkler through an Ajax travelling irrigator. The additional irrigation applied in treatments $I_{(H)}$, $I_{(L\rightarrow H)}$ and $I_{(H\rightarrow L)}$ was applied through a fixed sprinkler system. The exact quantity of water delivered by each system was not determined. However, the approximate quantity was determined from manufacturers' performance guides. For example, Pope "Lowthrow Premier" sprinkler was reported to deliver 2.92 inches/hour when the discharge pressure was 30 p.s.i. Both irrigation systems were fitted with pressure gauges and both the pressure and the duration of irrigation were controlled.

The approximate input of water from both irrigation and rainfall, in each irrigation treatment, throughout the season, is presented in Figure IV B 3.1. Figure IV B 3.2 compares the 1978-79 rainfall with the mean long-term rainfall (24 years) at "Rotherwood", Ouse.

Nitrogen. Four treatments involving rates of applied fertiliser nitrogen were used:

50kg N/ha, $N_{0.5}$; 100kg N/ha, N_1;

200kg N/ha, N_2; 300kg N/ha, N_3.

The fertiliser nitrogen was applied as ammonium nitrate ("Nitram") in split applications. A basal dressing of 100kg K/ha as murate of potash and 50kg P/ha as high grade superphosphate was applied to each plot. Fertilisers were applied to all plots on 1 December 1978 and irrigation commenced immediately. The commercial irrigation level (25mm) was applied to all plots once weekly and the additional irrigation received by some plots four days later. On 13 January 1979 the second application of nitrogen was applied to all plots and the irrigation treatments were altered according to the previously mentioned programme. The experimental layout and allocation of treatments to plots is given in Figure IV B 3.3.

c. Pest and Disease Control

Spraying to control peppermint rust (*Puccinia menthae*) and bud worms (*Heliothis* sp.) was conducted on 17 February 1979. This spray programme consisted of 260g of Plantvax and 260g of Orthene 75 per 200ℓ with an application rate of 16ℓ/100m^2.

d. Harvesting

Throughout the growing season samples of herb were taken from areas adjacent to the experimental area at weekly intervals to establish the stage of maturity of the crop. Harvesting of the experimental area was conducted on 16 February 1979. On this date the main commercial planting had reached a stage at which the oil extracted contained 45

Figure IV B 3.1

Figure IV B 3.1.

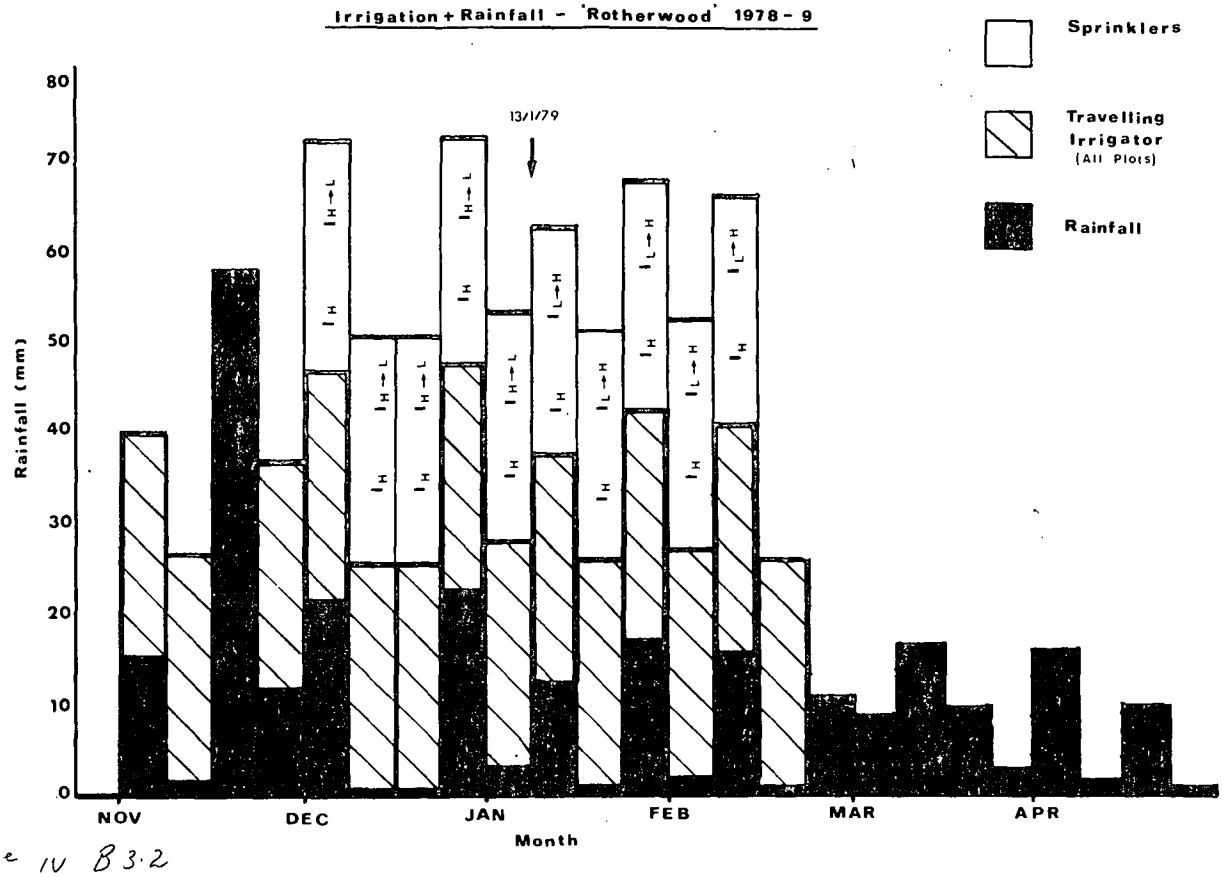


Figure IV B 3.2

Figure IV B 3.2

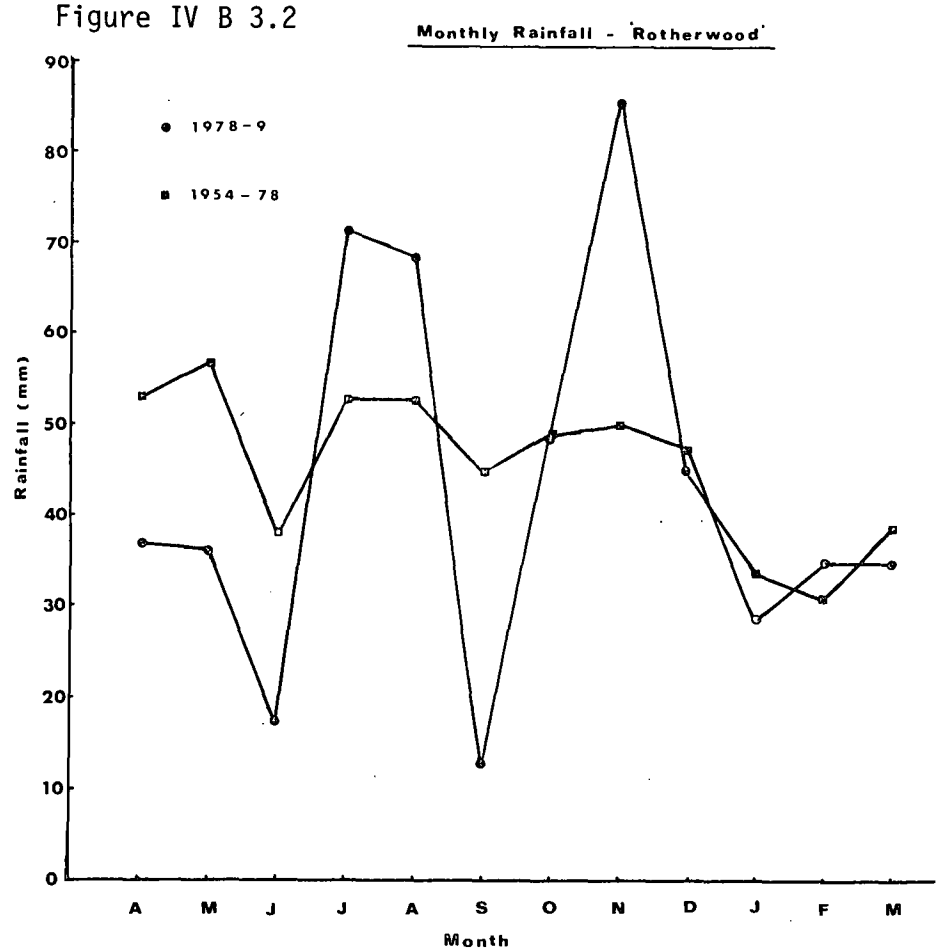
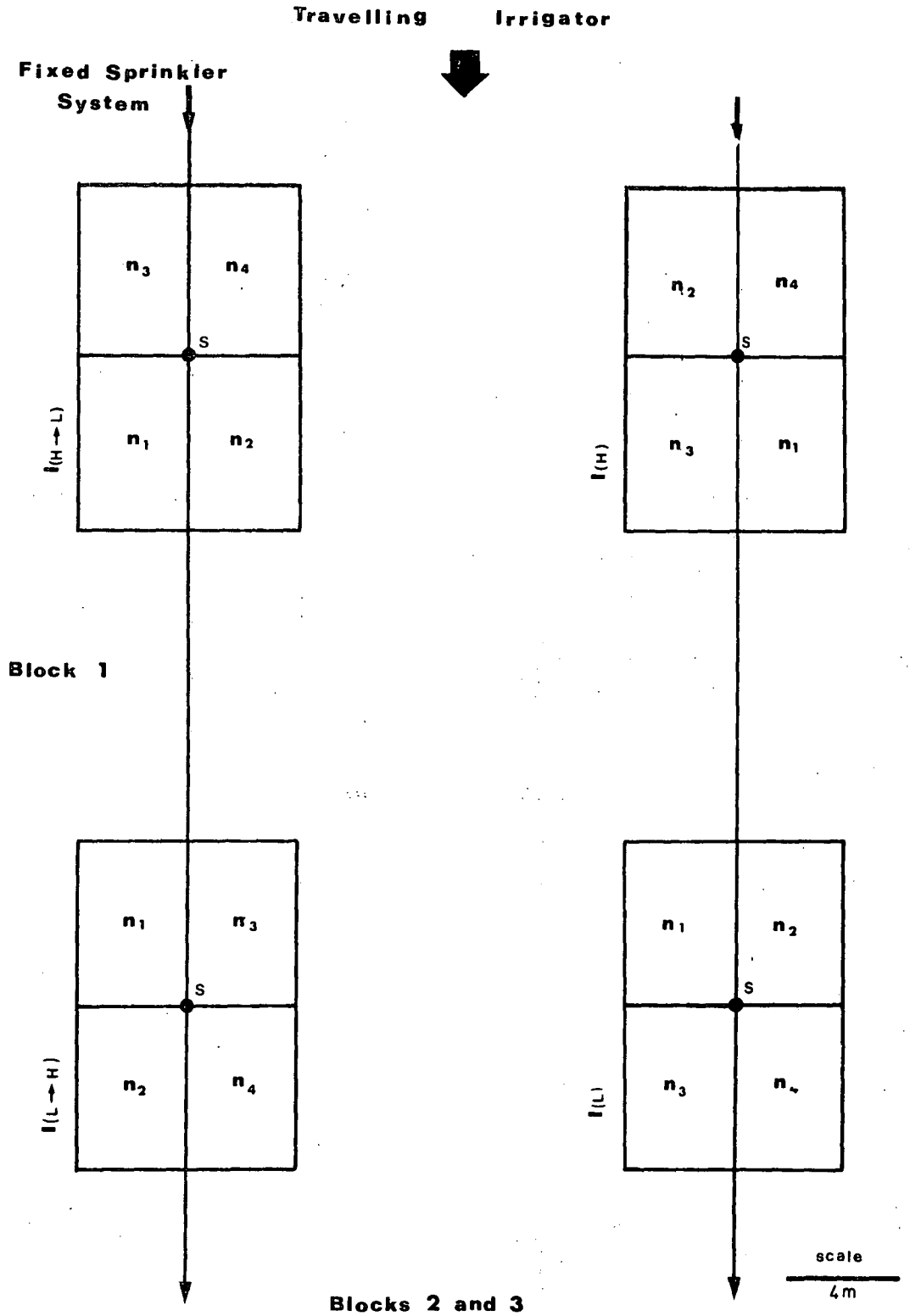


Figure IV B 3.3.



percent menthol. Maximum oil yield per unit area and optimum oil composition have been observed to correspond to this stage of maturity (Section IV B 2).

At harvest all plots were cut with a swath mower, herb weighed and subsamples of 2kg taken for dry matter determination and steam distillation. The material was air dried for one day, steam distilled, and oil yield, dry matter yield, percentage oil yield and oil composition determined.

e. Porometry

On several occasions throughout the growing season, leaf diffusive resistance measurements were conducted on plants from each irrigation treatment. These measurements were taken at midday, on both the abaxial and adaxial leaf surfaces of the first fully expanded leaf, using a Lambda L1-65 autopormeter fitted with a L1-20S sensor.

f. Regrowth

Following the first harvest (16 February 1979) all plots received 25mm of irrigation, after which no irrigation was applied. On 25 April 1979 three quadrat samples (0.6 x 0.3m) of regrowth plant material were harvested from each plot. The subsequent determinations made on these samples were the same as outlined above (d).

g. Analysis of Results

The results were analysed as a split plot in time and space. Since there were no significant differences between the whole unit errors and the sub unit errors, these error terms were pooled and the experiment analysed as a factorial design (Steel and Torrie, 1960). The statistical significance of all data is based on LSD (5%).

3.3 Results

In the following discussion, harvest 1 will refer to plants harvested on 16 February 1979 and harvest 2 (or regrowth) will refer to regrowth plants harvested on 25 April 1979.

a. Dry Matter Yield (g/m^2)

Dry matter at harvest 1 increased with a nitrogen rate of 100kg N/ha in irrigation treatments $I_{(H)}$ and $I_{(H\rightarrow L)}$ (Figure IV B 3.4). In the above irrigation treatments, increasing nitrogen to 300kg N/ha had no effect on dry matter yield. There was no effect of nitrogen on the dry matter yield obtained from irrigation treatments $I_{(L)}$ and $I_{(L\rightarrow H)}$. At each nitrogen level, irrigation treatments $I_{(L)}$ and $I_{(H\rightarrow L)}$ yielded less than $I_{(H)}$ and $I_{(L\rightarrow H)}$.

At harvest 2, dry matter yield from $I_{(H)}$ and $I_{(L\rightarrow H)}$ increased with nitrogen to 300kg N/ha, and with the exception of yields at 50 kg N/ha, yielded significantly more than $I_{(L)}$ and $I_{(H\rightarrow L)}$. There was no significant effect of nitrogen on dry matter yield response when the nitrogen rate was increased from 100kg N/ha to 200kg N/ha. With the exception of $I_{(L\rightarrow H)}$ at 300kg N/ha, each irrigation-nitrogen treatment yielded highest at harvest 1.

b. Oil Yield (g/m^2)

Oil yield increased with increased nitrogen in irrigation treatments $I_{(H)}$ and $I_{(L\rightarrow H)}$ at both harvest dates with the maximum yield at 300kg N/ha, except in treatment $I_{(L\rightarrow H)}$ at harvest 1 where the highest yield was reached at 200kg N/ha (Figure IV B 3.5). At harvest 1, $I_{(L\rightarrow H)}$ yielded more oil than $I_{(H)}$ at 50kg N/ha and 200kg N/ha. However, at harvest 2 both irrigation treatments had similar yields at each level of nitrogen.

The response of oil yield to nitrogen was less pronounced in irrigation treatments $I_{(L)}$ and $I_{(H\rightarrow L)}$. At harvest 1 the oil yield resulting from irrigation treatment $I_{(L)}$ was highest at 200 kg N/ha.

This also applied to harvest 2, although no significant decrease in oil yield resulted from increasing nitrogen to 300kg N/ha in the later harvest treatment. In irrigation treatment $I_{(H\rightarrow L)}$ at harvest 1, oil yield was highest at 200kg N/ha and was not significantly altered by increasing nitrogen to 300kg N/ha. At harvest 2, oil yields obtained from irrigation treatment $I_{(H\rightarrow L)}$ were not influenced by nitrogen fertiliser.

Oil yields resulting from irrigation treatments $I_{(H)}$ and $I_{(L\rightarrow H)}$ were higher than from irrigation treatments $I_{(L)}$ and $I_{(H\rightarrow L)}$, at both harvests when 300kg N/ha was applied. Such differences became less pronounced at low levels of fertiliser nitrogen.

c. Percentage Oil Yield

Overall, the percentage oil yield was highest at harvest 1 when 200 to 300 kg N/ha was applied (Table IV B 3.1). Irrigation treatments had no pronounced effects on percentage oil yield.

Oil Composition

In general, treatment effects on oil composition were most pronounced at harvest 2 and the oil composition varied with harvest date.

The percentage α-pinene and β-pinene was highest at harvest 2 (Figures IV B 3.6 and 3.7). Neither irrigation nor nitrogen treatments resulted in any overall effect on the percentage α-pinene or β-pinene.

The percentage limonene was significantly higher at harvest 2 than harvest 1, and this was most pronounced at the higher levels of nitrogen (Figure IV B 3.8). Overall, there was no significant change in limonene with increased nitrogen at harvest 1. At harvest 2, increased nitrogen resulted in an overall increase in limonene.

Cineole decreased from harvest 1 to harvest 2 and showed no response to nitrogen (Figure IV B 3.9). Menthone was lower at harvest 2 than harvest 1 (Figure IV B 3.10). At harvest 1 nitrogen had no significant

Figure IV B 3.4

The effect of irrigation and nitrogen on the dry matter peppermint; 2 harvests.

Figure IV B 3.5

The effect of irrigation and nitrogen on the oil yield of peppermint; 2 harvests.

Key to Figures

Harvest 1

$I_{(L)}$ ○——○

$I_{(H \to L)}$ ▲——▲

$I_{(L \to H)}$ ■——■

$I_{(H)}$ ●——●

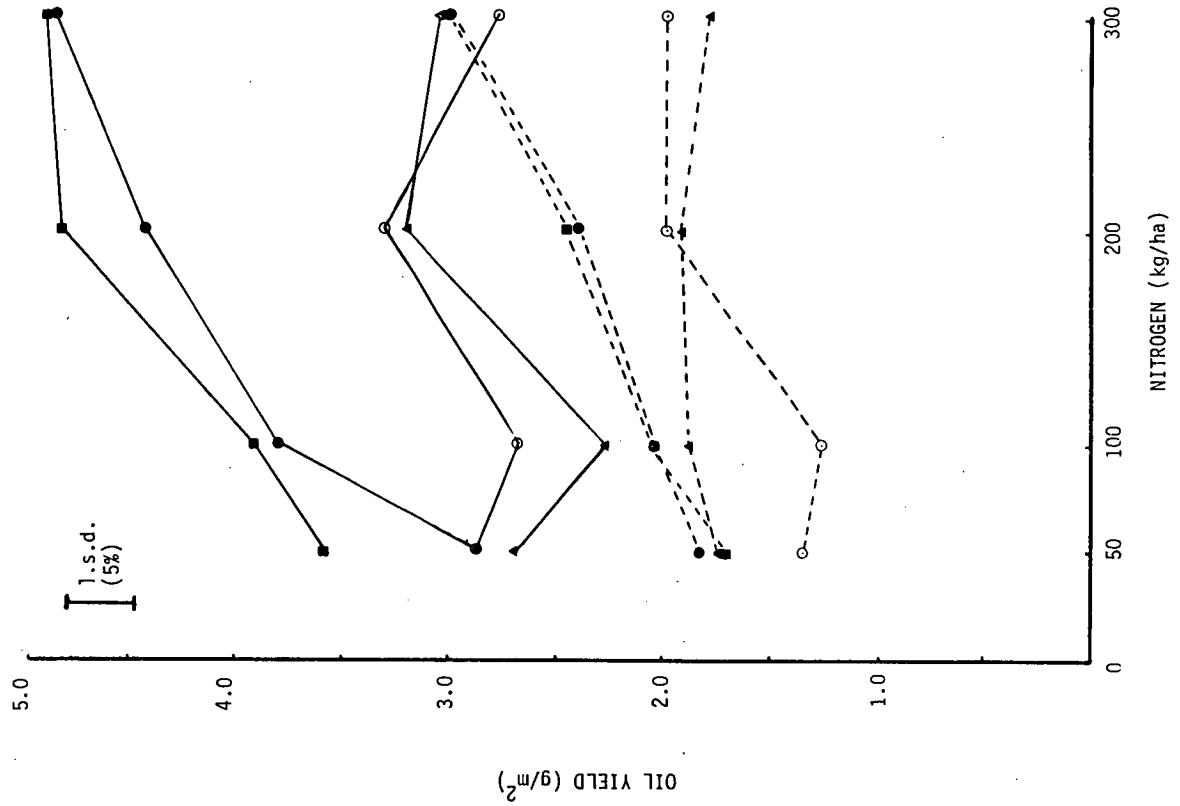
Harvest 2

$I_{(L)}$ ○- - - - - - - ○

$I_{(H \to L)}$ ▲- - - - - - - ▲

$I_{(L \to H)}$ ■- - - - - - - ■

$I_{(H)}$ ●- - - - - - - ●


OIL YIELD (g/m²)
5.0
4.0
3.0
2.0
1.0
0
50
100
200
300
NITROGEN (kg/ha)
l.s.d.
(5%)

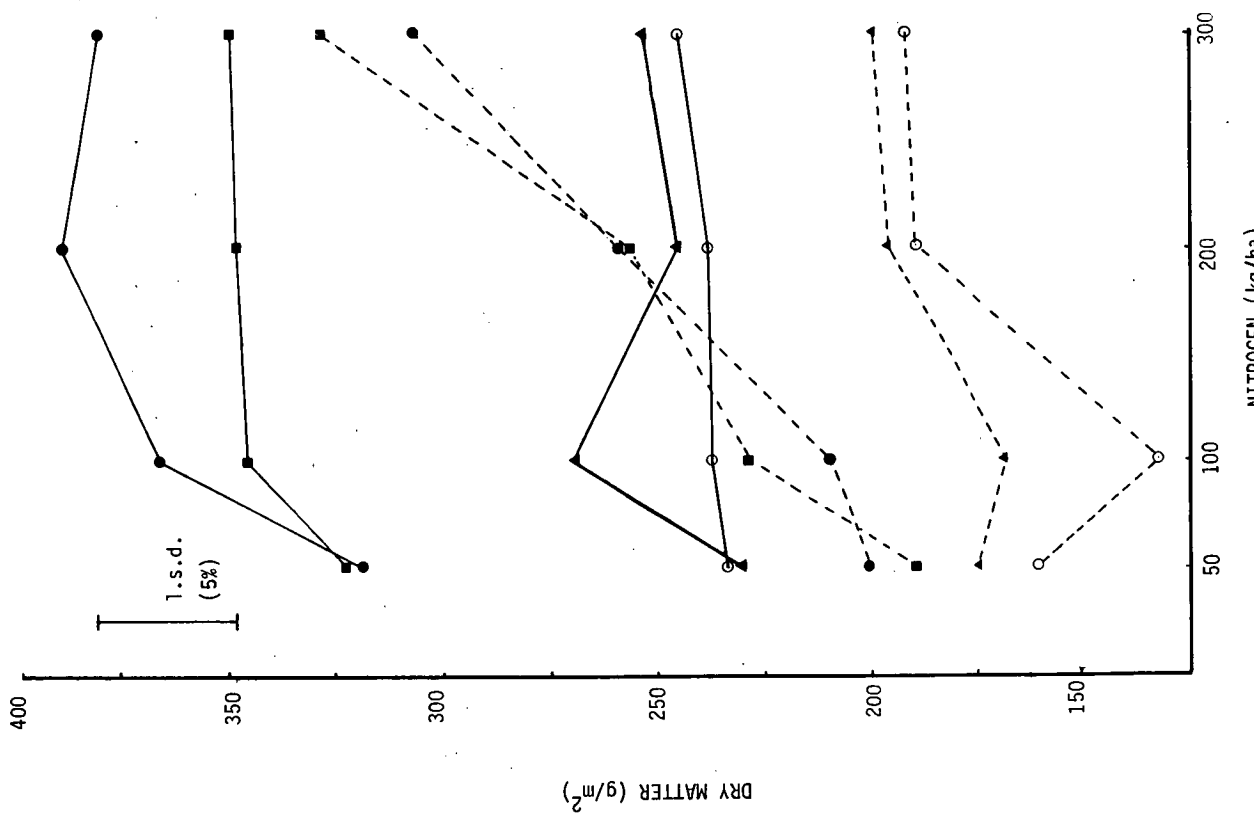

DRY MATTER (g/m²)
400
350
300
250
200
150
50
100
200
300
NITROGEN (kg/ha)
l.s.d.
(5%)

Table IV B 3.1. Percentage oil yield (dry matter basis).

Nitrogen	N1		N2		N3		N4	
Harvest No.	1	2	1	2	1	2	1	2
Irrigation								
$I_{(L)}$	1.247	0.843	1.130	0.943	1.403	1.053	1.140	1.070
$I_{(H \to L)}$	1.177	1.013	0.850	1.123	1.313	1.000	1.223	0.900
$I_{(L \to H)}$	1.110	0.930	1.133	0.900	1.397	0.957	1.407	0.920
$I_{(H)}$	0.903	0.927	1.047	1.003	1.147	0.930	1.283	0.993

LSD (t = 0.05) = 0.168 (nitrogen x harvest date x irrigation).

effect on menthone concentration, with the exception of the low menthone concentration in treatment $I_{(L)}$ at 200 kg N/ha. At harvest 2, irrigation treatments $I_{(L)}$ and $I_{(H\rightarrow L)}$ had similar menthone concentrations, both of which were significantly lower than irrigation treatments $I_{(H)}$ and $I_{(L\rightarrow H)}$. These effects were most pronounced at high levels of nitrogen. In irrigation treatments $I_{(H)}$ and $I_{(L\rightarrow H)}$, the concentration of menthone increased in response to nitrogen rate in the range 100 to 300kg N/ha.

The percentage menthofuran was higher at harvest 2 than harvest 1 (Figure IV B 3.11). Neither irrigation nor nitrogen treatments affected menthofuran at harvest 1. At harvest 2, irrigation treatments $I_{(L)}$ and $I_{(H\rightarrow L)}$ had similar menthofuran concentrations at 300kg N/ha as did irrigation treatments $I_{(H)}$ and $I_{(L\rightarrow H)}$. The latter irrigation treatments were significantly lower in menthofuran. This effect was less pronounced when lower levels of nitrogen fertiliser were applied. The highest percentage isomenthone occurred at harvest 1, with the exception of treatment $I_{(H\rightarrow L)}$ 50kg N/ha, at harvest 1. Irrigation and nitrogen treatments had little effect on the percentage of isomenthone (Figure IV B 3.12).

Menthyl acetate was higher at harvest 2 than harvest 1 (Figure IV B 3.13). Neither nitrogen nor irrigation treatments significantly affected the percentage menthyl acetate at harvest 1. At harvest 2 there was a significant effect of irrigation treatments on menthyl acetate. An increase in menthyl acetate with irrigation treatments occurred in the following order: $I_{(H)} < I_{(L\rightarrow H)} < I_{(H\rightarrow L)} < I_{(L)}$. Menthol was highest at harvest 2 (Figure IV B 3.14). At harvest 1, all irrigation treatments resulted in similar concentrations of menthol, when 300kg N/ha was applied. At harvest 2 irrigation treatments $I_{(H)}$ and $I_{(L\rightarrow H)}$ had similar menthol concentrations, which were considerably lower than $I_{(L)}$ and $I_{(H\rightarrow L)}$.

The percentage of neomenthol and pulegone in oil from each treatment is presented in Tables IV B 3.2 and 3.3, respectively. Neither nitrogen nor irrigation treatments had any pronounced effects on either compound. However, the analysis of variance data included in Appendix IV B 3.2 indicates that harvest date had a significant overall effect on both compounds, neomenthol was highest at harvest 1, and pulegone was highest at harvest 2.

e. Porometry

Leaf diffusive resistance measurements taken during the first half of the growing season (31 December 1978, 7 January 1979) indicated no significant difference in the degree of stomatal opening between irrigation treatments (Table IV B 3.4). On 28 January 1979 and 11 February 1979 the degree of stomatal opening was highest in irrigation treatments $I_{(H)}$ and $I_{(L \rightarrow H)}$. (This assumes that a lower leaf diffusive resistance indicates a higher degree of stomatal opening.)

3.4 Discussion

Increasing the level of irrigation from $I_{(L)}$ to $I_{(H)}$ increased dry matter and oil yield at both harvests. The timing of irrigation was important, increased application rate during the last half of the growing season being most effective. The additional irrigation received by treatment $I_{(H)}$ relative to $I_{(L \rightarrow H)}$ had no adverse effect on dry matter or oil yield. This does not support the suggestion made by Loomis (1977) that water stress induced early in summer to produce small, drought-tolerant leaves, may increase oil yields. The differences which existed between irrigation treatments at harvest 1 were evident in the subsequent regrowth, even though irrigation treatments were terminated at the time of first harvest.

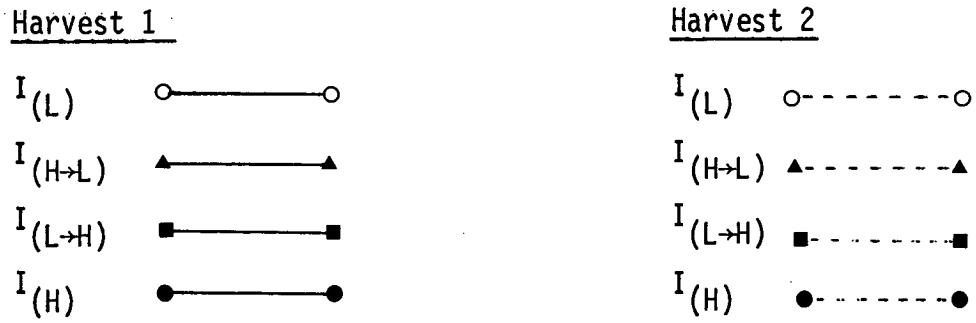
Figure IV B 3.6

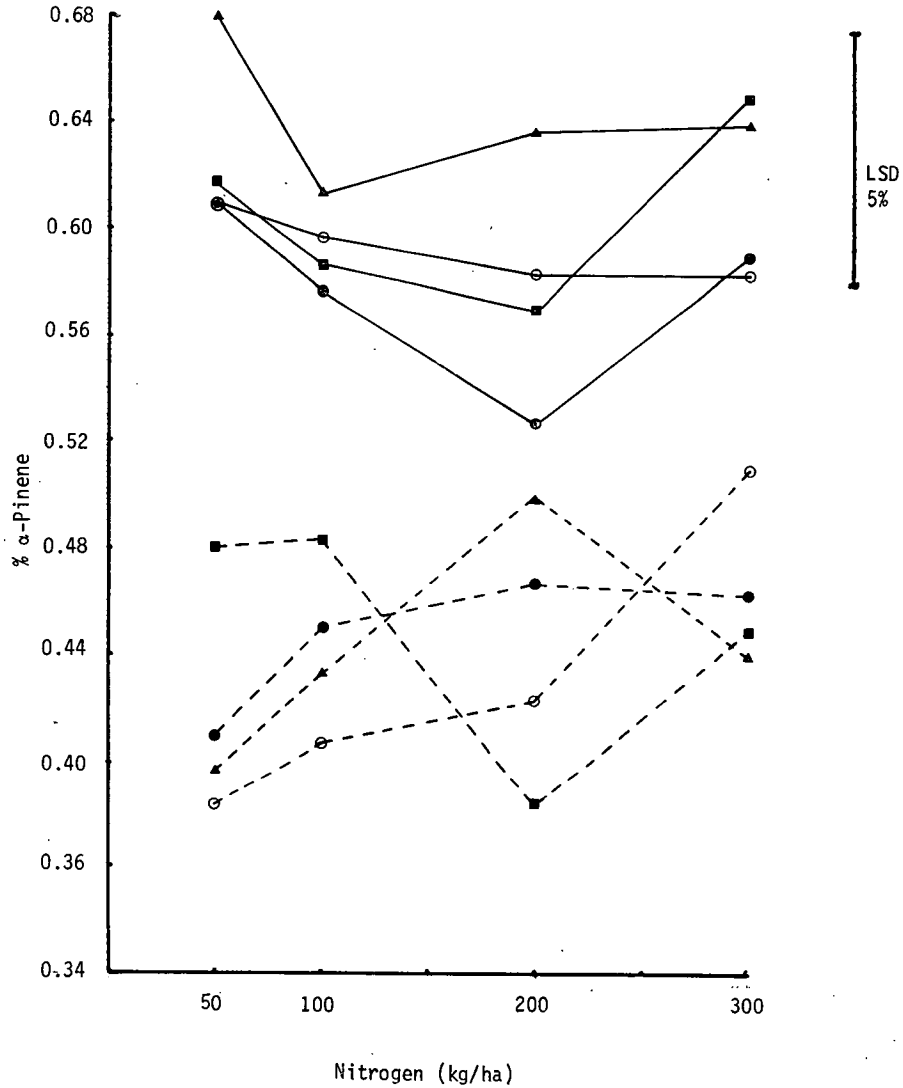
The effect of irrigation and nitrogen on the percentage of α-pinene in peppermint oil; 2 harvests.

Figure IV B 3.7

The effect of irrigation and nitrogen on the percentage of β-pinene in peppermint oil; 2 harvests.

Key to Figures




0.68
0.64
0.60
0.56
0.52
0.48
0.44
0.40
0.36
0.34
% α-Pinene
LSD
5%
50
100
200
300
Nitrogen (kg/ha)

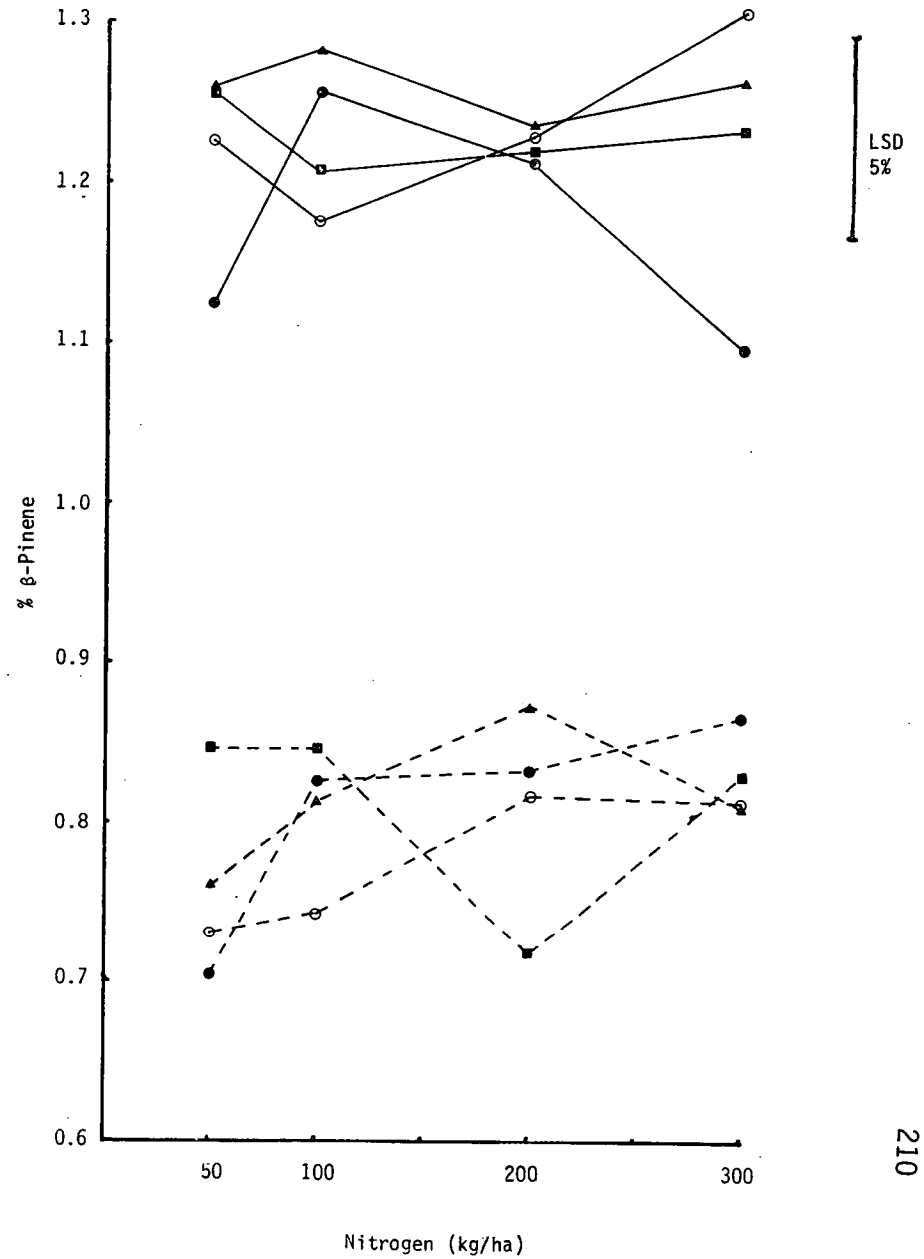

1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
% β-Pinene
LSD
5%
50
100
200
300
Nitrogen (kg/ha)

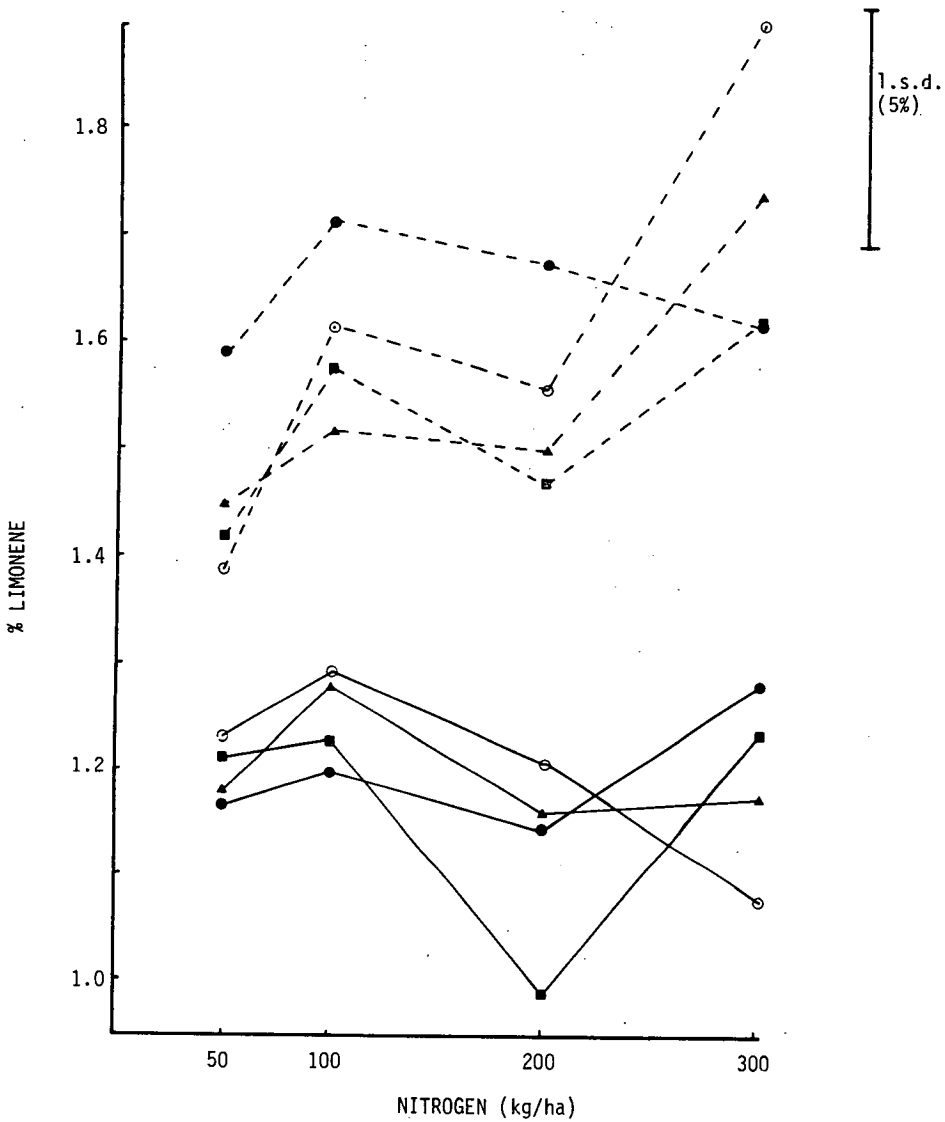
Figure IV B 3.8

The effect of irrigation and nitrogen on the percentage limonene in peppermint oil;
2 harvests.

Figure IV B 3.9

The effect of irrigation and nitrogen on the percentage cineole in peppermint oil;
2 harvests.

Key to Figures


% LIMONENE
1.8
1.6
1.4
1.2
1.0
50
100
200
300
NITROGEN (kg/ha)
l.s.d.
(5%)

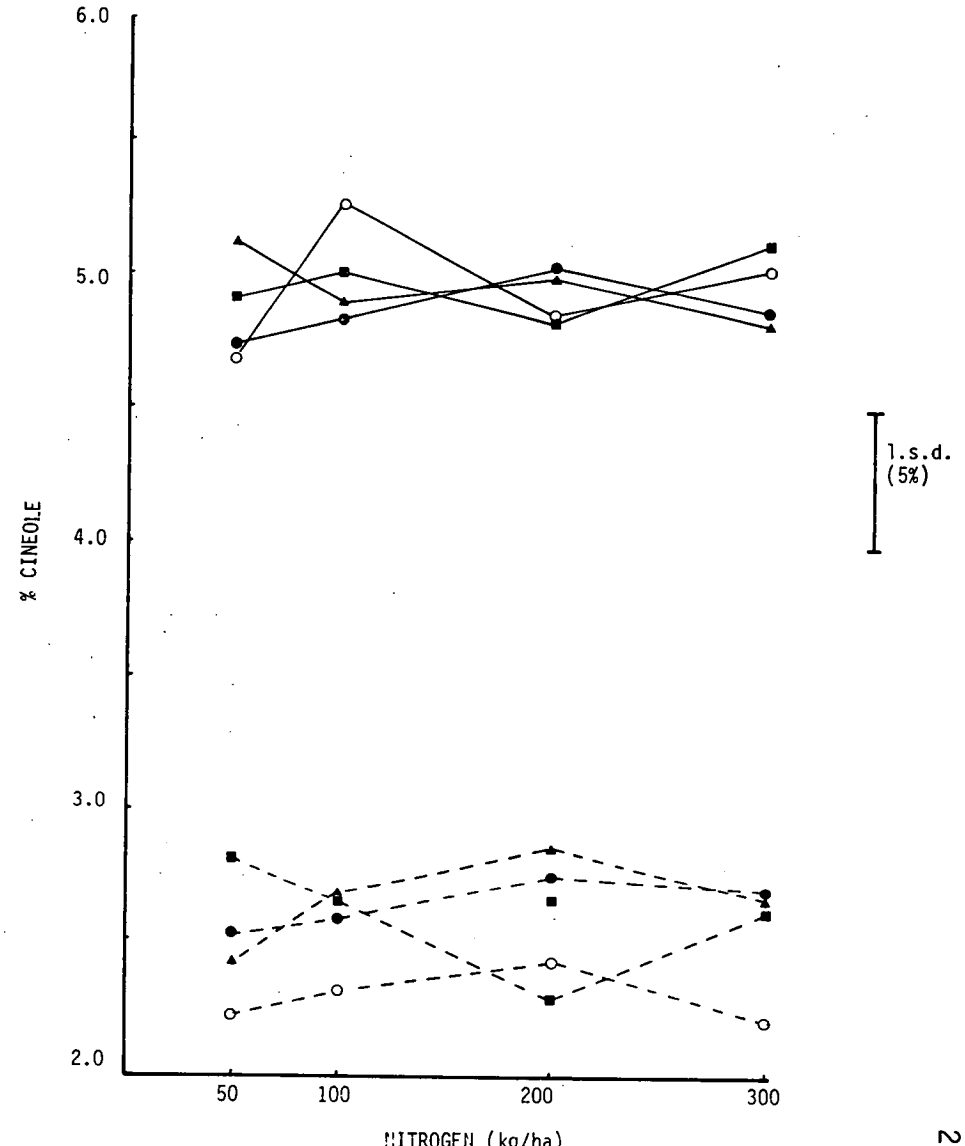

% CINEOLE
6.0
5.0
4.0
3.0
2.0
50
100
200
300
NITROGEN (kg/ha)
l.s.d.
(5%)

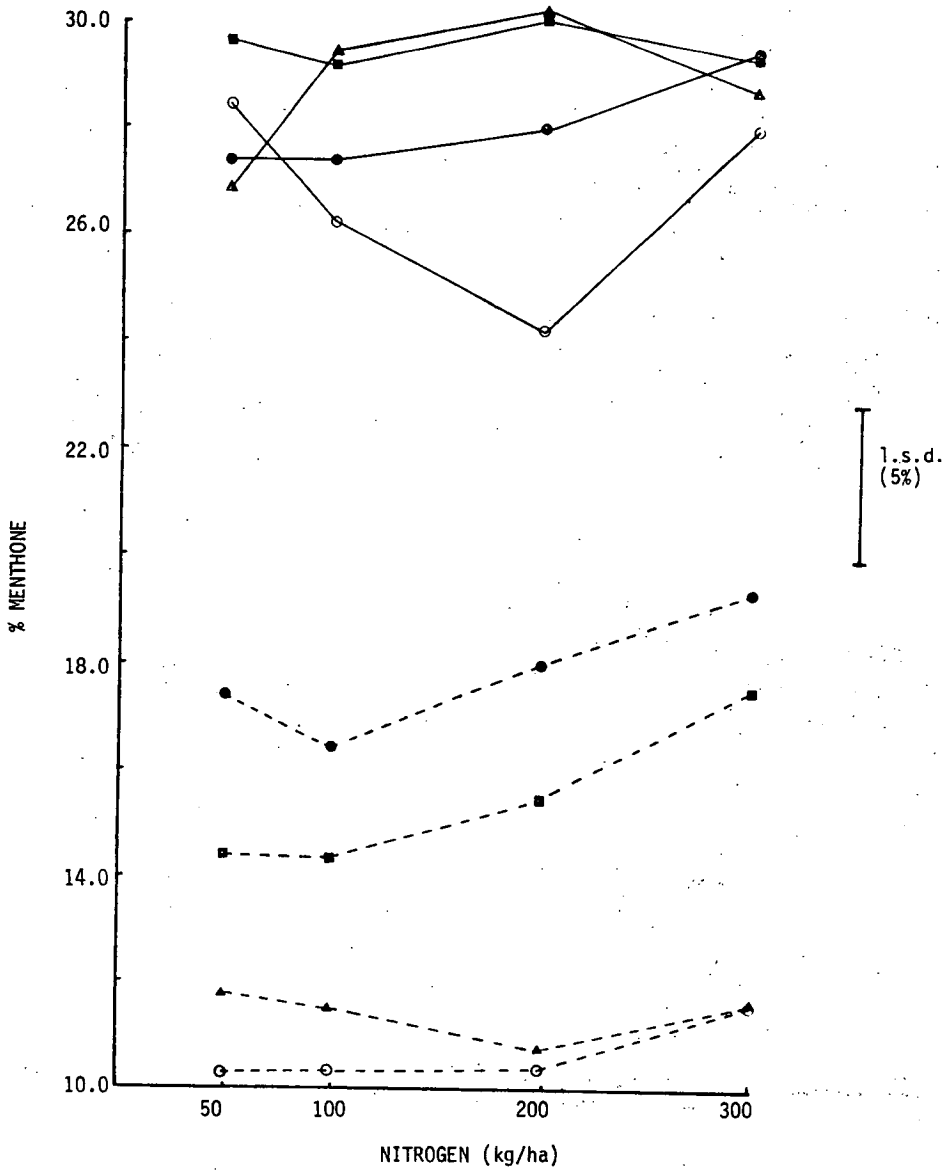
Figure IV B 3.10

The effect of irrigation and nitrogen on the percentage menthone in peppermint oil; 2 harvests.

Figure IV B 3.11

The effect of irrigation and nitrogen on the percentage menthofuran in peppermint oil; 2 harvests.

Key to Figures


30.0
26.0
22.0
18.0
14.0
10.0
% MENTHONE
l.s.d.
(5%)
50
100
200
300
NITROGEN (kg/ha)

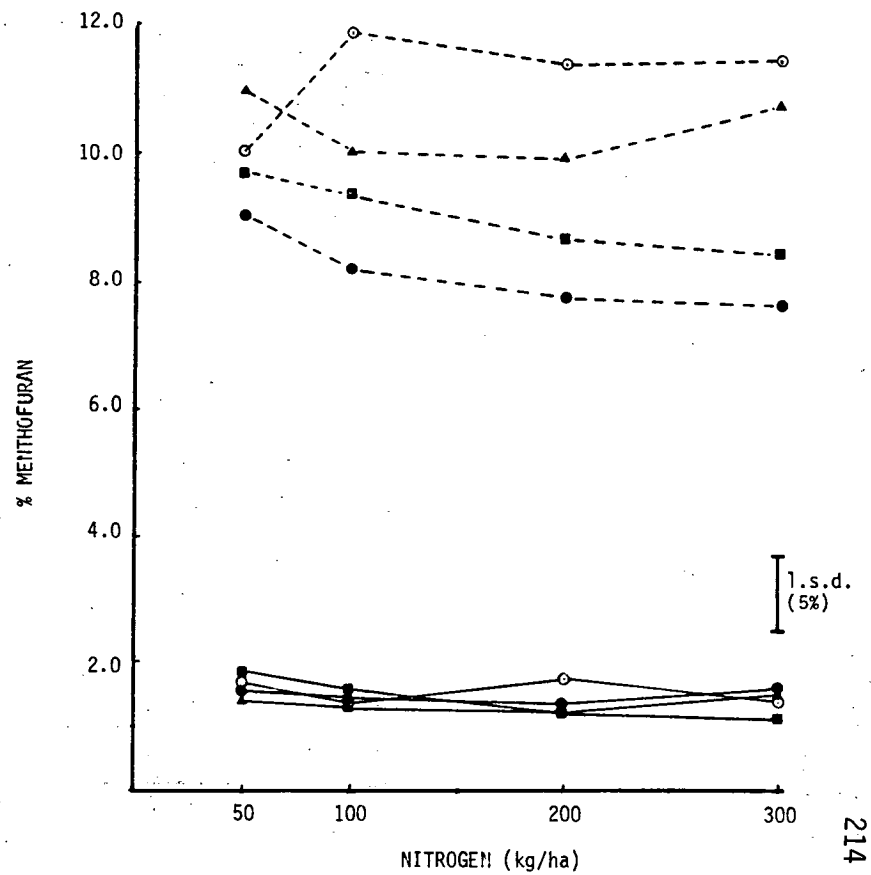

12.0
10.0
8.0
6.0
4.0
2.0
% MENTHOFURAN
l.s.d.
(5%)
50
100
200
300
NITROGEN (kg/ha)

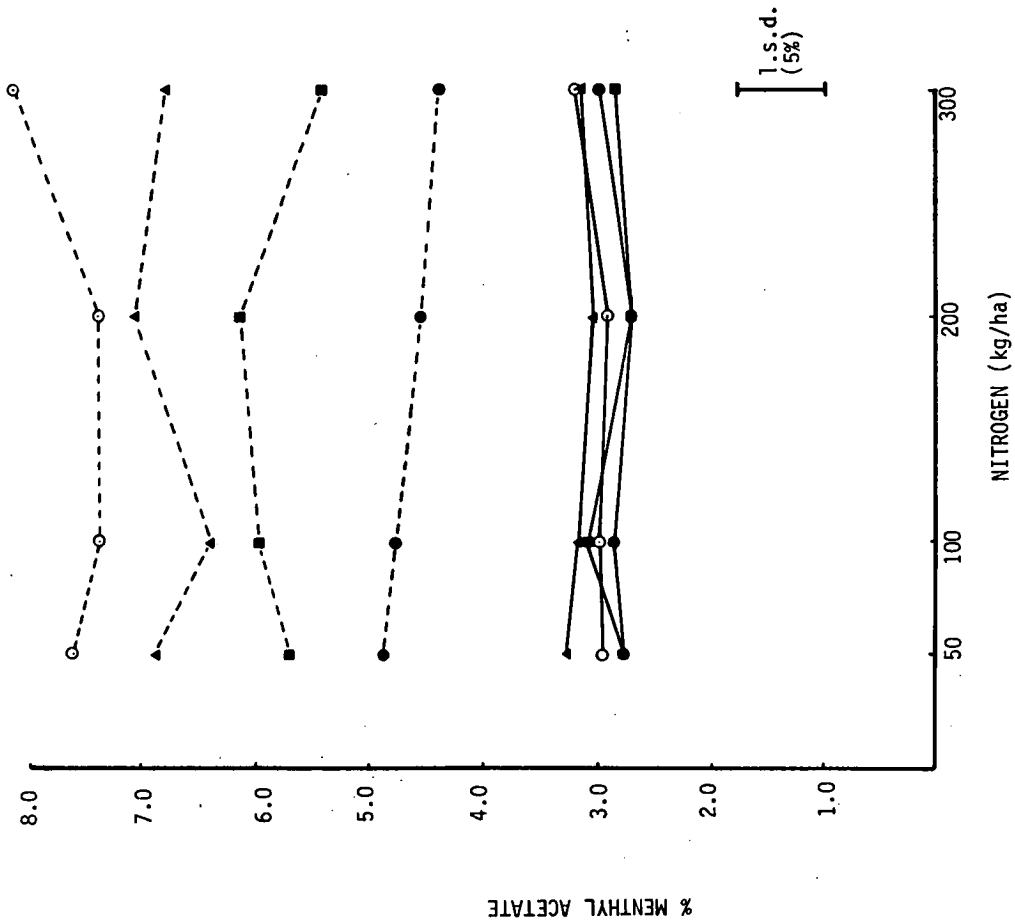
Figure IV B 3.12

The effect of irrigation and nitrogen on the percentage of isomenthone in peppermint oil; 2 harvests.

Figure IV B 3.13

The effect of irrigation and nitrogen on the percentage of menthyl acetate in peppermint oil; 2 harvests.

Key to Figures


% MENTHYL ACETATE
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
l.s.d. (5%)
50
100
200
300
NITROGEN (kg/ha)

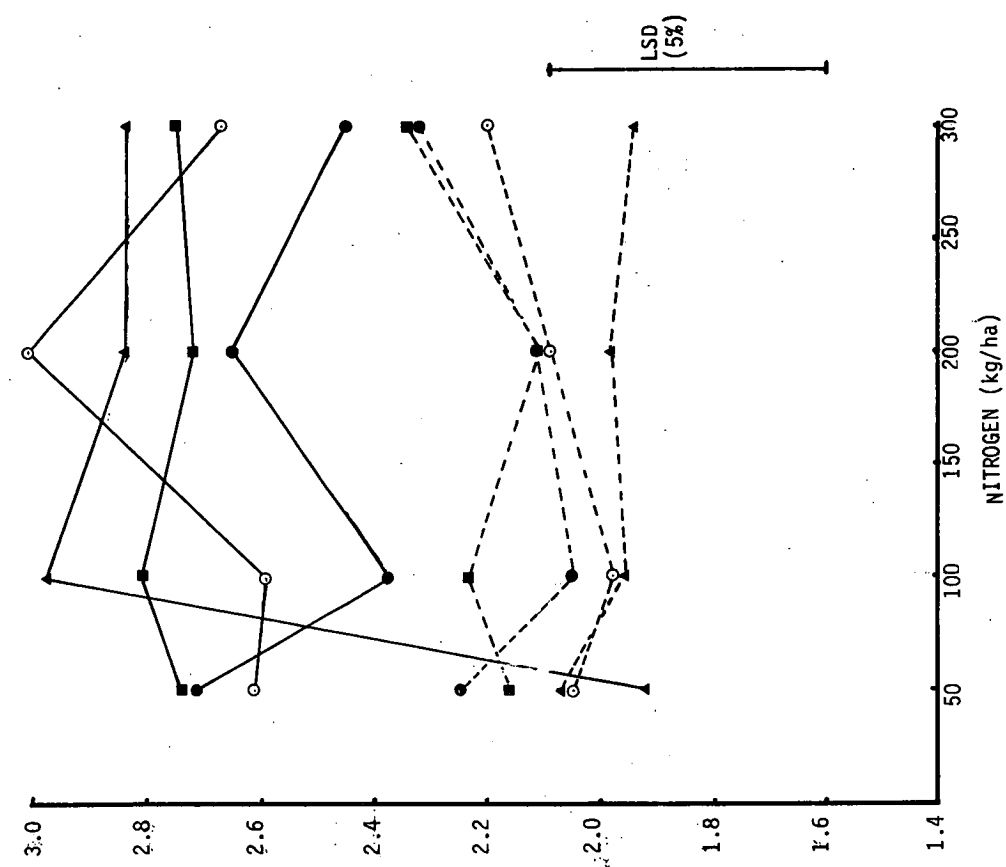

% ISOMENTHONE
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
LSD (5%)
50
100
150
200
250
300
NITROGEN (kg/ha)

Figure IV B 3.14

The effect of irrigation and nitrogen on the percentage menthol in peppermint oil;
2 harvests.

Key to Figure

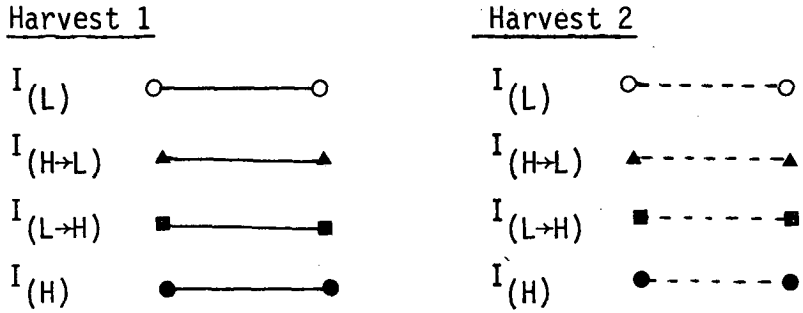


Table IV B 3.2

The effect of irrigation and nitrogen on the percentage neomenthol in peppermint oil;
2 harvests.

Table IV B 3.3

The effect of irrigation and nitrogen on the percentage of pulegone in peppermint oil;
2 harvests.

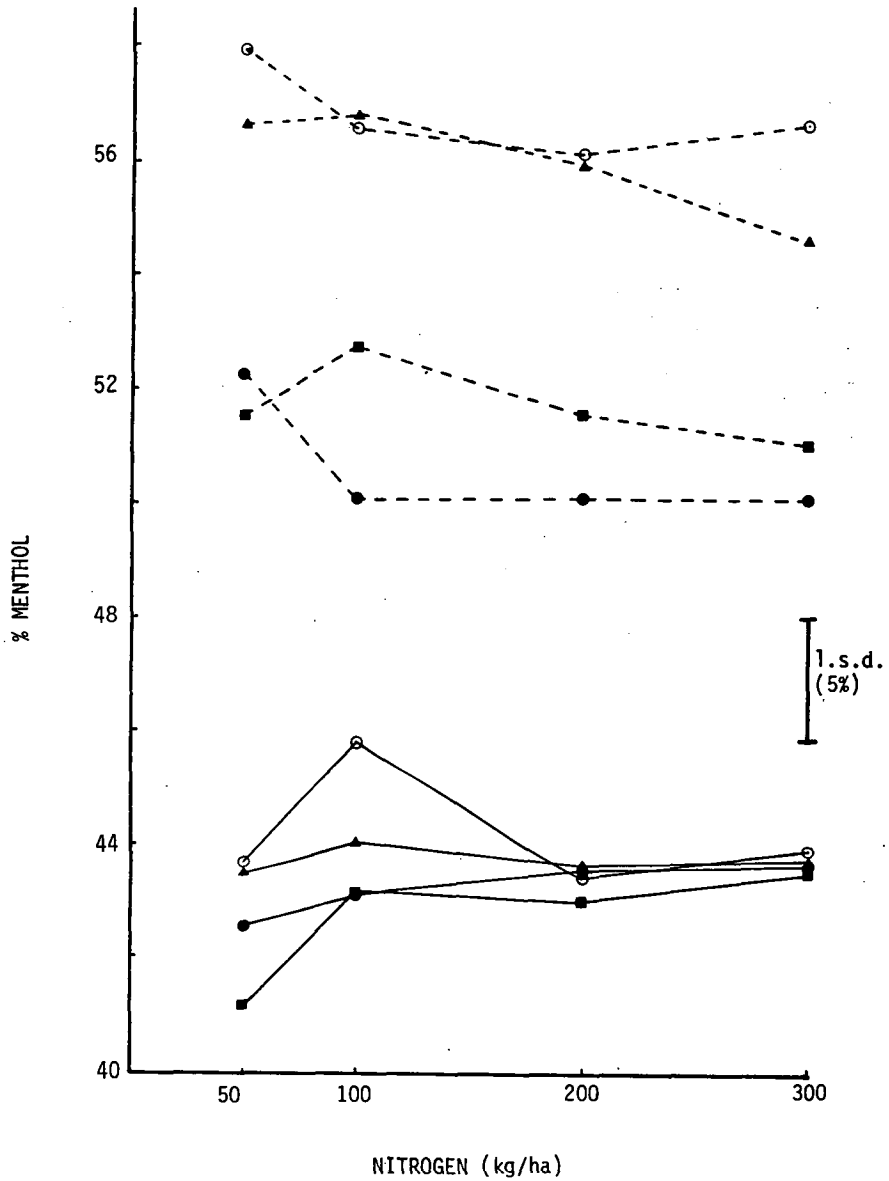


Table IV B 3.2. % Neomenthol.

Nitrogen	N_1		N_2		N_3		N_4	
Harvest No.	1	2	1	2	1	2	1	2
Irrigation								
$I_{(L)}$	3.470	3.270	3.707	3.103	3.837	3.107	3.250	3.590
$I_{(H\rightarrow L)}$	3.310	3.150	3.443	3.260	3.337	3.717	3.970	3.093
$I_{(L\rightarrow H)}$	3.350	3.113	3.420	3.593	3.697	3.383	3.413	3.553
$I_{(H)}$	3.383	2.937	3.473	2.927	3.583	2.783	3.473	3.340

LSD (t = 0.05) = 0.560 (nitrogen x harvest date x irrigation).

Table IV B 3.3 % Pulegone.

Nitrogen	N_1		N_2		N_3		N_4	
Harvest No.	1	2	1	2	1	2	1	2
Irrigation								
$I_{(L)}$	1.330	1.760	1.243	1.683	1.300	1.623	1.323	1.813
$I_{(H\rightarrow L)}$	1.347	1.710	1.200	1.820	1.660	1.440	1.553	1.763
$I_{(L\rightarrow H)}$	1.630	1.443	1.473	1.827	1.317	1.897	1.323	1.930
$I_{(H)}$	1.243	1.537	1.100	1.670	1.353	1.857	1.317	1.200

LSD (t = 0.05) = 0.427 (nitrogen x harvest date x irrigation).

Table IV B 3.4. Leaf diffusive resistance* (s cm^{-1}).

Date	Irrigation treatment				LSD (5%)
	$I_{(H)}$	$I_{(L\rightarrow H)}$	$I_{(H\rightarrow L)}$	$I_{(L)}$	
Abaxial surface					
31/12/78	1.45	1.35	1.40	1.43	0.563
7/1/79	1.64	1.58	1.50	1.59	0.602
28/1/79	1.65	1.62	6.34	7.87	1.367
11/2/79	1.78	1.83	7.53	6.72	0.967
Adaxial surface					
31/12/78	64.98	62.62	66.18	64.79	9.26
7/1/79	67.17	68.07	66.77	70.60	11.43
28/1/79	71.99	68.12	96.07	94.02	9.13
11/2/79	69.14	71.16	98.98	97.86	12.16

*Average result of five measurements in each of 3 blocks - 300kg N/ha subplots. Measurements conducted at the end of the weekly irrigation regime.

From leaf diffusive resistance measurements it appeared that irrigation treatments $I_{(L)}$ and $I_{(L\rightarrow H)}$ provided sufficient moisture to allow the plants to maintain a degree of stomatal opening, similar to treatments $I_{(H)}$ and $I_{(H\rightarrow L)}$ during the first half of the growing season. Since the degree of stomatal closure was greater in treatments $I_{(L)}$ and $I_{(H\rightarrow L)}$ relative to $I_{(H)}$ and $I_{(L\rightarrow H)}$during the latter half of the growing season, this indicated that plants receiving lower amounts of irrigation at this stage were experiencing water stress. The importance of irrigation in the latter half of the season may result from the alleviation of water stress which does not seem to develop until this latter stage. The fact that plants in irrigation treatment $I_{(L)}$ did not show signs of water stress in the early growing season would suggest that the lower irrigation regime was adequate and this may explain the previously mentioned, apparent disagreement with Loomis (1977a).

The response of oil yield to increased application of nitrogen was most pronounced in irrigation treatments receiving high amounts of irrigation late in the growing season. That is, highest yields of oil can only be obtained when both nitrogen and irrigation are increased.

Increased irrigation [$I_{(L)}$ to $I_{(L\rightarrow H)}$], increased the yield of oil from 28kg/ha to 48kg/ha when 300kg N/ha was applied. The yield of oil at harvest 2 [$I_{(L\rightarrow H)}$, 300kg N/ha] was similar to that obtained under present commercial growing conditions [$I_{(L)}$, 50kg N/ha]. However, it should be noted that a heavy, uniform infestation of rust occurred prior to harvest 1, resulting in the unusually low yields of oil at this harvest. The high oil yields from regrowth in response to increased irrigation and nitrogen introduce the possibility of obtaining a second commercial harvest provided oil quality is satisfactory.

From the overall results, it appeared that neither nitrogen nor irrigation treatments had any pronounced effects on oil composition at

harvest 1. The composition of oil at harvest 1 was typical of that obtained under commercial conditions. The oil obtained from regrowth (harvest 2) was significantly different from that obtained at the normal commercial harvest date (harvest 1). Generally, the regrowth oil had higher concentrations of limonene, menthofuran, menthyl acetate and menthol and lower concentrations of cineole and menthone.

The possibility of a second commercial harvest is directly related to oil quality. The regrowth herb contained an oil which satisfies the British Pharmacoepia (1968) with respect to oil composition. This requires that the oil should contain at least 45 percent menthol and 4-9 percent menthyl acetate. However, regrowth oil contained more of the undesirable menthofuran than is typical of Tasmanian peppermint oil. This higher level of menthofuran does not exceed levels reported for oils produced in several major oil producing areas of the U.S.A. (Smith and Levi, 1961).

The theoretical composition of oil obtained by combining oils from harvest 1 and 2 [$I_{(L \rightarrow H)}$, 300kg N/ha] is given in Table IV B 3.5.

It has been reported that two harvests of peppermint in one year has adverse effects on growth in subsequent seasons (Guenther, 1949b; Watson and St. John, 1955). This may be avoided if the regime of increased nitrogen and irrigation were employed. Therefore, it would appear that by manipulating irrigation, nitrogen and harvest date, substantial increases in oil yields are possible under Tasmanian conditions. Such increases in oil yield need not adversely affect oil quality.

Table IV B 3.5. Blend of oils from Harvest 1 and 2 [$I_{(L \rightarrow H)}$, 300kg N/ha]*.

Compound (%)	Harvest 1	Harvest 2	Combined
Limonene	1.24	1.63	1.39
Cineole	5.15	2.61	4.18
Menthone	29.34	17.52	24.83
Menthofuran	1.20	8.48	3.98
Menthyl Acetate	2.88	5.49	3.88
Menthol	43.57	51.23	46.50

*Oil Yield (g/m^2) - Harvest 1 = 4.932

- Harvest 2 = 3.046.

4. Changes in Oil Yield and Oil Composition during the Post-Commercial Harvest Regrowth of Peppermint

4.1 Introduction

In most areas where peppermint is grown commercially, the final stages of oil maturation have been associated with the onset of flowering. Croteau and Hooper (1978) considered that the reduction of menthone to menthol and the subsequent synthesis and accumulation of menthyl acetate were enzymatic processes associated with maturation and onset of reproductive growth in peppermint. Under Southern Tasmanian conditions satisfactory menthol concentrations (45%) in oil extracted from commercial plantings has been associated with flowering. In contrast, the results obtained from Section IV B 3 suggested that oil maturation may proceed without any associated onset of flowering. Therefore, the aim of the present work was to continue the examination of regrowth plant material until winter dormancy commenced. The changes in yield and composition of oil during this period are of particular interest when planning operations to obtain two harvests of peppermint per year in these areas.

4.2 Materials and Methods

Regrowth plant material was obtained from $I_{(H)}$ N_3 plots of Section IV B 3 on three harvest dates, 25 April 1979, 15 May 1979 and 19 June 1979. At each harvest date three quadrat samples (0.6m x 0.3m) were harvested from each of the above irrigation nitrogen subplots, in each block. Samples were harvested from representative areas within each plot (i.e. three quadrats were placed at random within the area and the area most vigorous selected, the same procedure was repeated to select an area intermediate and low in vigour). The three quadrat samples per subplot were pooled and oil composition determined in the usual manner, after steam distillation.

4.3 Results

The change in percentage composition of oil with time is presented in Table IV B 4.2. From these results it appeared that harvest date had no effect on the percentage of α-pinene, β-pinene or menthofuran. The concentration of limonene at the last harvest date was significantly higher and the concentration of cineole significantly lower than obtained on either of the first two harvest occasions. The percentage menthone and isomenthone decreased continuously with time and menthyl acetate increased with time. Neomenthol and pulegone were highest at the last harvest date. Menthol increased from the first to the second harvest date and then decreased significantly on the last harvest. A gas chromatogram, illustrating the composition of oil obtained at the last harvest date, is included in Figure IV B 4.2.

Dry matter yield, oil yield and percentage oil yield decreased from the first to the last harvest date (Table IV B 4.1).

4.4 Discussion

Dry matter production of peppermint regrowth and net oil accumulation by the crop had ceased by the first harvest date. From 25 April 1979 to 15 May 1979 there was no evidence of crop growth, either from dry matter results or from the general appearance of the crop. However, on 15 May 1979 the typical short day growth habit of peppermint (recumbent shoots) was evident. On the last harvest date considerable loss of leaves had occurred from the bottom of plants. This loss of leaves may have contributed to the decrease in dry matter yield, oil yield and percentage oil yield observed on 19 June 1979. However, on the basis of the above results, it is not possible to discount possible metabolic depletion of oil as a result of decreasing daylength.

Table IV B 4.1. Dry Matter, Oil and Percentage Oil Yield - Mean Results.

Harvest Date / Yield Component	25th April 1979	15th May 1979	19th June 1979	LSD (5%)
Dry Matter Yield (g/m^2)	308.18	288.32	260.28	43.01
Oil Yield (g/m^2)	3.033	2.445	1.928	0.52
Percentage Oil Yield (Dry Matter Basis)	0.99	0.85	0.74	0.22

Table IV B 4.2. Mean Compositional Changes in Oil Extracted from Regrowth.

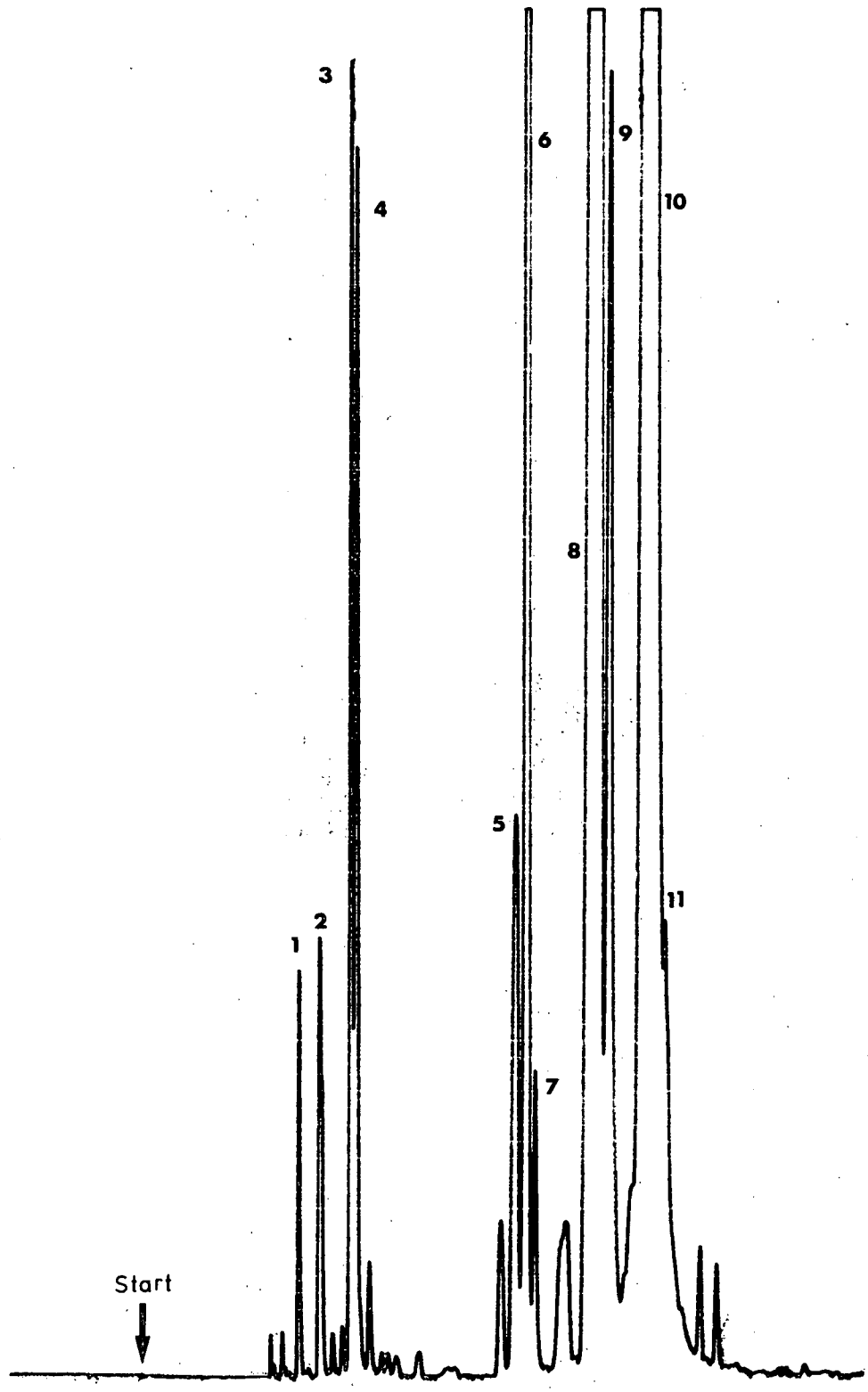
Harvest Date / Compound (% w/w)	25th April 1979	15th May 1979	19th June 1979	LSD (5%)
α-Pinene	0.46*	0.42*	0.48*	0.1281
β-Pinene	0.87*	0.80*	0.85*	0.1884
Limonene	1.62*	1.50*	2.01	0.3663
Cineole	2.72*	2.77*	2.13	0.1900
Menthone	19.39	9.71	2.01	1.3715
Menthofuran	7.58*	6.55*	7.41*	3.3124
Isomenthone	2.32	1.92	0.72	0.3680
Menthyl Acetate	4.42	8.48	23.59	0.8245
Neomenthol	3.34*	3.32*	5.10	0.5659
Menthol	50.23*	59.25	49.35*	3.6107
Pulegone	1.54*	1.27*	2.67	0.9797

*Results not significantly different (LSD, 5%).

Figure IV B 4.1

Gas chromatograph of peppermint oil extracted from material harvested on 19/6/79 [F.F.A.P., S.C.O.T. column. Chart Recorder = 30 cm/hr $80^{o}C \rightarrow 160^{o}C$ at $5^{o}C$/min.].

Peak No.	Retention Time (sec.)	Compound
1	275	α-Pinene
2	313	β-Pinene
3	368	Limonene
4	377	Cineole
5	661	Menthone
6	683	Menthofuran
7	697	Isomenthone
8	813	Menthyl Acetate
9	830	Neomenthol
10	910	Menthol
11	928	Pulegone


3
6
9
10
4
8
5
11
2
1
7
Start

The oil that was present on 25 April 1979 appeared to undergo the normal maturation process with respect to menthol, menthone, isomenthone and menthyl acetate. That is, menthone and isomenthone decreased and menthol increased at first and then decreased while menthyl acetate increased continuously. These changes are in accordance with the biosynthetic scheme proposed by Reitsema (1958) for the monoterpenes of peppermint: menthone → menthol → menthyl acetate. The extent to which this maturation occurred was greater than observed during the normal commercial growing season. For example, menthyl acetate seldom exceeds 4 percent and menthone seldom decreases below 15 to 20 percent in oil produced under Tasmanian conditions. Unlike the situation in most commercial crops, it was not the commencement of a reproductive stage that triggered the maturation of oil. This maturation of oil may have resulted from the cessation of crop growth due to the onset of dormancy. As mentioned previously, it is not possible to discount possible metabolic depletion of oil during the period of decreasing oil yield per unit area. Croteau and Martinkus (1979) reported that in flowering peppermint a major portion of menthone was converted to the non-volatile metabolite neomenthyl glucoside, in the midstem leaves. Such a mechanism could have been operative in plants under the conditions of the regrowth period. That is, metabolic conversion of menthone to non-volatile metabolites would have decreased oil yield as well as menthone concentration in the oil. However, on the basis of the present results, the decrease in oil yield could be explained equally as well by the observed loss of leaves, and the decrease in menthone by conversion to menthol and menthyl acetate.

5. The Manipulation of Nitrogen, Irrigation and Harvest Date A method of increasing the commercial yield of peppermint oil under Southern Tasmanian conditions

5.1 Introduction

Under Southern Tasmanian conditions oil yield per unit area from commercial plantings of peppermint (30-60 plants/m^2) increased initially and remained constant for approximately 6 weeks before harvest (Section IV B 2). Although harvesting at the beginning of the period of maximum yield would seem advisable with respect to oil yield per unit area, the oil composition was not considered satisfactory at this stage due to the low menthol concentration. During the 6 weeks of maximum yield, menthol increased from 40 to 45 percent. Harvesting commenced at the 45 percent menthol stage.

In addition to the increased oil yields resulting from inputs of irrigation and nitrogen, significant regrowth of peppermint occurred after harvest. Subsequent determination of oil yield and composition from post-harvest regrowth suggested the possibility of obtaining two commercial harvests of peppermint per season, under Southern Tasmanian conditions. When the first crop was harvested at 45 percent menthol, approximately 50kg of oil/ha were obtained. The oil yield arising from regrowth ($I_{(H)}$, 300kg N/ha) approached 30kg/ha. Furthermore, the oil extracted from regrowth was very mature, having high menthol and low menthone concentrations.

From a knowledge of oil yield and composition arising from the above two harvest system, the following management programme is suggested. It may be possible to harvest the first crop of peppermint before 45 percent menthol is reached but at maximum oil yield per unit area (early January). This early harvest would lengthen the regrowth growing season

and may have a desirable influence on regrowth oil yields. By combining oils from the two harvests, a high yield of oil having acceptable composition could be expected.

Therefore, the aim of the present work was to investigate the possibility of substantially increasing oil yield per unit area under Southern Tasmanian conditions by manipulating the inputs of nitrogen and irrigation, and harvesting two crops during the growing season. The effect of this practice on oil composition was also investigated.

5.2 Materials and Methods

a. Site

This experimental work was conducted in the same field at "Rotherwood", Ouse, as used in 1978-79 (Section IV B 3). The experiment was located in a 2 year old planting of meadow mint.

b. Treatments

Irrigation. Irrigation commenced in early November 1979, and two irrigation treatments were involved:

$I_{(L)}$: 25mm of irrigation weekly, throughout the growing season.

$I_{(L\rightarrow H)}$: 25mm of irrigation weekly during the first half of the growing season and twice weekly during the last half of the growing season.

All irrigation was applied through an Ajax travelling irrigator.

Nitrogen. Three treatments involving rates of applied nitrogen were used:

$N_{0.5}$: 50kg N/ha - applied at the commencement of growth of crop 1 (22 October 1979)

N_2 : 200kg N/ha - applied at the commencement of growth of crop 1 (22 October 1979)

N_{2+1} : 200kg N/ha - applied at the commencement of growth of crop 1

(22 October 1979) and 100kg N/ha applied after first harvest (21 January 1980).

The fertiliser nitrogen was applied as ammonium nitrate and a basal dressing of 100kg K/ha as muriate of potash and 50kg P/ha as high grade superphosphate, were applied to nitrogen treatment plots, N_2 and N_{2+1}, on 22 October 1979.

Harvest Date. Throughout the growing season oil yield/ha and oil composition were monitored. When one harvest per season was intended, this harvest was timed to coincide with 45 percent menthol. In the case of two harvests per season (2H), the first harvest (H1) was timed to coincide with maximum oil yield/ha and approximately 40 percent menthol and the second harvest (H2) was based on considerations of both oil yield and composition.

The selected combination of irrigation, nitrogen and harvest treatments were as follows:

$I_{(L)}\ N_{0.5}$: this treatment represented the irrigation-nitrogen-harvest date regime, used by commercial producers, prior to the 1978-79 season.

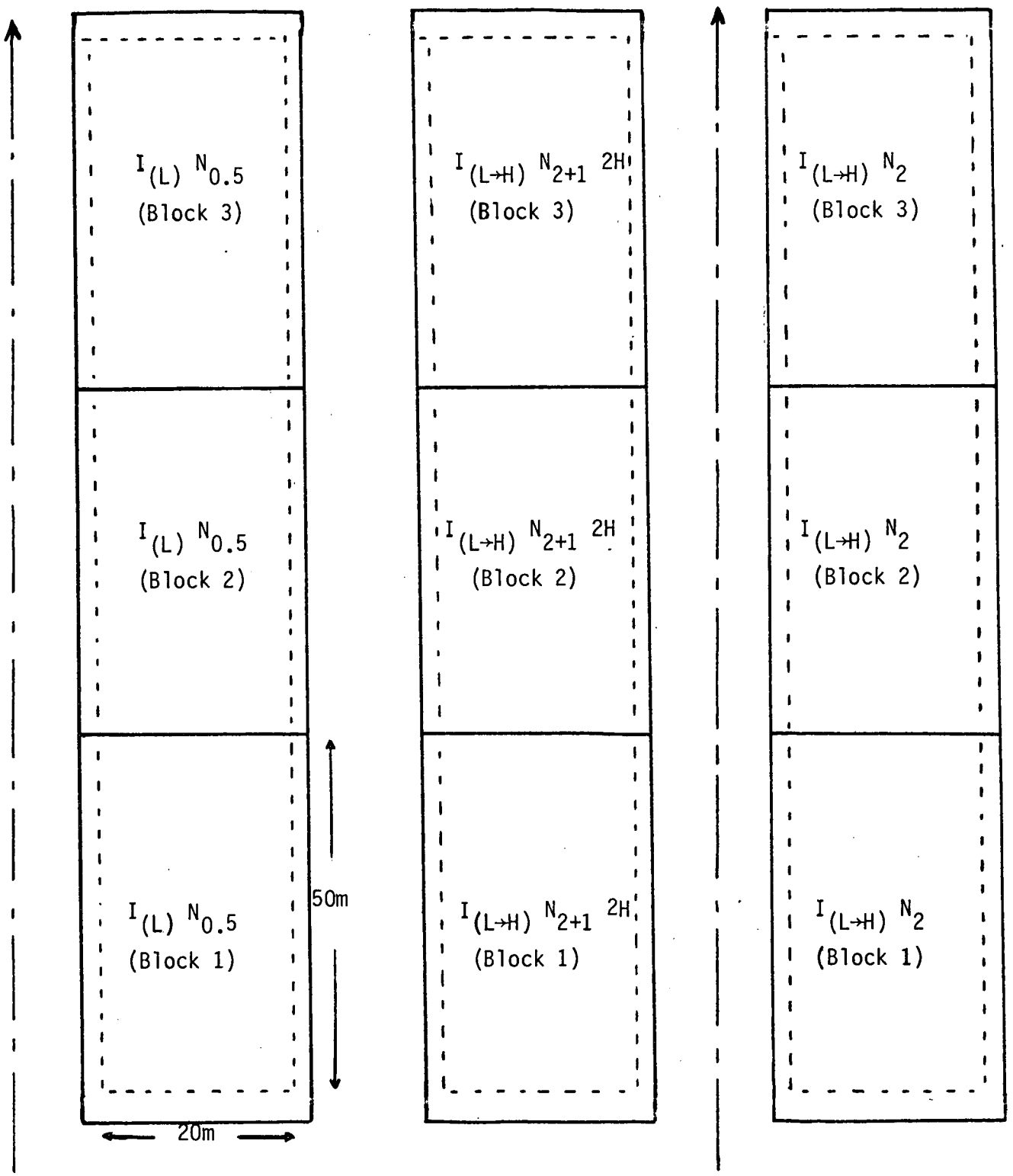
$I_{(L\rightarrow H)}\ N_2$: this treatment combined the highest irrigation and nitrogen treatments used in 1978-79 (harvest 1) (Section IV B 3).

$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$: in addition to treatment $I_{(L\rightarrow H)}\ N_2$ this treatment involved two harvests and an additional application of 100kg N/ha after harvest 1 (H1).

c. Layout and Experimental Technique

All plots were 20m x 50m in size and three replications were used. A 2.5m buffer area was established around all plots. Details of layout and allocation of treatments to plots are included in Figure IV B 5.1.

Figure IV B 5.1.



Travelling irrigator (delivering 25mm of irrigation weekly, throughout the growing season).

Travelling irrigator (delivering 25mm of irrigation twice weekly during the last half of the growing season).

At weekly intervals, $1m^2$ quadrat samples of plant material were harvested at random from within the larger 20m x 50m plots. These samples were dried in the glasshouse, steam distilled and analysed in the normal manner (Section III).

With respect to the large plots (20m x 50m), all operations were conducted using commercial production techniques. Fertiliser application, rust control, weed control, irrigation, harvesting and distillation were conducted using the normal equipment associated with large-scale commercial production. The requirement that plots should be managed on a commercial scale imposed some restrictions on the layout of plots and allocation of treatments to plots. All replications within each irrigation treatment were restricted to an area adjacent to the same travelling irrigator pathway.

5.3 Results

a. Weekly Samples

Results indicating the changes in percentage menthol, percentage menthone and oil yield are included in Figures IV B 5.2 to 5.4, respectively.

The menthol content of oil from treatments $I_{(L)}\ N_{0.5}$ and $I_{(L\rightarrow H)}\ N_2$ increased initially until 14 January, decreased to a mid-season low level at the end of January, and finally increased to 45 percent on 25 February. When a significant difference existed in the menthol content of the above treatments, $I_{(L)}\ N_{0.5}$ yielded oil with the highest menthol content. Such differences were most pronounced at the beginning of the growing season and no significant difference existed between the treatments during the final period of increasing menthol. Oil from treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ increased in menthol from 19 December to 14 January and decreased on 21 January. The subsequent regrowth oil from the latter treatment

increased in menthol content with time from 25 February until 31 March.

Changes in percentage menthone with harvest date were the reverse of those changes observed for menthol. That is, the high level of menthone present at the beginning of the experimental period decreased until mid-January, increased to early February and finally decreased until the end of February. The changes in percentage menthone in regrowth oil as well as the differences between treatments, paralleled the changes in menthol with a decrease in menthol reflecting an increase in menthone.

The oil yield resulting from treatment $I_{(L)}\ N_{0.5}$ increased until 21 January after which no significant change was observed with time. The maximum oil yield from the above treatment was approximately 6.5g/m^2. A similar initial increase in oil yield was observed in treatment $I_{(L\rightarrow H)}\ N_2$ with the plateau value of 7.5-8.0g/m^2 being reached towards the end of January. Where significant differences in oil yield existed between treatments $I_{(L)}\ N_{0.5}$ and $I_{(L\rightarrow H)}\ N_2$, the latter treatment yielded most oil. Oil yield from treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ increased continuously from 19 December to 21 January, with a maximum oil yield approaching 8.0g/m^2 being obtained on the last harvest occasion. In regrowth arising from treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$, oil yield increased significantly until 7 March after which a plateau value of approximately 3.5-4.0g/m^2 was maintained until 31 March.

b. Commercially Harvested Samples

Results indicating the commercially harvested yield of oil and the composition of this oil from the various treatments, are included in Figure IV B 5.3 and Table IV B 5.1, respectively.

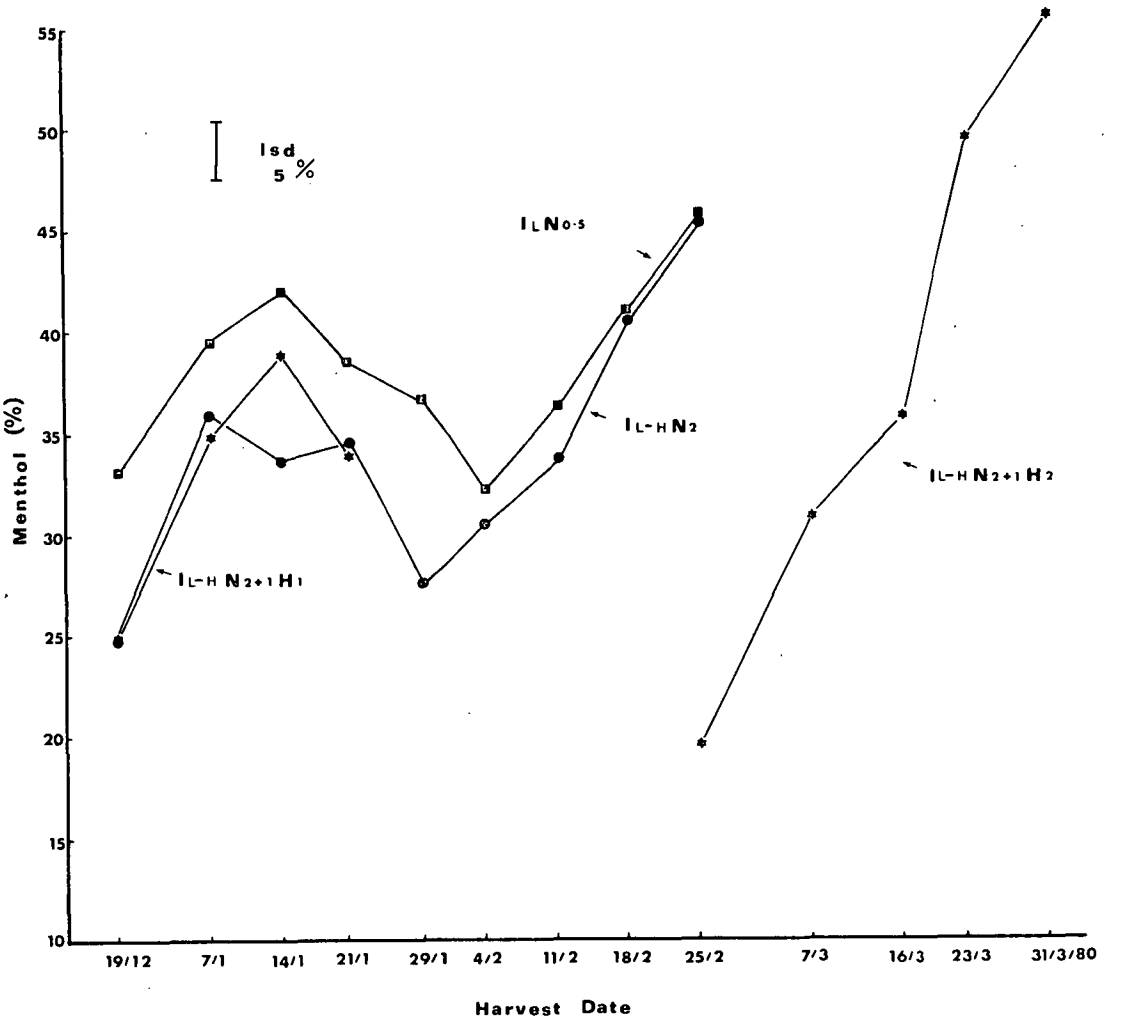
The oil yield obtained from treatment $I_{(L)}\ N_{0.5}$ was significantly lower than that obtained from treatments $I_{(L\rightarrow H)}\ N_2$ and $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$.

Figure IV B 5.2.

The effect of nitrogen, irrigation and harvest date on the change in percentage menthol with time.

Figure IV B 5.3

The effect of nitrogen, irrigation and harvest date on the change in percentage menthone with time.


lsd
5 %
$I_L N_{0\cdot5}$
$I_{L-H} N_2$
$I_{L-H} N_{2+1} H_1$
$I_{L-H} N_{2+1} H_2$
Menthol (%)
55
50
45
40
35
30
25
20
15
10
19/12
7/1
14/1
21/1
29/1
4/2
11/2
18/2
25/2
7/3
16/3
23/3
31/3/80
Harvest Date

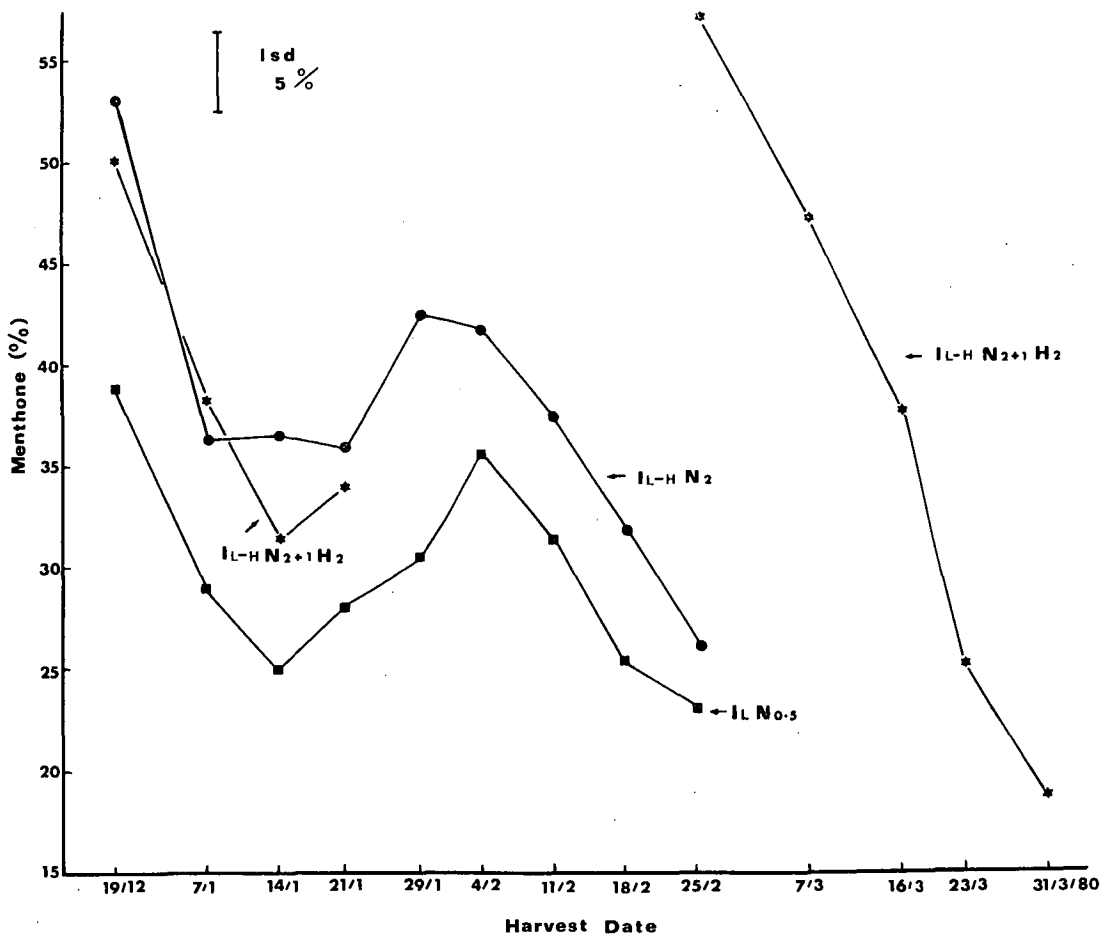
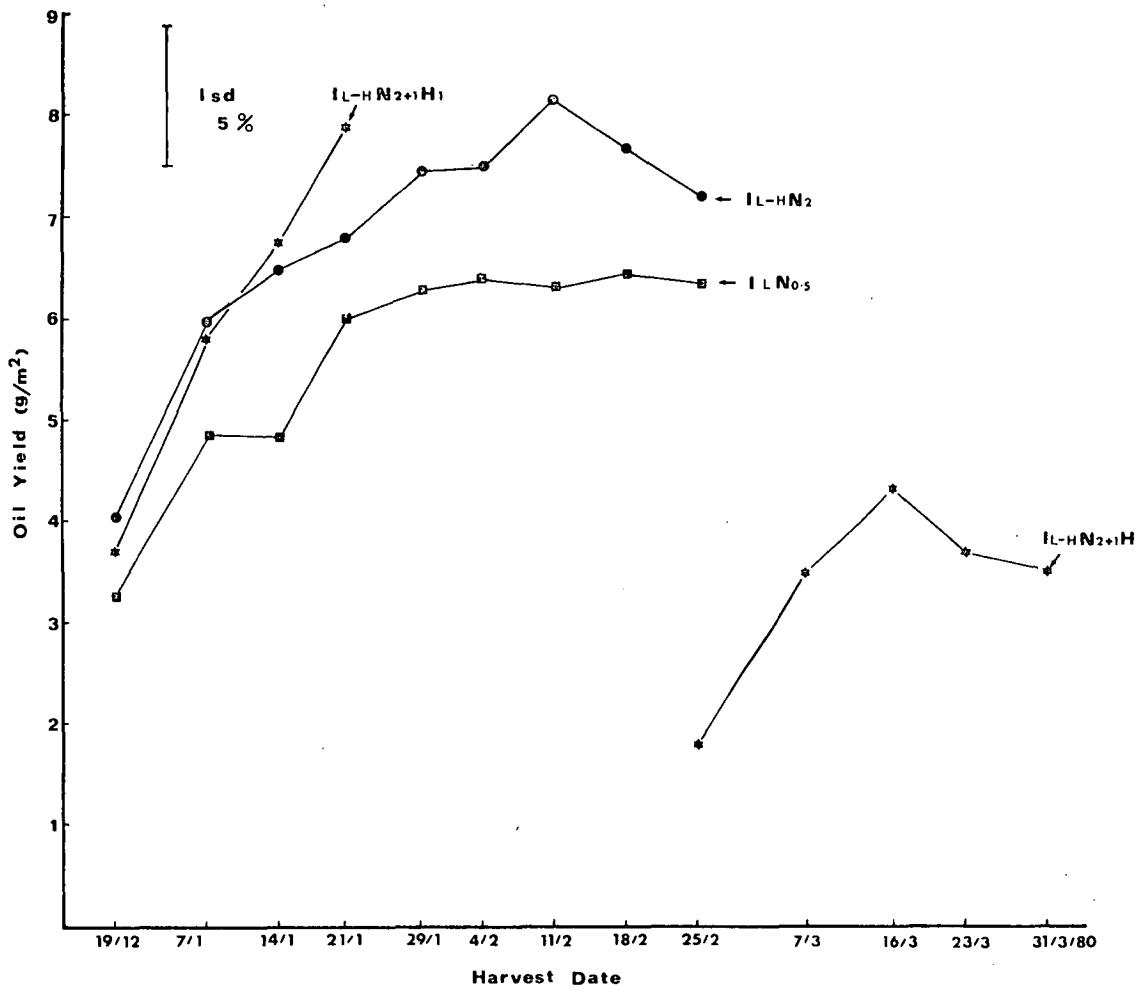

lsd
5 %
$I_{L-H} N_{2+1} H_2$
$I_{L-H} N_2$
$I_L N_{0\cdot5}$
$I_{L-H} N_{2+1} H_2$
Menthone (%)
55
50
45
40
35
30
25
20
15
19/12
7/1
14/1
21/1
29/1
4/2
11/2
18/2
25/2
7/3
16/3
23/3
31/3/80
Harvest Date

Figure IV B 5.3

The effect of nitrogen, irrigation and harvest date on the change in oil yield with time.

Figure IV B 5.4

The effect of nitrogen, irrigation and harvest date on the commercial yield of oil.


lsd
5 %
$I_{L-H}N_{2+1}H_1$
$I_{L-H}N_2$
$I_LN_{0\cdot5}$
$I_{L-H}N_{2+1}H_2$
Oil Yield (g/m²)
Harvest Date
19/12
7/1
14/1
21/1
29/1
4/2
11/2
18/2
25/2
7/3
16/3
23/3
31/3/80

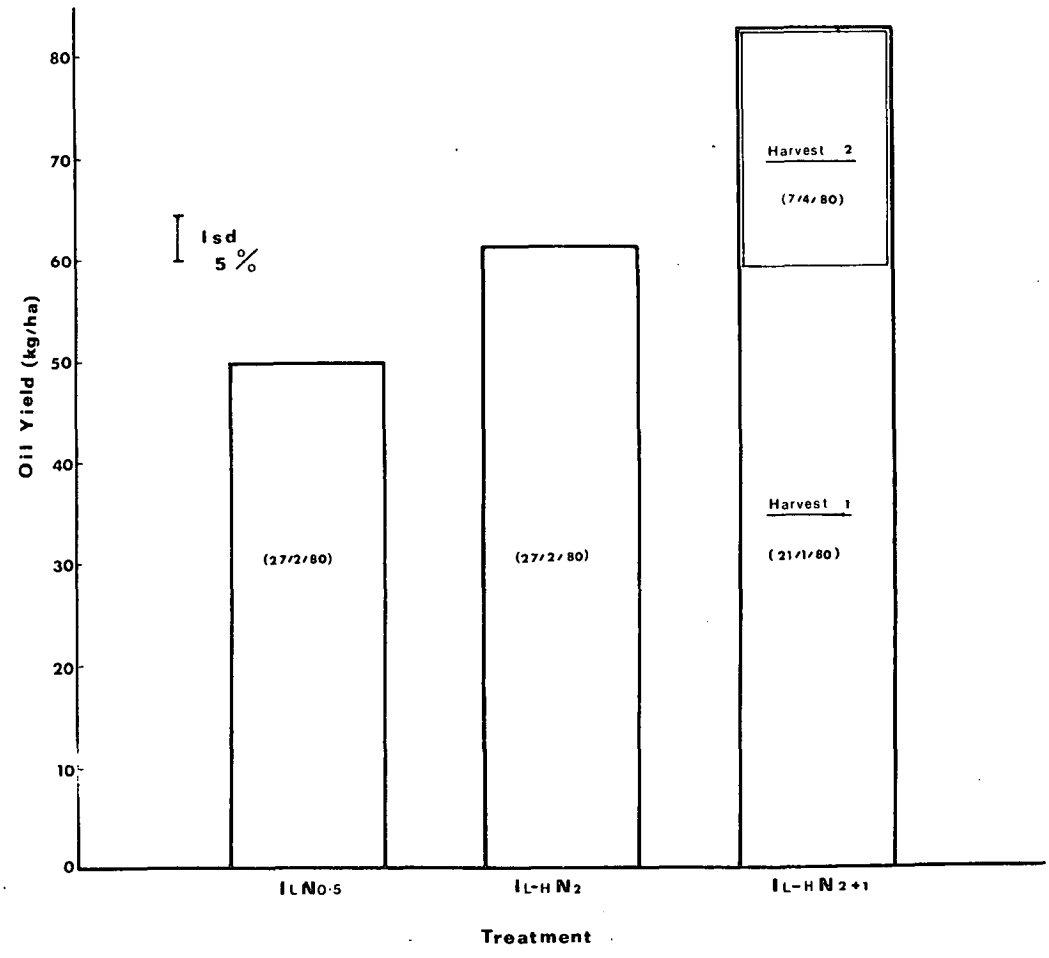

lsd
5 %
Harvest 2
(7/4/80)
Harvest 1
(21/1/80)
(27/2/80)
(27/2/80)
Oil Yield (kg/ha)
$I_LN_{0\cdot5}$
$I_{L-H}N_2$
$I_{L-H}N_{2+1}$
Treatment

Table IV B 5.1.

The effect of harvest date and number, irrigation and nitrogen on the commercial yield and composition of peppermint oil.

Treatment	Mean Values					
	$I_{(L)}\ N_{0.5}$	$I_{(L\rightarrow H)}\ N_2$	$I_{(L\rightarrow H)}\ N_{2+1}$ Combined $H_1 + H_2$	$I_{(L\rightarrow H)}\ N_{2+1}$ Harvest 1 (H_1)	$I_{(L\rightarrow H)}\ N_{2+1}$ Harvest 2 (H_2)	LSD (5%)*
(1) Oil Yield (g/m^2)	49.84	61.10	82.02	58.37	23.67	4.43
(2) Oil Composition (%)						
α-Pinene	0.71	0.66	0.69	0.70	0.66	0.06
β-Pinene	1.56	1.45	1.55	1.62	1.36	0.14
Limonene	1.89	1.86	2.36	2.58	1.86	0.41
Cineole	5.38	5.55	5.76	6.53	3.87	0.55
Menthone	18.52	18.87	28.34	33.01	16.67	2.85
Menthofuran	2.72	1.56	1.93	0.74	4.80	0.56
Isomenthone	2.18	2.72	2.85	2.79	3.04	0.90
Menthyl Acetate	3.12	3.15	2.52	1.75	4.51	0.47
Neomenthol	5.26	5.22	3.94	3.91	3.99	0.66
Menthol	50.83	51.34	43.42	38.88	54.67	5.03

*LSD (5%) - calculated for treatments $I_{(L)}\ N_{0.5}$, $I_{(L\rightarrow H)}\ N_2$, $I_{(L\rightarrow H)}\ N_{2+1}$ (Combined $H_1 + H_2$)

No significant difference existed between yields obtained from the first harvest (21 January) of treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ and treatment $I_{(L\rightarrow H)}\ N_2$ harvested on 27 February. The additional yield of oil obtained from treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ on 7 April resulted in a significantly higher overall oil yield from this treatment relative to all other treatments. The final oil yields per hectare from treatments $I_{(L)}\ N_{0.5}$, $I_{(L\rightarrow H)}\ N_2$ and $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ were approximately 50kg, 60kg and 80kg, respectively.

The only significant difference in composition of oil obtained from treatments $I_{(L)}\ N_{0.5}$ and $I_{(L\rightarrow H)}\ N_2$ was a higher concentration of menthofuran in oil from the former treatment. In comparison with the above treatments, oil obtained at harvest 1, treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$, contained higher concentrations of limonene cineole and menthone and lower concentrations of menthofuran, menthyl acetate, neomenthol and menthol. Oil obtained at harvest 2 (7 April) contained lower cineole and neomenthol and higher menthofuran and menthyl acetate concentrations than oil obtained from treatments $I_{(L)}\ N_{0.5}$ and $I_{(L\rightarrow H)}\ N_2$.

5.4 Discussion

The maximum oil yield per unit area was obtained from treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ when harvest 1 was conducted on 21 January. Subsequent changes in oil yield from treatment $I_{(L\rightarrow H)}\ N_2$ with harvest date indicated that no significant increase in oil yield would have resulted from delaying harvest 1 [$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$] after 21 January.

In addition to the requirement that harvest 1 should coincide with the period of maximum oil yield, consideration was also given to oil composition at harvest. Rapid oil maturation occurred from 19 December until 14 January, resulting in menthol levels approaching 40 percent on 14 January. The 40 percent menthol level was selected as being a suitable stage of maturity to conduct harvest 1. The acceptance of this

stage of oil maturity was based on considerations of oil yield and composition obtained from harvest 1 and 2 during the 1978-79 season (Section IV B 3). From the results of trial distillations it appeared that after 14 January, oil reverted to an immature composition with decreasing levels of menthol and increasing levels of menthone. This period of decreasing maturity in extracted oils corresponded to a period of rapid lateral shoot growth. The increased proportion of young to old leaves, associated with the commencement of lateral shoot production, most likely accounted for the observed changes in oil composition.

Therefore, by monitoring oil yield and composition during the late December-January period it was possible to time the first harvest of treatment $I_{(L \rightarrow H)}\ N_{2+1}\ 2H$ to coincide with the period of maximum oil yield per unit area and a period during which menthol levels approached 40 percent. (The menthol level in commercially harvested plants was approximately 39 percent.) However, although the period of maximum yield continued well into February, menthol levels and hence oil maturity decreased during mid-January, as a result of lateral shoot growth. The early season peak menthol levels were only exceeded when harvesting was delayed until mid-late February. Therefore, the early harvesting of peppermint to achieve maximum oil yield per unit area, with menthol levels approaching 40 percent, was limited to a short period between the time when yield reached a maximum level and before significant lateral shoot growth occurred. Since treatments receiving high levels of nitrogen and irrigation tended to yield less mature oil during the early growing season, relative to treatment $I_{(L)}\ N_{0.5}$, the period during which menthol levels approached 40 percent was considerably shorter in the former treatments relative to the latter treatment .

Harvesting of the second crop arising from treatment $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ was timed to coincide with a menthol content of approximately 50 percent in extracted oils. From trial distillation results this stage was reached on 31 March. Oil yield from the second crop increased initially and reached a plateau value on 7 March, after which no significant change occurred. A severe rust infestation occurred during the regrowth period and this may have prevented oil yields from increasing after 16 March. (The increase in severity of this infestation from block 3 to block 1 was reflected in the lower oil yields obtained from block 1.) From the results presented in Section IV B 4, delaying harvest after the menthol level reached 55-60 percent may have resulted in a decrease in menthol with an associated increase in menthyl acetate.

With respect to the commercially harvested material from treatments $I_{(L)}\ N_{0.5}$, $I_{(L\rightarrow H)}\ N_2$ and $I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ the resultant oil yields were 50, 60 and 80kg/ha, respectively. Therefore, within the commercially operated system, increasing nitrogen to 200kg/ha and increasing irrigation during the latter half of the growing season significantly increased oil yield per unit area. In addition, associating the two harvest systems with increased nitrogen and irrigation resulted in substantial increases in oil yield. Regrowth of plants from treatments $I_{(L)}\ N_{0.5}$ and $I_{(L\rightarrow H)}\ N_2$ following harvest (27 February), was not sufficient to allow a second commercial harvest from these plots.

In relation to oil yield, two aspects of the present results require some explanation. Firstly, a considerable difference existed between oil yields obtained from trial distillation and oil yields obtained from commercial distillation. Consistently trial harvesting and distillation yielded 10-15kg of oil/ha more than obtained when the same areas were commercially harvested and distilled, at approximately the same time.

It could be suggested that this inconsistency resulted from the trial samples not being representative of the larger areas. However, quadrat samples were taken at random within the larger areas which were extremely uniform. In addition, the inconsistency existed in all treatments including the regrowth crop which was uniquely uniform in growth. Oil losses resulting from the commercially operated system would seem more plausible. Avenues of oil loss during such commercial operations may have included loss of oil from glands and loss of leaves during field curing, a failure to harvest and collect all material, oil and leaf loss during collection, and/or inefficient distillation, condensing or separation. The exact nature of the significant difference in yield between trial distillations and commercial distillations will be the subject of future research.

The second aspect of oil yield requiring some comment is the apparently lower increase in oil yield which resulted from adding nitrogen (200kg/ha) and irrigation (50mm during the latter half of the growing season) during 1979-80, compared with the response recorded in 1978-79 (Section IV B 3). That is, the increase in oil yield obtained from $I_{(L\rightarrow H)}\ N_2$ relative to $I_{(L)}\ N_{0.5}$ was approximately 30kg/ha (harvest 1) during 1978-79, but only 10kg/ha from similar treatments during 1979-80. From a consideration of results reported in Section IV B 3, Figure IV B 5.3 and 5.4, it is apparent that the smaller yield difference between treatments $I_{(L\rightarrow H)}\ N_2$ and $I_{(L)}\ N_{0.5}$ during 1979-80 was largely a reflection of higher yields from $I_{(L)}\ N_{0.5}$, rather than lower yields from $I_{(L\rightarrow H)}\ N_2$. It is possible that the higher oil yield from treatment $I_{(L)}\ N_{0.5}$ during 1979-80 resulted from a residual effect of the 1978-79 nitrogen and irrigation regime. That is, prior to the 1978-79 season commercial production was associated with low inputs of nitrogen and irrigation

[$I_{(L)}\ N_{0.5}$]. However, as a result of the significant benefit associated with increasing nitrogen and irrigation, which became apparent during the latter half of the 1978-79 season, additional irrigation (including post-harvest irrigation) and nitrogen were applied to the field at "Rotherwood", towards the end of the 1978-79 season. Therefore, due to the different past history of nitrogen and irrigation, treatment $I_{(L)}\ N_{0.5}$ during 1978-79 may not be equivalent to treatment $I_{(L)}\ N_{0.5}$ during 1979-80.

As mentioned previously, the success of the two harvest programme is largely dependent on the overall composition of oil. Data obtained from combining oils from harvest 1 and 2, giving consideration to the respective oil yields at each harvest, is included in Table IV B 5.1. Generally, the compositional profile of the combined oil is similar to oils produced from Southern Tasmania, under conventional production systems. The major difference in composition between the combined oil and oil from treatments $I_{(L)}\ N_{0.5}$ and $I_{(L\rightarrow H)}\ N_2$, lies in the increased maturity of the latter oils. This increased maturity is reflected in increased levels of menthol and menthyl acetate and decreased levels of menthone and isomenthone. Within a commercial operation, any problems which may arise from the lower menthol levels in combined oil samples, could be overcome by increasing the proportion of second harvest oil within the final oil blend.

Therefore, it would appear that a potential exists to substantially increase the oil yield per unit area under Southern Tasmanian conditions, by manipulating harvest date, irrigation and nitrogen. Such increases in oil yield need not necessarily have adverse affects on oil composition. However, the successful operation of the two harvest programme would require careful quality control at both harvests.

V GENERAL DISCUSSION

This study attempted to define some of the factors which determine the yield and composition of peppermint oil. By investigating the effect of these factors on individual plants, an attempt was made to understand the factors controlling oil yield and composition under field conditions. The manipulation of the field variables to increase oil yield per unit area without adversely affecting oil composition, was investigated.

The results presented support previous reports of a true photoperiodic effect on oil yield, growth habit and flowering of peppermint. Furthermore, there exists a true photoperiodic effect on monoterpene composition of peppermint oil. Unlike the results of Grahle and Holtzel (1963), the photoperiodic effect on oil composition observed in the present study as well as the effect suggested from results of Burbott and Loomis (1967), was less pronounced. Although the results of Grahle and Holtzel (1963) suggested photoperiod as the sole determinant of oil composition in peppermint, evidence now exists to implicate other factors in the control of monoterpene metabolism. Firstly, the results of Burbott and Loomis (1967) suggested light and temperature were important determinants of oil composition. The influence of these environmental conditions was attributed to an effect on the photosynthate status of monoterpene producing cells. Secondly, the results obtained in the present work indicate that photoperiod, day temperature, night temperature, light intensity and daylength are all important interacting factors controlling monoterpene composition.

Whether photoperiod has an independent effect on monoterpene metabolism or has its effect through a modifying influence on the availability of photosynthate to monoterpene producing cells, as envisaged for other environmental conditions, remains largely unknown.

If photoperiod has its influence through an effect on photosynthate availability, it follows that long photoperiodic conditions must favour the maintenance of photosynthate supply to monoterpene producing cells which in turn favours the maintenance of adequate supplies of reduced respiratory co-enzymes, necessary for the interconversion of pulegone to menthone. For example, when plants were grown at 20^{o}C days and high light intensities, neither decreasing photoperiod nor increasing night temperature influenced monoterpene composition, relatively low concentrations of menthofuran being accumulated under all conditions. However, at low light intensity a pronounced effect of photoperiod was observed, long photoperiods favouring lowest concentrations of menthofuran. Assuming that photoperiod has its influence on photosynthate availability, it follows that high light intensity was sufficiently conducive to the maintenance of adequate levels of available photosynthate, regardless of either photoperiod or night temperature. Decreasing light intensity would have decreased the availability of photosynthate within the plant and therefore photoperiod through its effect on photosynthate balance, became an important determinant of oil composition. Likewise, when conditions were such that the maintenance of an adequate level of photosynthate was not possible even under long photoperiods, the photoperiodic effect had little influence on oil composition. That is, in Section IV A 3 high concentrations of menthofuran accumulated under low light intensity conditions and neither daylength and/or night temperature had any pronounced influence on the concentration of this compound.

Therefore, the photoperiodic effect on monoterpene composition in peppermint would appear to be dependent on other environmental conditions. This interaction between environmental conditions and photoperiod may

account for the apparent disagreement in the reported effect of photoperiod on oil composition. In this context, it becomes difficult to understand how photoperiod could have such a pronounced influence on oil composition as was reported by Grahle and Holtzel (1963); photoperiod being only one of several interacting conditions determining final composition.

Burbott and Loomis (1969) reported that conditions which favoured the accumulation rather than metabolic turnover of monoterpenes, were those favouring maintenance of high levels of photosynthate. Similarly, high levels of photosynthate favoured the relatively reduced compound menthone rather than menthofuran and pulegone (Burbott and Loomis, 1967). Since long photoperiodic conditions have been found to favour the accumulation of menthone, photoperiod may have an influence on oil yield through an effect on photosynthate availability as well as by the reported effect on the number of oil glands per unit leaf area (Langston and Leopold, 1954).

The effect of pre-treatment growing conditions on growth habit and oil composition (Section IV A 5), oil yield, gland number and inflorescence initiation (Langston and Leopold, 1954) is particularly important with respect to photoperiodic investigations. That is, to avoid a confounding influence from pre-treatment conditions, propagating material for photoperiodic investigations should be selected from plants growing under photoperiods identical to those to be used in the subsequent investigation.

The photosynthate model proposed by Burbott and Loomis (1967) stated that "the oxidation-reduction level of the monoterpenes reflects the general oxidation-reduction state of the respiratory co-enzymes of the terpene producing cells and that this is dependent on the balance between daytime photosynthesis and night time utilization of photosynthate".

In general, the effect of daylength, night temperature, day temperature and light intensity on pulegone, menthone and menthofuran, are explainable within the context of this model. That is, conditions favouring the maintenance of high levels of reduced respiratory co-enzymes (e.g. $NADPH_2$) are seen as favouring conversion of pulegone to menthone. In this way, long days and saturating light intensity (light intensities greater than $500\mu\Sigma m^{-2}s^{-1}$) are considered to favour conversion of pulegone to menthone by increasing the duration of the photosynthetic period and maintaining maximum rates of photosynthesis, respectively. The effect of day temperature and night temperature on monoterpene composition can also be explained by an effect on photosynthate status, within the photosynthate model, if consideration is given to the net CO_2 exchange characteristics of peppermint.

From such net CO_2 exchange characteristics it is apparent that increasing night temperature would increase the night time utilization of photosynthate by dark respiration. The dependence of monoterpene composition on day temperature arises from the effect of temperature on 'true' photosynthesis, dark respiration (which is assumed to continue in the light) and photorespiration. As a consequence of changes in these net CO_2 exchange characteristics, net CO_2 fixation is maximal at 20^oC. That is, 20^oC days favours production rather than utilization of photosynthate. Whereas increasing day temperature from 5^oC to 20^oC favours production of photosynthate by increasing day time photosynthesis, further increasing temperature above 20^oC favours utilization of photosynthate largely as a result of the rapid increase in photorespiration between 15^oC and 25^oC. Therefore, the importance of photorespiration as a means of photosynthate utilization should be recognised within the photosynthate model. That is, the oxidation-reduction state of the

respiratory co-enzymes is not only dependent on the balance between day time photosynthesis and night time utilization of photosynthate but is also dependent on day time utilization of photosynthate, especially by photorespiration.

The effect of increasing day temperature above 20°C decreased net CO_2 fixation and increased the dependence of oil composition on conditions such as night temperature, daylength and light intensity. Cooler nights, longer days and higher light intensities were required at temperatures above 20°C, to promote the accumulation of menthone relative to menthofuran and pulegone. Furthermore, it would appear from net CO_2 exchange characteristics that within the photosynthate model, day temperature may be a more important determinant of oil composition than night temperature. Whereas increased day temperature increased both photorespiration and dark respiration, increased night temperature increased only dark respiration. Since photorespiration is greater than dark respiration and most peppermint production is confined to areas having relatively long days rather than long nights, a small change in day temperature may have a much more pronounced effect on photosynthate balance and monoterpene composition, than a similar change in night temperature.

As previously mentioned, conditions favouring the accumulation of photosynthate (high light intensity, 20°C days, cool nights, long days) favoured reduction of pulegone to menthone rather than oxidation of pulegone to menthofuran. The nature of the significant interactions between environmental conditions on composition were also supportive of the photosynthate model and of the fact that all conditions were influencing a common mechanism of monoterpene metabolism.

In addition to the effect of environmental conditions on pulegone, menthone and menthofuran, the present study reported an effect on several

other monoterpenes of peppermint oil. Conditions favouring the accumulation of menthone also favoured accumulation of cineole (e.g. high light intensity, cool nights, long days and long photoperiodic treatments). Limonene was favoured by short days, short photoperiodic treatments, high light intensity and cool nights. Within the photosynthate model, decreasing daylength is envisaged as having the opposite effect on photosynthate balance to increasing light intensity and decreasing night temperature. In Section IV A 3, it was proposed that night temperature and light intensity were affecting limonene via a photosynthetic mechanism whilst daylength was affecting limonene via a photoperiodic mechanism. However, in the subsequent discussion of the effect of photoperiod on pulegone, menthone and menthofuran, an indirect effect of photoperiod, through an influence on photosynthate availability, was suggested. No obvious explanation exists to account for this apparent inconsistency.

Menthol and menthyl acetate appeared to accumulate under conditions which also favoured the oxidation of pulegone to menthofuran. Such a result would not be expected within the photosynthate model. However, it is possible that environmental conditions may exert direct and indirect control over monoterpene metabolism and biosynthesis. Daylength, photoperiod, light intensity, night temperature and day temperature have a direct effect on monoterpene composition through an influence on the availability of the required co-factors involved in the reduction of pulegone to menthone (e.g. $NADPH_2$). On the other hand, environmental conditions may influence oil composition indirectly through an effect on growth habit, extent of flowering, the proportion of immature to mature leaves, and the extent of oil maturation (menthone → menthol → menthyl acetate).

When whole plants were harvested (Sections IV A 2 and 3) the composition of oil reflected both the indirect influence of environmental conditions on the differing ratios of mature and immature leaves, and the direct effects of environmental conditions on the direction and extent of oil biosynthesis in leaves of equivalent maturity. An indication of the extent to which differences between environmental conditions might reflect differing ratios of mature and immature leaves can be obtained from experiments in which individual leaves were harvested. Although a marked difference in oil composition between mature and immature leaves is evident in Section IV A, it should be noted that this difference is most pronounced in the compounds menthol, menthone and to a lesser extent menthyl acetate. The percentage menthofuran and pulegone did not vary to the same extent with leaf maturation. Therefore, although a change in the ratio of mature to immature leaves may be reflected in the extent of oil maturation (menthone → menthol → menthyl acetate) when whole plants are harvested, a change in this ratio would have a much less pronounced influence on the balance between menthofuran, pulegone and menthone. Evidence indicating a direct effect of environmental conditions on monoterpene composition, independent of leaf position (stage of maturity), is provided in Section IV A, where a pronounced effect of pre-treatment growing conditions on the concentration of menthofuran in leaves of equivalent age was reported.

Therefore, it becomes necessary to recognise at least three factors or groups of factors which affect monoterpene composition in peppermint oil. These factors exert their influence through photosynthetic mechanisms, photoperiodic mechanisms and indirect mechanisms involving maturity dependent conversions. The extent to which the three factors are related remains largely unknown, and this aspect requires further

development as a result of progressive experimentation.

Ameluxen (1964, 1965) reported that peppermint glands mature, fill with oil and the secretory cells degenerate at a very early stage of leaf development, prior to significant leaf expansion. If it is assumed that these degenerative cells no longer synthesise oil, then oil accumulation would be expected to be confined to very young leaves. In contrast, $^{14}CO_2$ tracer studies and periodic analyses of monoterpenes in peppermint leaves (Burbott and Loomis, 1969) indicated that accumulation continued at least whilst leaf expansion was occurring. The latter reports are supported by the findings presented in Section IV A 1. The concern that has arisen from this apparent disagreement has led to several possible explanations. Burbott and Loomis (1969) suggested that either secretory cells remain functional and continue to synthesise oil, after the degeration observed by Ameluxen (1964, 1965), or that synthesis of oil may not be confined to glandular structures. From the results presented in Section IV A 1, it is apparent that although the observations made by Ameluxen (1964, 1965) may have been representative of individual glands on young peppermint leaves, they were certainly not representative of the whole leaf. That is, although mature glands (fully distended subcuticular space) were observed on young leaves, only a small proportion of the final number of glands were present, many being very immature. The appearance of new glands and the filling of immature glands with oil continued long after the stage suggested by Ameluxen (1964, 1965). Such observations may explain the previous apparent disagreement between the results of Ameluxen (1964, 1965) and Burbott and Loomis (1969). Lemli (1963) also observed that gland filling continued long after the stage suggested by Ameluxen (1964, 1965).

Gas chromatographic analysis of oil isolated from individual glands by Ameluxen *et al.* (1969), suggested that the glandular trichomes on

very young leaves (less than 1.5cm in length), contained principally menthol and menthyl acetate, whereas glandular hairs contained menthone. Given that glandular trichomes were present on leaves 2-4cm in length and significant amounts of oil were accumulated in such structures, the oil extracted from these leaves should contain significant proportions of menthol and menthyl acetate. Compositional data presented in Section IV A 1 as well as by numerous workers (Reitsema *et al.*, 1957; Battaile and Loomis, 1961) indicated that young leaves (2-4cm) contained principally menthone, with menthol and menthyl acetate being associated with considerably more mature leaves.

Although oil accumulation corresponded to the period of leaf expansion, during which glandular trichomes were observed to fill with oil, the maximum amount of oil accumulated per leaf was dependent upon leaf ontogeny and environmental conditions. The lower yield of oil from basal leaves relative to midstem leaves was largely a reflection of the lower maximum amount of oil accumulated by these leaves, and was not associated with a rapid loss of oil from basal leaves with time. However, a significant loss of oil did occur in basal and midstem leaves from plants growing under long day-low night temperature conditions. Whilst the specific mechanism of oil loss was not investigated, it is possible that several avenues of oil loss were involved. The metabolic turnover of oil components, the conversion of components into non-volatile metabolites, and the loss of oil glands from lower leaves are suggested as possibilities. However, any proposed mechanism of oil loss from basal leaves needs to be consistent with the observed changes in oil composition in these basal leaves with time.

The lower quantities of oil accumulated under short day-high night temperature conditions, is in agreement with observations of Burbott and Loomis (1969). However, the lower yield from short day-high night

temperature treatments did not appear to be associated with a limited supply of photosynthate, since oil maturation and interconversions, processes known to be dependent on the availability of photosynthate, proceeded to the same extent under both conditions. Alternatively, the lower oil accumulation may be attributed to the fewer glands per unit leaf area on short day plants (Langston and Leopold, 1954). Both short photoperiodic conditions and short daylengths were observed to decrease oil yield per plant through a decreasing effect on both dry matter production and percentage oil yield. That is, although a decrease in percentage oil yield under short days may have resulted in an increase in the ratio of leaf to stem tissue, this change is also consistent with a decrease in the number of glands per unit leaf area under short days. Finally, Burbott and Loomis (1967), when reporting the effect of photosynthate balance on monoterpene composition, stated "The increased amount of essential oil formed under long day conditions appear to be largely a reflection of increased growth". From the preceding discussions of factors affecting oil accumulation in peppermint, the above statement would seem to over-simplify the situation. That is, both photosynthate status and photoperiodic effects have been implicated as important determinants of oil accumulation.

From an understanding of factors influencing oil yield and composition on an individual plant basis and under glasshouse-growth room conditions, it becomes possible to attempt an explanation of changes in oil yield and composition under field conditions. However, extreme caution is required when extrapolating to the field situation and the many limitations should be realised.

Under Tasmanian conditions, provided relatively high plant densities were considered, oil yield per unit area increased initially and then remained constant for a considerable period prior to the appearance of

inflorescences. During the period of increasing yield per unit area, both the number of leaves per unit area and the oil content of these leaves would be expected to have increased. Since there is an upper limit to the leaf area which is capable of being supported per unit surface area of ground (due to shading), it could be proposed that this upper limit of leaf area was achieved at the time oil yield per unit area reached the plateau value. This is consistent with the observation that yield per unit area continued to increase throughout the growing season when low density plantings were considered.

During the period of maximum oil yield per unit area, it follows that oil lost must equal oil produced. Loss of oil could have resulted from loss of oil from glands (metabolic depletion, conversion of oil compounds to non-volatile metabolites and/or evaporation), loss of glands from leaves and/or loss of lower leaves. Production of oil may have involved the formation of new leaves and/or increased oil content of existing leaves. From individual plant studies, the production of oil and the loss of oil were considered to be confined mainly to apical and basal leaves, respectively. Due to limits on leaf area, the production of new leaf area in the apical region would result in the loss of an approximately equal leaf area from the basal region. If leaf production and loss were the only factors involved, a steady increase in oil yield per unit area would have been expected, since apical leaves tend to accumulate more oil per unit leaf area than basal leaves. Such an increase was not observed under field conditions and this may have been due to oil losses from midstem or basal leaves.

Since harvesting in Tasmania coincides with the appearance of inflorescences, any rapid increase in the essential oil content of midstem leaves at this stage, as reported by Burbott and Loomis (1969),

would result in a rapid increase in oil yield per unit area. This assumes no drastic changes in loss of oil from apical and basal leaves at this time. An increase in oil yield per unit area was not associated with the appearance of inflorescences, suggesting that no rapid increase occurred in the oil content of midstem leaves. The decreased oil yield per unit area following inflorescence appearance may have been associated with either a decreased rate of oil production and a constant rate of oil loss or an increased rate of oil loss from midstem and basal leaves.

A characteristic of the Tasmanian production areas is the rapid increase in yield per unit area during the early growing season, a relatively long period of maximum yield followed by a gradual decrease in yield per unit area. This increase followed by a decrease in oil yield is reported to be more rapid under other environmental conditions (Embong *et al.*, 1977), with the maximum yield being associated with inflorescence appearance. An increased followed by a decrease in oil content of midstem leaves at the time of inflorescence appearance could account for changes in yield per unit area with time, reported by Embong *et al.* (1977). That is, the magnitude of the increase and decrease in oil content of midstem leaves may be dependent on environmental conditions. This dependence of changes in oil content on environmental conditions may explain the apparent disagreement in results reported in Section IV A 1 and by Burbott and Loomis (1969).

From the proceding discussion, it is apparent that environmental conditions such as daylength, light intensity, day temperature and night temperature are important determinants of oil yield and composition. Within the limits to oil yield and composition imposed by the particular environmental conditions experienced in Tasmania, the potential to increase oil yield per unit area whilst maintaining the required oil composition was investigated by optimising and manipulating several

cultural practices.

With respect to harvest date, provided relatively high plant densities were considered, maximum oil yield per unit area and satisfactory oil composition (>45% menthol, <3% menthofuran) were obtained when harvesting commenced as soon as the 45 percent menthol content was achieved in extracted oils. In this respect a compromise between oil yield and oil composition was not required, under Tasmanian conditions. That is, not only were photosynthetic and photoperiodic conditions conducive to the conversion of pulegone to menthone, the required balance between mature and immature leaves (which is a reflection of the maturity dependent conversion of menthone to menthol and menthol to menthyl acetate) was such as to allow the appropriate balance between menthol, menthone and menthyl acetate and minimum menthofuran (small proportion of oil from inflorescences), within the period of maximum oil yield per unit area.

The 45 percent menthol level was consistently associated with crops in which most plants had formed a terminal infloresence which was 1-2cm in length. If harvest date was delayed until the full bloom stage, as is common practice in many world production areas, this would result in further increases in menthol but at the expense of increased menthofuran and possible decreased oil yields. A 'rule of thumb' based on the appearance of inflorescences, may represent a valuable guide to the timing of harvest and should reduce the number of sequential harvests and trial distillations necessary. However, since such methods may be subject to variations between areas, seasons and cultural practices, 'rules of thumb' should only be used with their limitations in mind and in conjunction with trial distillations and oil analyses.

At low plant densities (e.g. 10 plants/m^2), considerable benefit may result from delaying harvest, well after the 45 percent menthol

content is exceeded. Since menthofuran is reported to decrease after the full bloom stage and provided that oil yield per unit area and menthol content continue to increase in low plant densities, a delay in harvest until after full bloom may result in improved oil yields and menthol contents.

Loomis (1977b), when considering the physiological manipulation of peppermint, stated "Could one induce early blooming and thereby get two crops? We have seen mint that looked ready to cut in June but was not considered mature until mid-August - Build-up of menthol does not start till growth stops. In the northwest this means at the time of flowering." Loomis (1977b) suggested the manipulation of flowering by controlling irrigation and fertiliser nitrogen.

When the effect of nitrogen and irrigation were investigated, it was found possible to increase oil yields under Tasmanian conditions. That is, the low yields commonly obtained in Tasmania are most likely a reflection of deficiencies in cultural techniques rather than a limit imposed by the Tasmanian environment. Furthermore, it appeared that high rates of irrigation during the latter part of the growing season, combined with high applications of fertiliser nitrogen, had the most pronounced effect on oil yield. Little benefit would result from increasing either nitrogen or irrigation alone. This is particularly the case within present commercial operations where low inputs of both irrigation and nitrogen are practised.

In addition to the above increase in oil yield per unit area, significant yields of oil resulted from a later harvest of regrowth herb arising from the high irrigation, high nitrogen treatments. Such significant yields of regrowth oil introduced the possibility of a double harvest, thereby increasing the total oil yield. Although the nitrogen-irrigation treatments had significant effects on oil

composition at both harvests, the most pronounced effect was the large difference in oil composition obtained at the different harvest times. The second crop (or regrowth) yielded more mature oil than the first harvest, having higher concentrations of menthol and menthyl acetate and lower concentrations of menthone. Therefore, in addition to the possibility of a double harvest, the mature nature of the regrowth oil introduced a degree of flexibility with respect to oil composition and harvest date. That is, by blending oil obtained from the two harvests, any immature characteristics in oil obtained from the first harvest could be compensated for by the mature characteristics of oil from the second harvest.

In the physiological manipulations suggested by Loomis (1977b), the need to shorten the growing season of the first crop was recognised. It was suggested that attention be given to inducing early flowering and therefore early maturation of oil. That is, from both the observations of Loomis (1977b) and results reported in Section IV B 2, it is apparent that any successful attempt at obtaining two harvests per season needs to overcome problems associated with the lengthy period of time required for the maturity dependent conversion of menthone to menthol, following achievement of maximum oil yield per unit area. Under Tasmanian conditions, several alternative management strategies are apparent from the findings of the present study. Such alternatives rely on increased inputs of irrigation (especially during the latter part of the growing season) and nitrogen, as well as the particular environmental condition prevailing during the growing season, in Tasmania.

Firstly, it is possible to tolerate the long period of growth required for oil maturation in the first crop, given that growing conditions which prevail during the subsequent regrowth induce rapid

maturation of oil. Secondly, the growing season of the first crop may be shortened by harvesting as soon as maximum oil yield per unit area is obtained, regardless of the immature nature of this first harvest oil.

The balance between immature and mature leaves, necessary to produce oil with 45 percent menthol, was associated with the formation of inflorescences in the first crop. Regrowth plants did not flower, and oil maturation was associated with the onset of dormancy. Environmental conditions prevailing during the later stages of regrowth appeared to favour rapid oil maturation. However, the shorter days may be responsible for the higher menthofuran and low cineole to limonene ratio.

By increasing the frequency and rate of nitrogen the severity of rust infestations in crops (harvested at 45 percent menthol concentration) was increased. Early harvesting (≈40% menthol, maximum oil yield/ha) avoided the damaging effects of rust in the first crop. However, rust infestations caused considerable damage in subsequent regrowth crops during 1980. From changes in oil yield and composition it is apparent that the loss of lower leaves due to a severe rust infestation would have more important consequences on oil composition than oil yield. That is, lower leaves contain less oil but oil higher in menthol and menthyl acetate, relative to midstem leaves. Severe rust infestations occurred towards the end of the growing season in crops harvested at 45 percent menthol, as well as regrowth crops. During the latter stages of crop growth it is important that the conversion of menthone to menthol should proceed as rapidly as possible, if the required level of menthol is to be achieved at harvest. Since the time required for the maturity dependent conversion of menthone to menthol is already considered a limitation within the two harvest programme,

any loss of menthol arising from the loss of lower leaves would have adverse effects on oil composition. With severe rust infestations the loss of menthol arising through the loss of lower leaves may exceed the production of menthol from menthone in the remaining leaves. Furthermore, the loss of lower leaves causes a reduction in oil yield per unit area as well as a reduction in the proportion of leaf oil to flower oil. Thus, severely rust infected plantings often result in low yields of oil having low menthol and high menthofuran (e.g. numerous King Island oils - Table IV B 1). Since one of the requirements of oil from regrowth is a high menthol content, it is obvious that rust control in regrowth will determine the success of the two harvest programme.

Arising from this study are several factors that require further development within an ongoing research and development programme. Firstly, before the suggested two harvest programme could be recommended for large-scale commercial application, an assessment of the influence of double harvesting on oil yields in subsequent seasons is required. The effect of the two harvest programme on the decline in vigour of established plantings after approximately four years, should also be assessed. Secondly, although the above increases in oil yield were achieved without any physiological manipulations (e.g. induction of early flowering), significant advantages may be associated with the incorporation of the latter manipulations into the two harvest programme. In the initial nitrogen-irrigation investigation an attempt was made to assess the effect of water stress induced during the early growing season on subsequent oil yields per unit area. To this end, irrigation treatments $I_{(H)}$ and $I_{(L \rightarrow H)}$ were included. However, from leaf diffusive resistance measurements conducted during the early part of the growing season, it was apparent that the low irrigation regime provided adequate moisture at this stage. No valid assessment of the influence of water

stress, early in the season, was therefore obtained.

Within the two treatment system, it may be possible to increase the plateau oil yield reached prior to harvest by inducing moisture stress early in the growing season. Loomis (1977a)suggested that moisture stress during the early season promotes smaller leaves and therefore more leaves per unit area. These smaller leaves were reported to contain similar amounts of oil as larger leaves. Furthermore, moisture stress induces early flowering and therefore earlier oil maturation. Increasing the maturity of oil obtained at harvest 1 would reduce the requirement for very mature oil at harvest 2.

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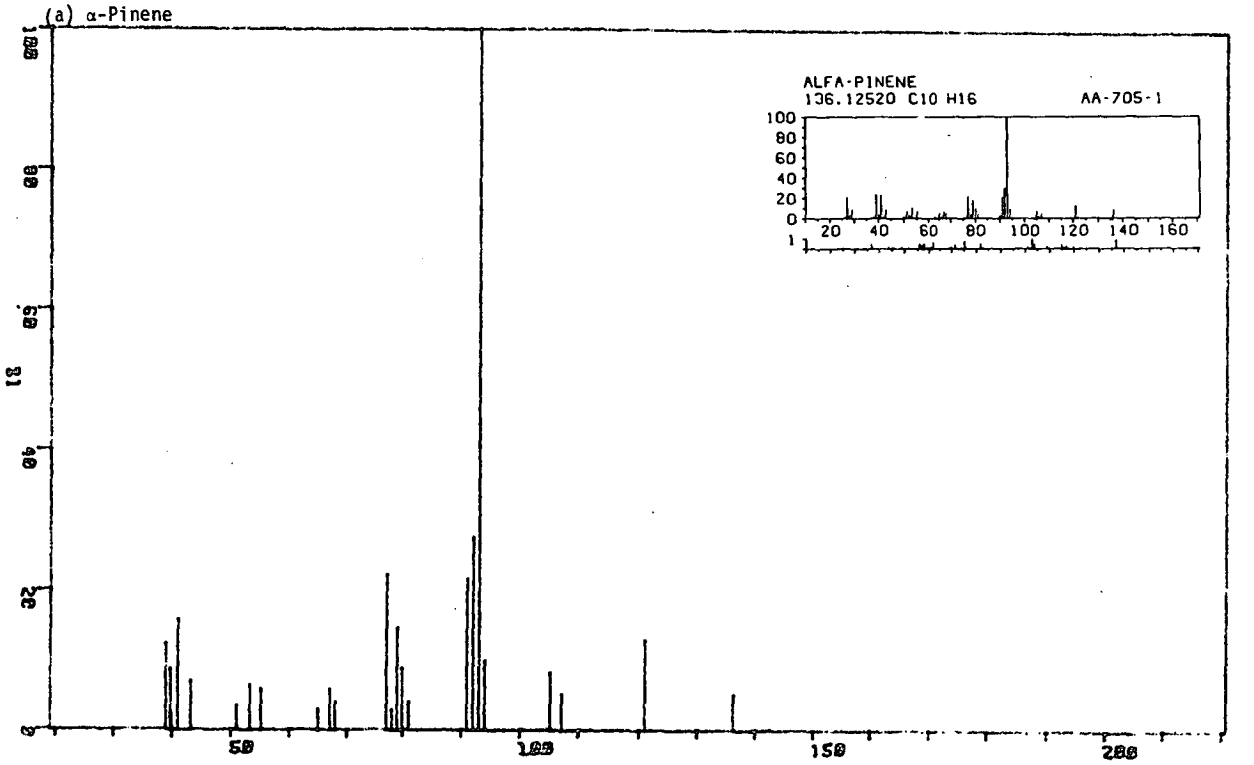
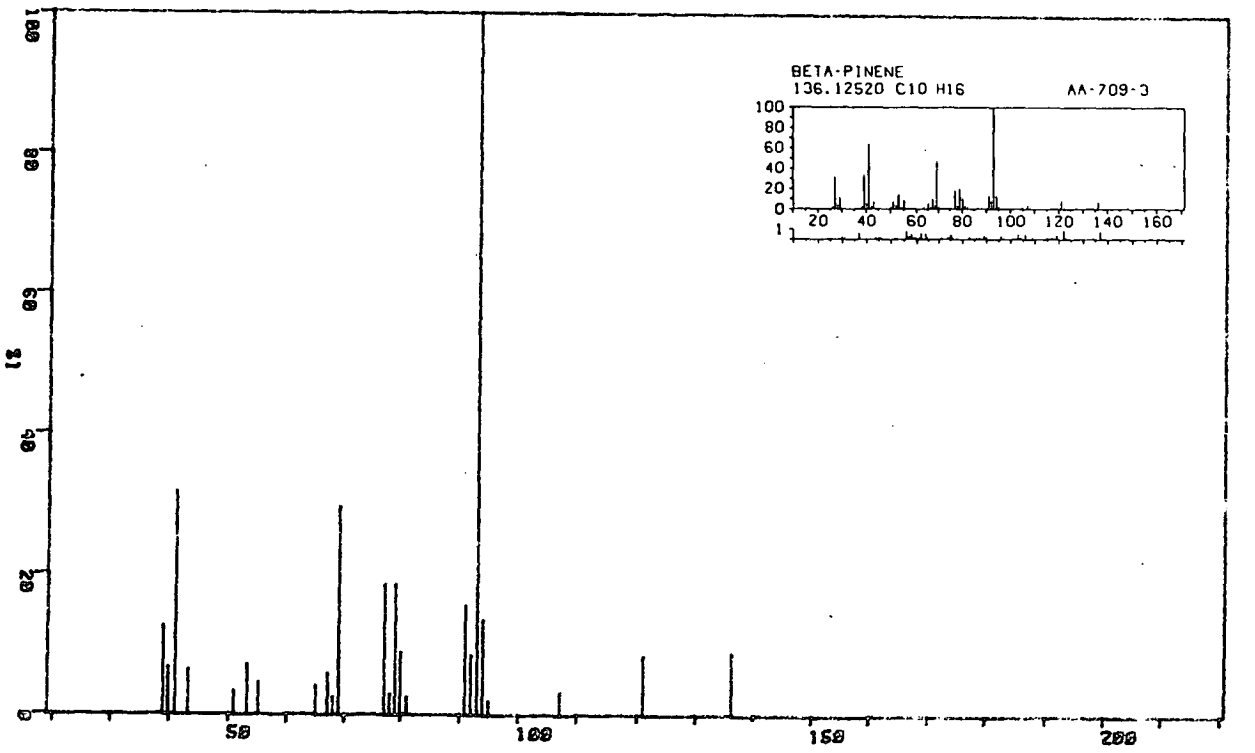
VII APPENDICES

Appendix III 4.1. Mass spectra of major peppermint oil components.
(Reference mass spectra taken from Stenhagen *et al.*, 1974).

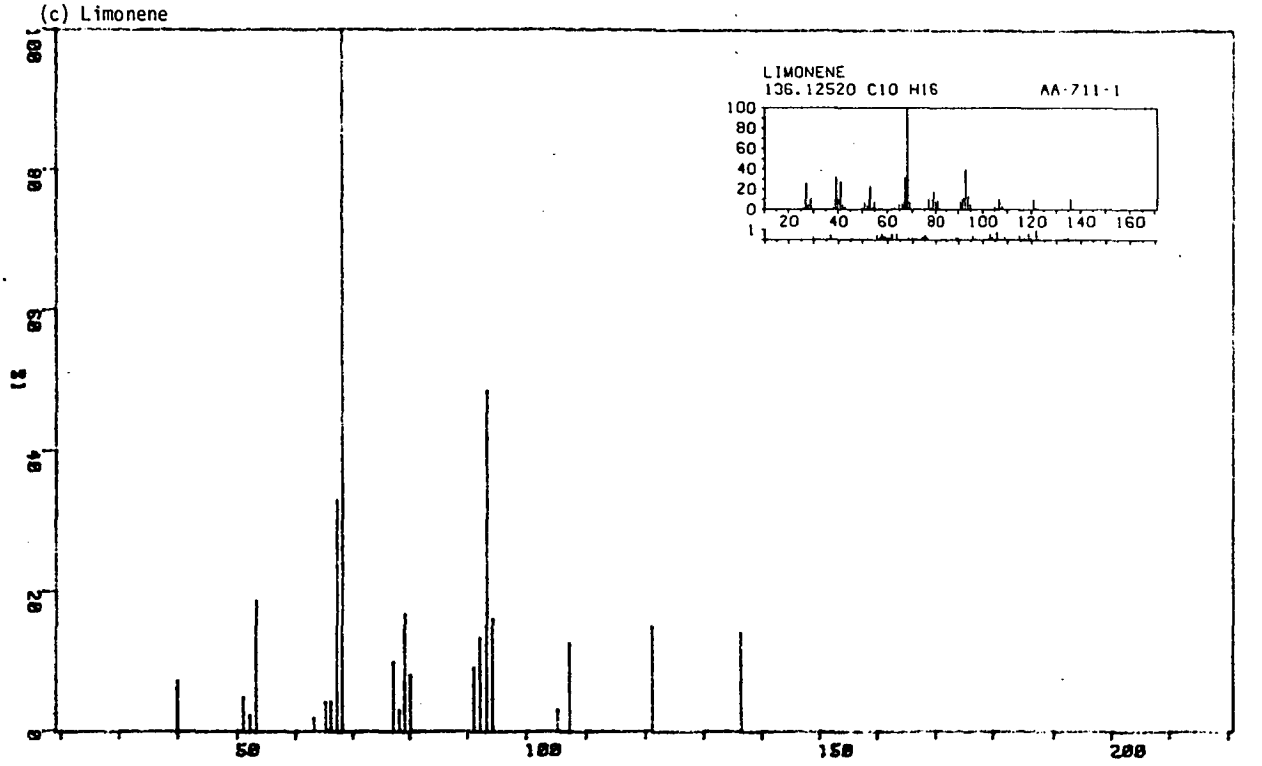
(a) α-Pinene



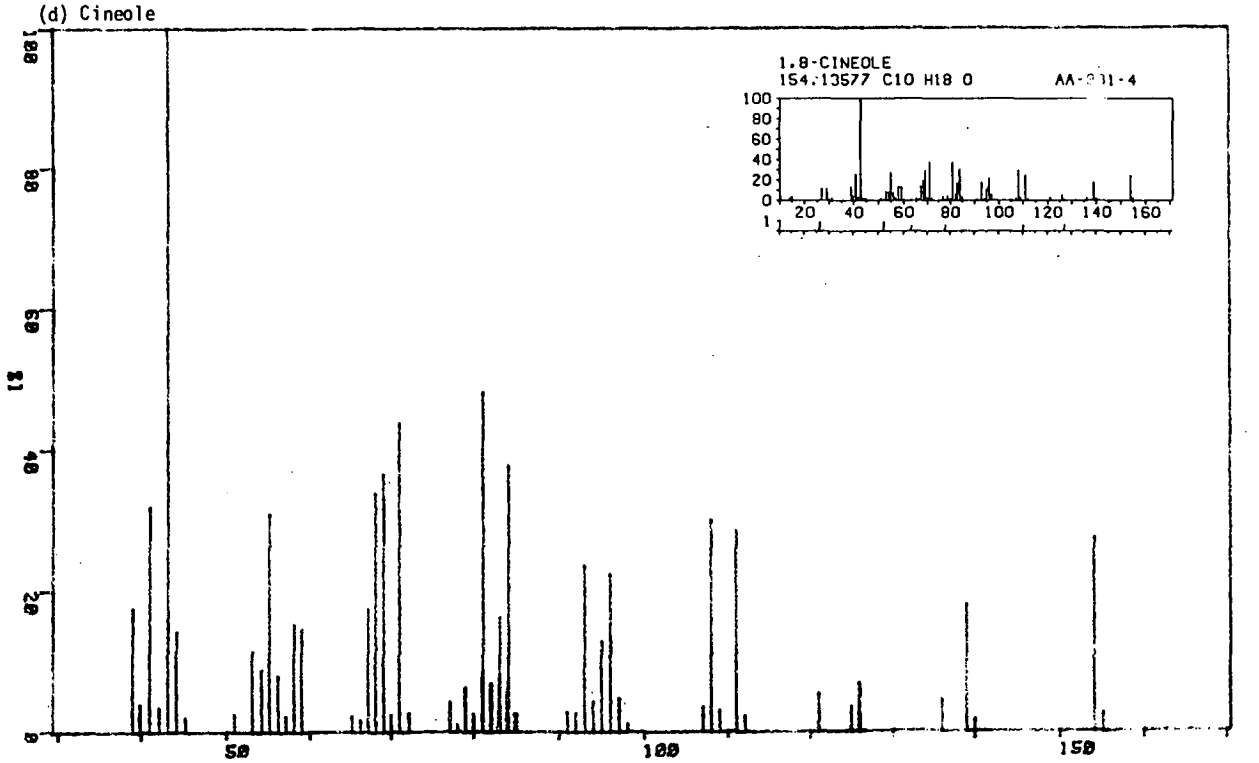
(b) β-Pinene



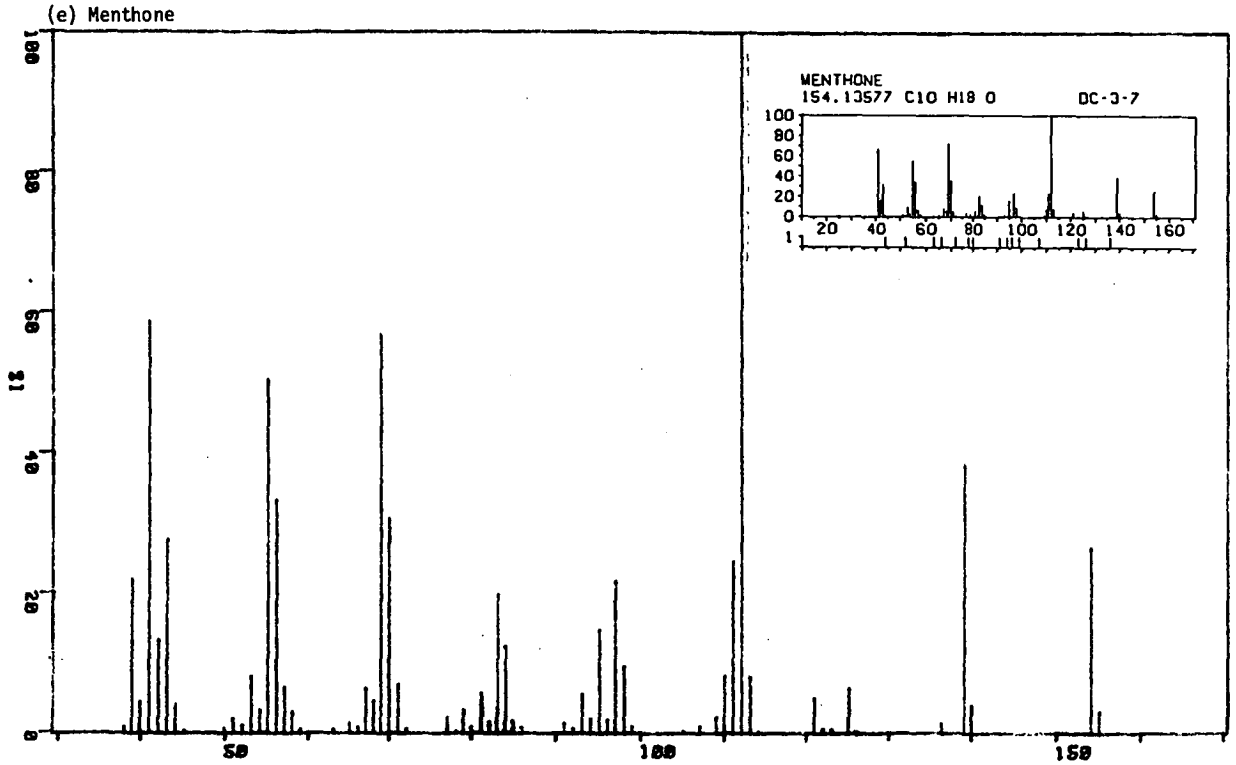
(c) Limonene



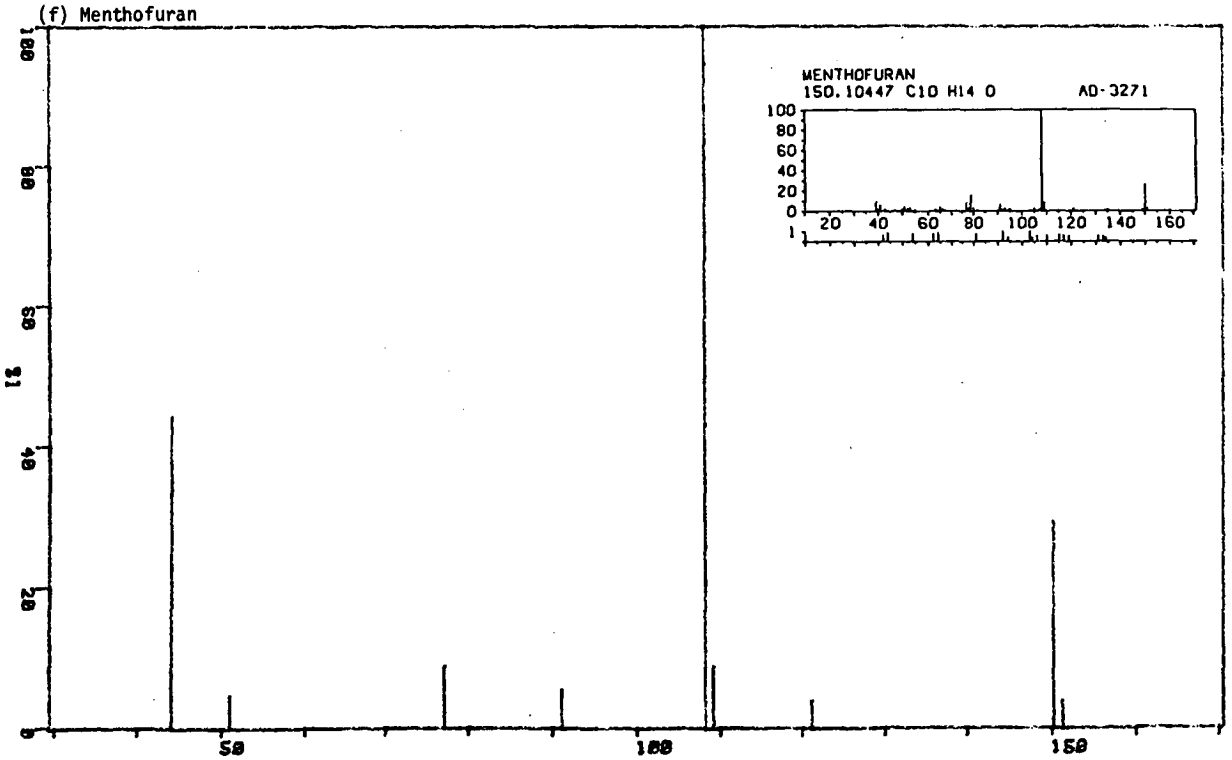
(d) Cineole



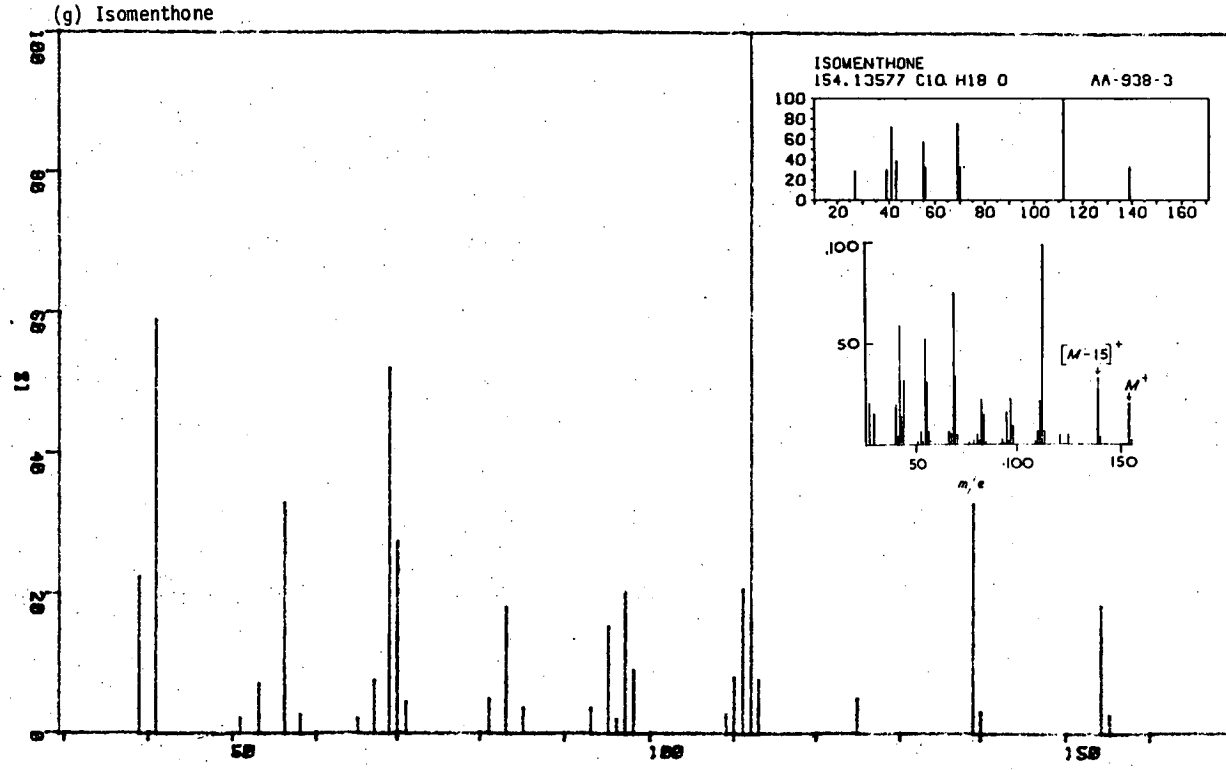
(e) Menthone



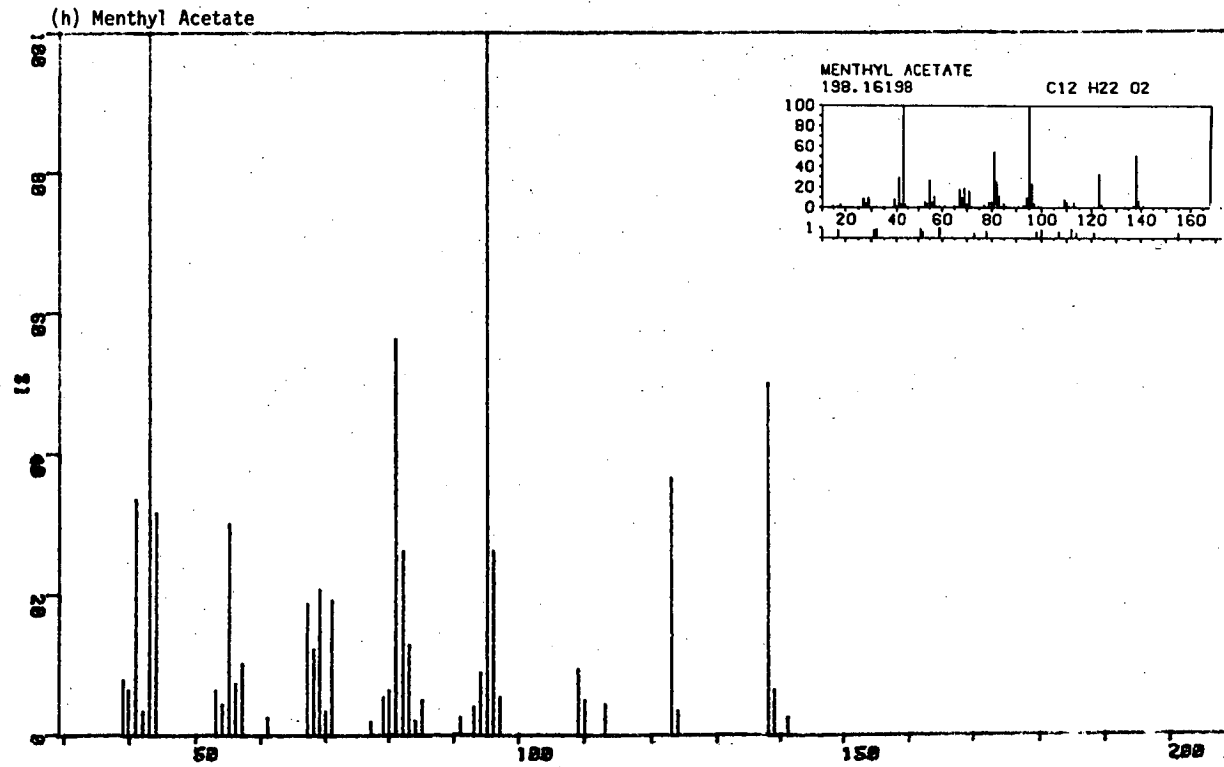
(f) Menthofuran



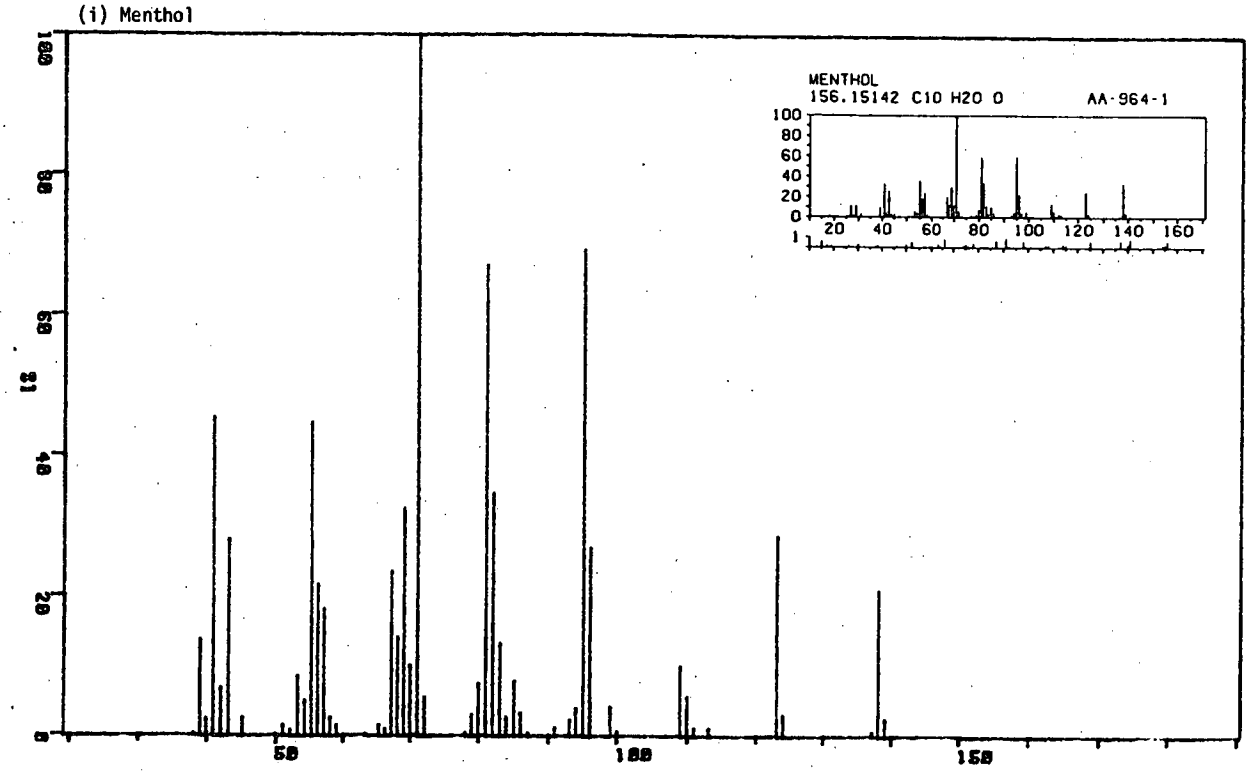
(g) Isomenthone



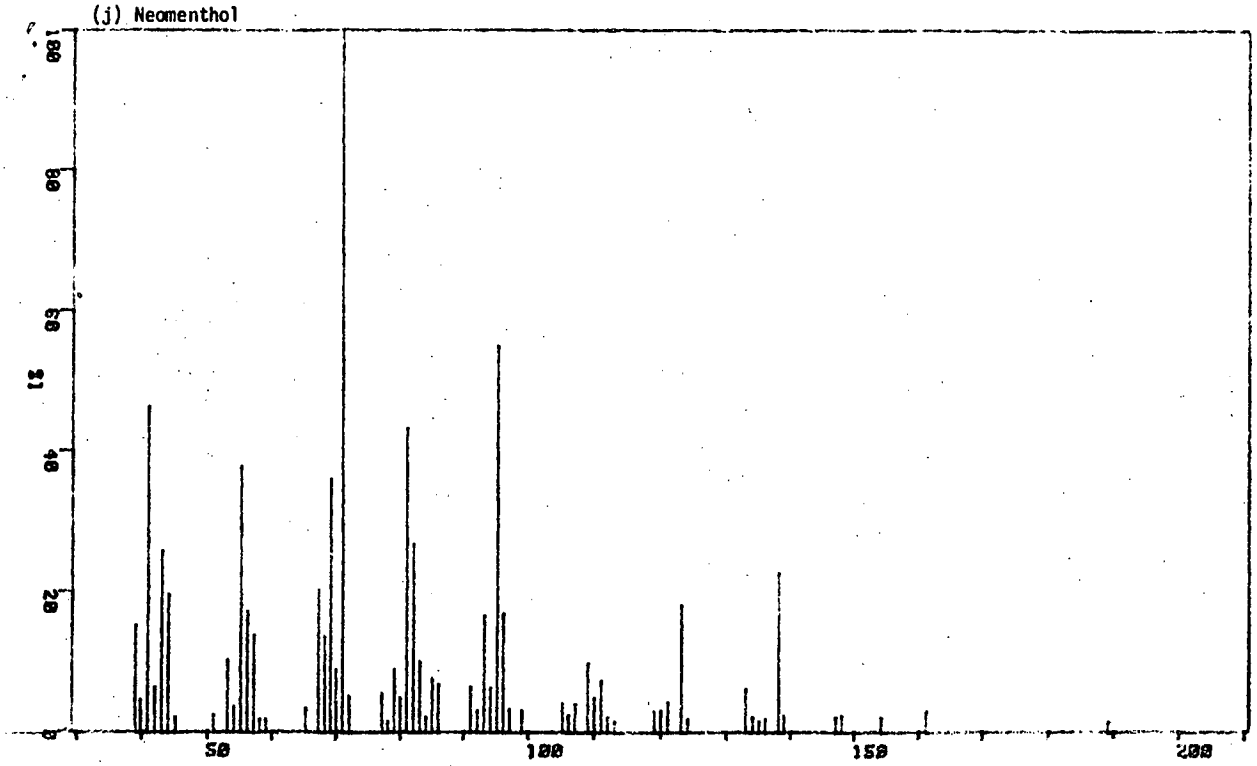
(h) Menthyl Acetate



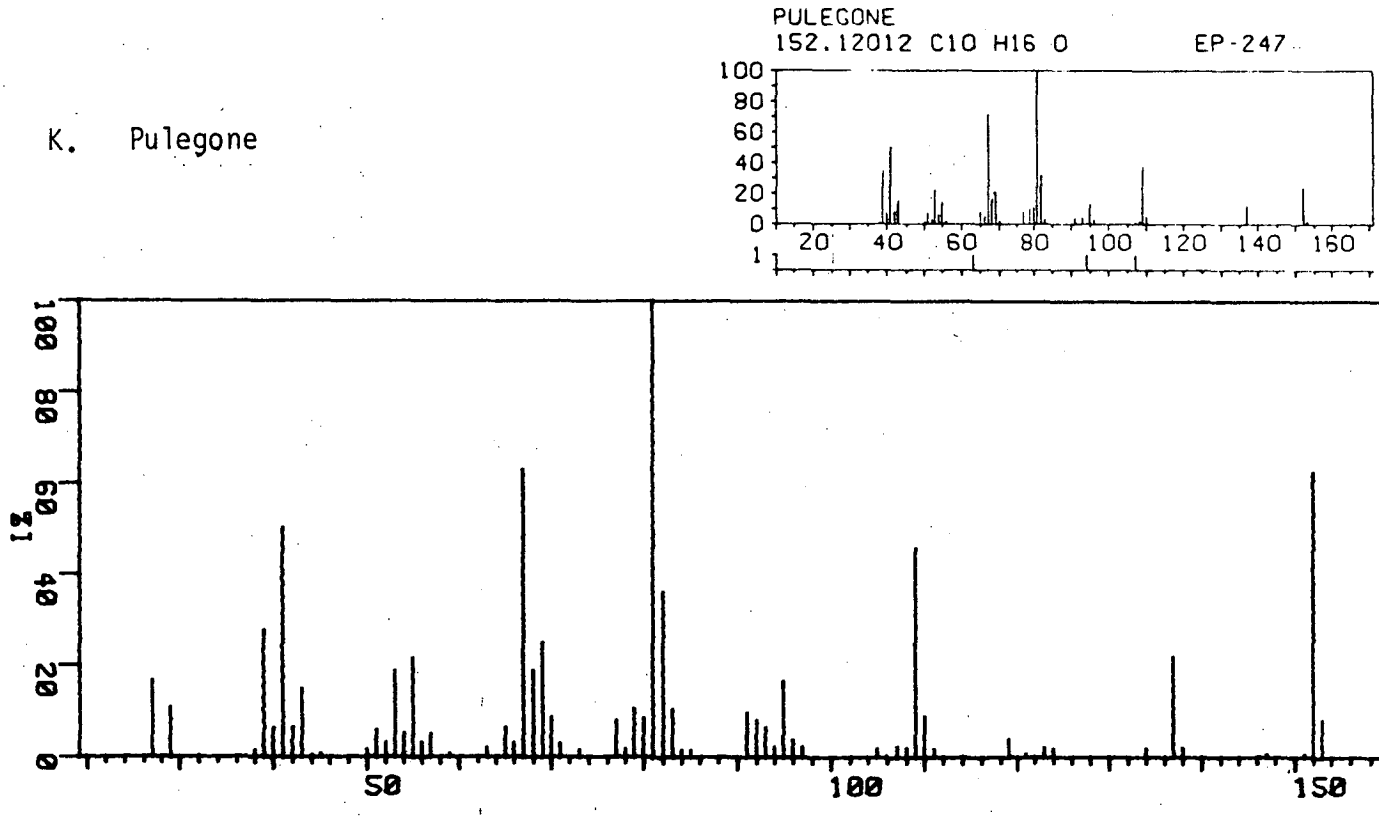
(i) Menthol



(j) Neomenthol



K. Pulegone


PULEGONE
152.12012 C10 H16 O
EP-247
100
80
60
40
20
0
1
20
40
60
80
100
120
140
160
%I
50
100
150

Appendix III 4.2. Fragmentation patterns of menthol isomers and unknown (Neomenthol)*.

m/e	Neoisomenthol	Neomenthol	Isomenthol	Unknown	m/e	Neoisomenthol	Neomenthol	Isomenthol	Unknown	m/e	Neoisomenthol	Neomenthol	Isomenthol	Unknown
39	13	11	12	15	77	2	2	3	6	110	5	5	4	5
40				5	78				2	111	2	1	6	7
41	42	36	40	46	79	4	3	4	9	112	5	2	3	2
42	6	6	7	6	80	5	5	6	5	113	3	1	1	1
43	32	30	32	33	81	52	42	54	43	119				3
44				20	82	29	25	17	26	120				3
45	3	3	3	2	83	11	8	10	11	121				4
51			3		84	3	2	3	2	122				
52					85	10	7	9	8	123	20	17	20	18
53	7	5	7	11	86	5	4	4	6	124	2	2	2	2
54	5	3	4	4	91	2	1	1	6	132				
55	42	35	39	38	93	3	2	3	16	133				6
56	20	17	18	17	94	4	3	3	6	134				2
57	24	21	23	14	95	57	54	52	55	135				1
58	3	2	3	2	96	19	19	12	17	136				2
59	1	1	1	2	97	5	3	4	3	137				
65				4	98	1		1		138				23
67	21	18	21	20	99	4	3	4	3	139				3
68	13	12	12	14	100	1		1		141	1			
69	32	26	29	36	105				4	147				2
70	12	10	11	9	106				3	148				2
71	100	100	100	100	107				4					
72	5	5	5	5	108									
73	1	1	2		109	11	8	8	11					

[*Fragmentation patterns for Menthols obtained from Thomas and Willhalm (1966).]

Appendix III 4.3. Flame Ionization Detector - Correction Factors[a]

(Gas chromatographic conditions used during the determination of correction factors were those outlined in Section III 4.)

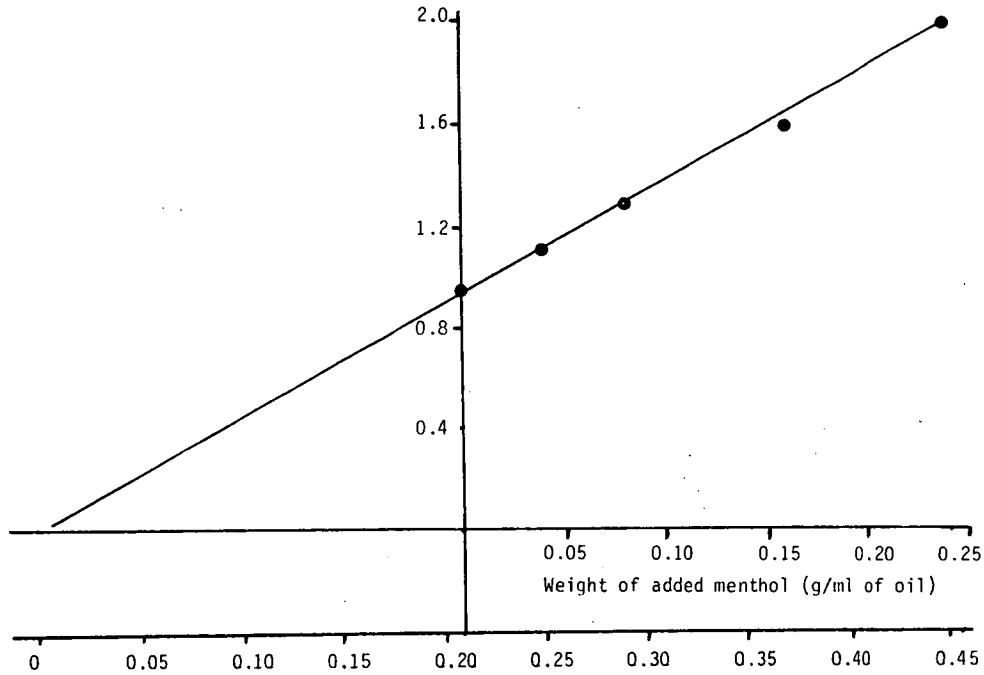
Reference Compound	Weight of compound added to mixture (mg/ml)	Peak area[b] (Standardized injection volume)	Calculated Peak area	Correction Factor
1. Limonene	42.5	343396	346182	1.01
2. Cineole	52.7	432690	429266	0.99
3. Menthone	63.5	476189	517237	1.09
4. Menthofuran	31.5	240294	256582	1.07
5. Isomenthone	26.5	190582	215855	1.13
6. Menthyl Acetate	91.9	674677	748569	1.11
7. Menthol	100.0	814547	814547	1.00
8. Pulegone	24.6	221704	200379	0.90

[a] Correction Factors were calculated using the technique of Smith and Levi (1961) - each peak area represents the mean of four determinations.

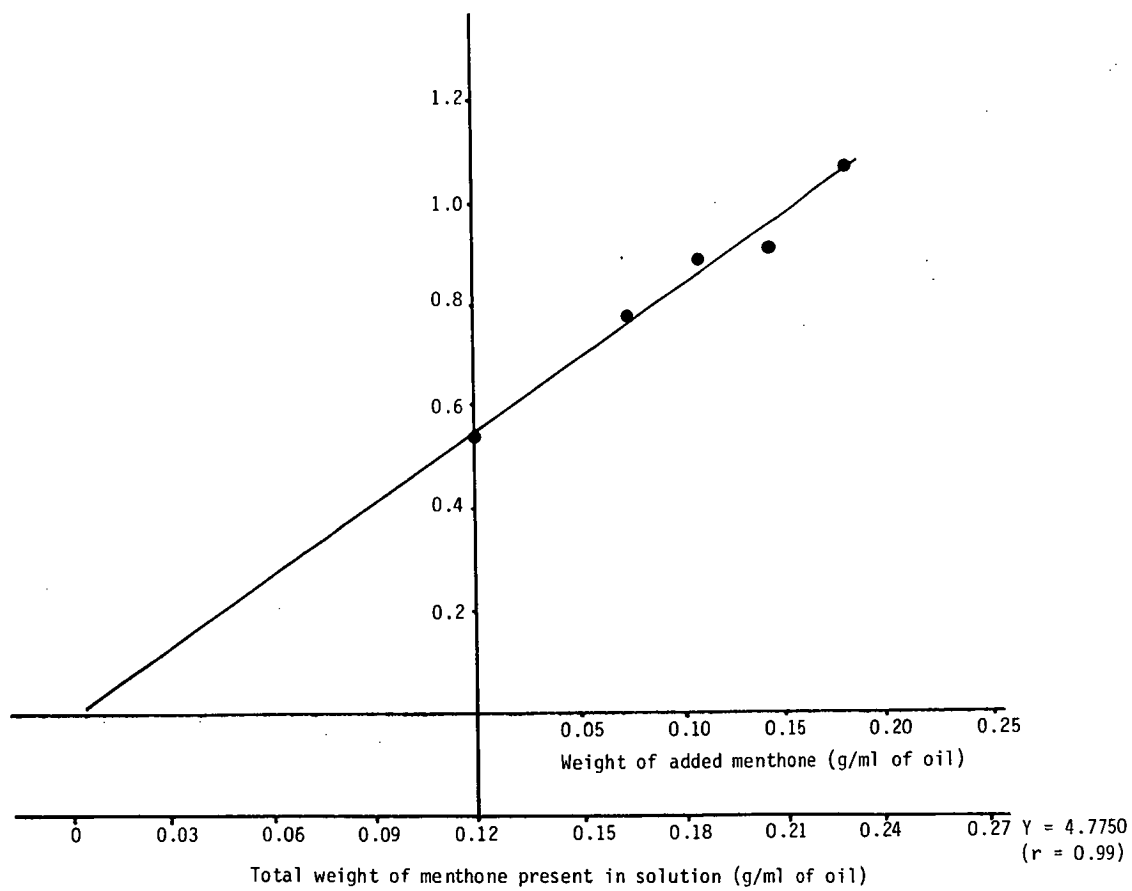
[b] Peak areas determined by the DP 88 Computing Integrator, were corrected for variations in volume injected by including an internal standard (β-methyl naphthalene) in the mixture.

Appendix III 4.4. Gas chromatograph-calibration curves (taken from Clark, 1976).

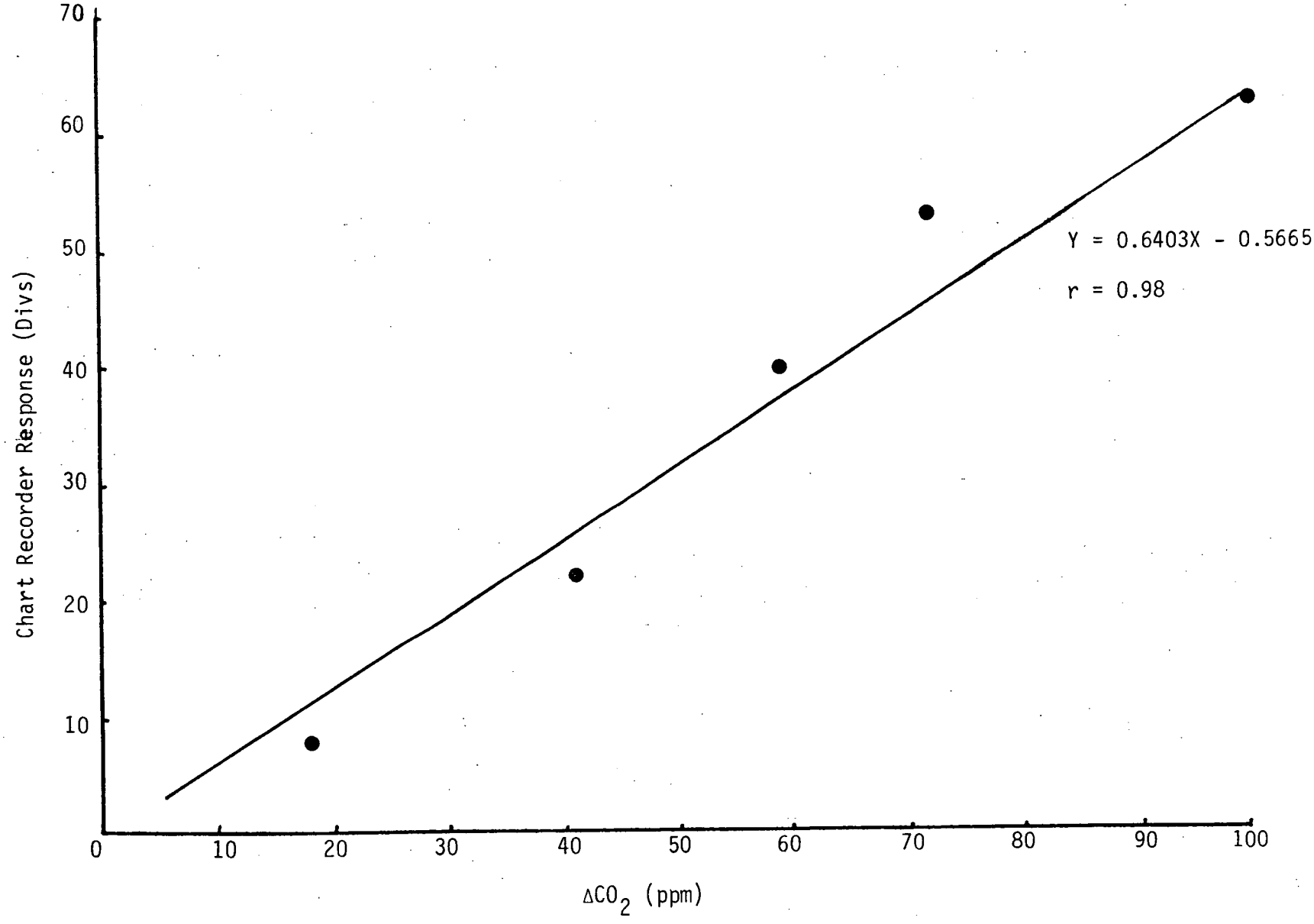
(a) Menthol.



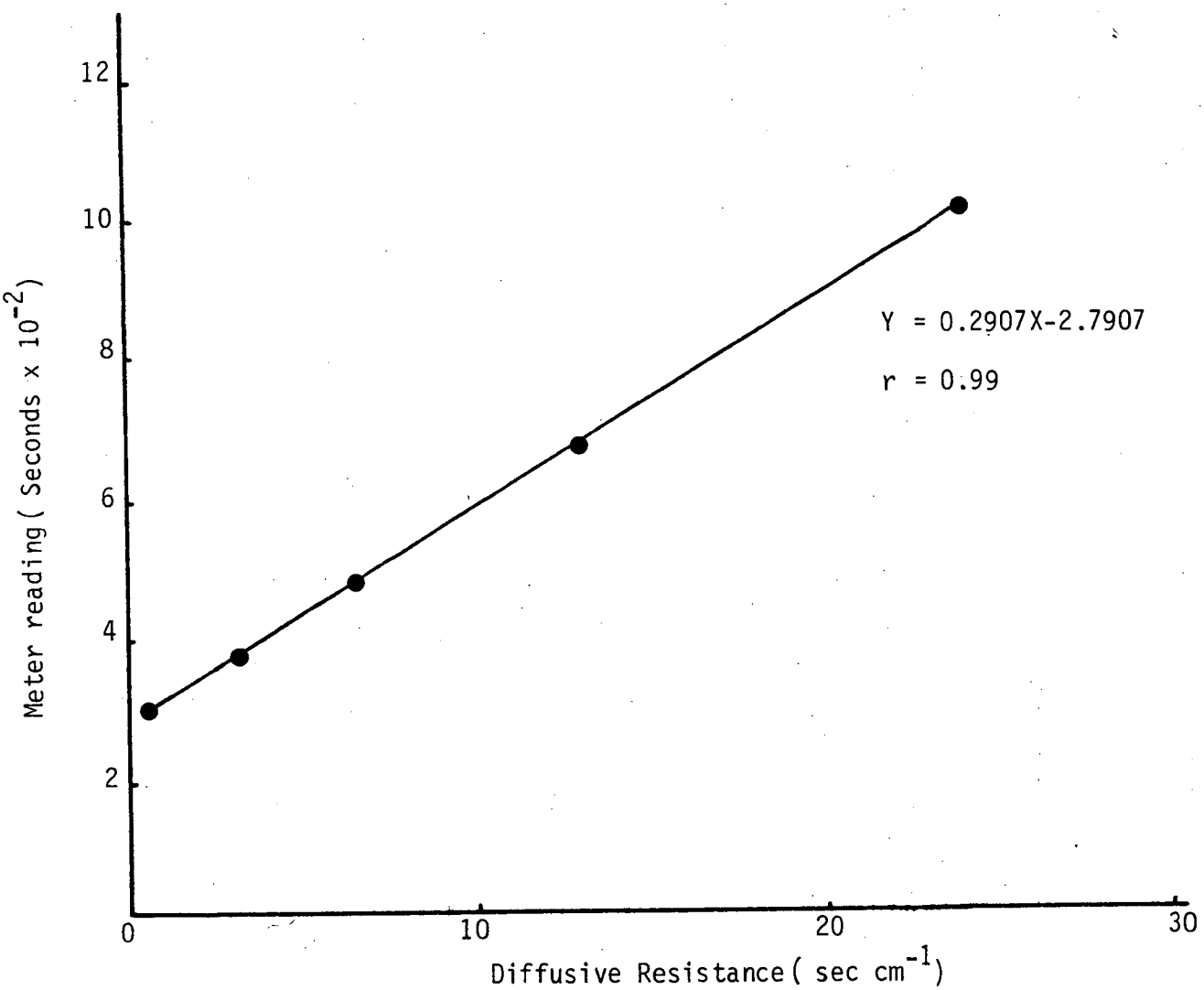
(b) Menthone.



Appendix III 5.1. Calibration Curve - Infra-red Gas Analyser.



Appendix III 7.1. Calibration Curve - Lambda L1-65 Autoporometer and L1-20S Sensor ($25^{o}C$).



Appendix III 7.2. Temperature Conversions.

Lambda L1-65 Autoporometer and L1-20S Sensor.

Temperature (^{o}C)	Conversion Factor
15	0.55
16	0.58
17	0.60
18	0.63
19	0.68
20	0.72
21	0.76
22	0.81
23	0.88
24	0.95
25	1.00
26	1.08
27	1.15
28	1.22
29	1.30
30	1.40
31	1.50
32	1.60
33	1.70

Appendix IV A 1.1 Relative Oil Yield.

Growing Conditions: LD x LNT

Leaf Pair	Ratio Total Peak Area:Peak Area of Internal Standard					
	Block No.	Harvest Date (17/8/77) 1	(24/8/77) 2	(1/9/77) 3	(18/9/77) 4	(4/10/77) 5
	1	4.45	4.40	1.22	1.44	
2	2	4.04	3.40	3.09	1.76	
	3	4.21	2.33	2.94	2.05	
	1	10.20	10.42	9.94	7.28	
4	2	12.04	12.39	5.08	3.21	
	3	9.02	10.92	3.56	5.29	
	1	19.41	19.44	25.38	16.70	10.70
6	2	24.08	24.93	36.10	24.39	9.73
	3	27.67	20.41	25.34	37.37	9.07
	1	3.29	22.73	27.25	52.98	42.37
8	2	10.26	29.26	42.17	40.29	38.98
	3	17.95	28.92	53.29	62.91	47.11
	1		3.27	23.21	47.99	40.11
10	2		4.74	13.98	39.20	42.73
	3		2.94	28.71	54.27	39.28
	1			7.12	31.29	37.17
12	2			6.21	26.93	40.29
	3			11.25	27.11	36.15
	1			3.29	17.11	31.29
14	2			2.17	12.11	35.17
	3			4.73	20.73	39.28
	1				3.16	11.97
16	2				6.20	18.29
	3				9.77	25.11

Growing Conditions: SD x HNT

Leaf Pair	Ratio Total Peak Area:Peak Area of Internal Standard					
	Block No.	Harvest Date (17/8/77) 1	(24/8/77) 2	(1/9/77) 3	(18/9/77) 4	(4/10/77) 5
	1	1.25	1.02	1.91	1.73	
2	2	1.73	1.59	1.27	1.80	
	3	2.01	1.98	1.62	2.59	
	1	2.10	1.86	1.93	2.76	
4	2	2.59	3.27	3.21	2.83	
	3	3.27	2.15	2.94	3.80	
	1	2.54	2.03	5.12	7.82	12.75
6	2	5.95	7.86	5.87	6.81	8.26
	3	7.35	8.27	6.23	9.22	8.64
	1	3.72	8.73	8.69	14.38	16.82
8	2	2.95	9.43	10.92	14.29	14.29
	3	5.29	9.29	10.27	15.79	17.73
	1		5.78	10.29	18.25	20.17
10	2		5.29	15.78	19.25	19.87
	3		6.21	12.64	29.55	23.17
	1			2.18	24.41	24.39
12	2			2.68	23.17	26.21
	3			5.11	25.11	25.98
	1			1.46	8.21	14.17
14	2			1.72	8.91	25.29
	3			2.39	10.23	23.91
	1				4.58	7.69
16	2				4.11	9.78
	3				2.11	8.11

Appendix IV A 1.2 Leaf Area (cm^2).

Growing Conditions: LD x LNT

Leaf Pair	Ratio Total Peak Area:Peak Area of Internal Standard					
	Block No.	Harvest Date (17/8/77) 1	(24/8/77) 2	(1/9/77) 3	(18/9/77) 4	(4/10/77) 5
	1	16.27	9.66	10.57	8.29	
2	2	9.37	7.18	8.29	11.55	
	3	11.26	10.42	7.91	8.34	
	1	28.24	30.49	27.32	31.98	
4	2	23.06	19.29	20.11	19.27	
	3	25.17	25.35	20.90	18.71	
	1	34.26	39.77	34.28	31.73	39.77
6	2	31.95	34.90	31.29	35.34	32.09
	3	32.86	32.64	38.27	35.90	38.11
	1	11.60	33.50	40.21	43.20	42.29
8	2	12.65	28.45	31.97	41.14	45.18
	3	12.51	22.67	32.01	42.72	44.39
	1		8.52	29.21	38.20	43.55
10	2		9.20	21.11	39.98	46.15
	3		6.98	16.43	39.19	44.22
	1			17.17	29.11	42.71
12	2			10.16	27.22	38.98
	3			5.68	23.11	37.73
	1			4.20	25.25	37.11
14	2			5.14	24.71	39.08
	3			3.27	12.17	28.10
	1				9.87	21.02
16	2				4.14	24.11
	3				5.25	29.73

Growing Conditions: SD x HNT

Leaf Pair	Ratio Total Peak Area:Peak Area of Internal Standard					
	Block No.	Harvest Date (17/8/77) 1	(24/8/77) 2	(1/9/77) 3	(18/9/77) 4	(4/10/77) 5
	1	7.29	6.43	8.27	5.99	
2	2	8.27	5.29	5.77	7.32	
	3	7.81	10.27	6.99	7.02	
	1	12.42	15.50	11.91	13.54	
4	2	6.53	12.57	9.21	12.19	
	3	10.21	13.55	13.49	12.00	
	1	10.91	14.62	25.39	25.02	24.11
6	2	11.29	17.32	21.02	22.17	19.15
	3	14.17	21.22	20.17	25.10	22.17
	1	6.33	17.13	24.20	26.29	28.29
8	2	2.51	12.55	19.18	27.33	26.25
	3	5.29	13.11	22.29	23.10	29.12
	1		4.56	14.11	15.11	24.17
10	2		6.29	16.87	29.27	27.19
	3		5.18	17.22	20.31	32.69
	1			8.92	13.07	20.29
12	2			4.29	14.11	22.13
	3			8.33	19.17	18.29
	1			4.14	8.11	12.10
14	2			2.10	9.07	13.77
	3			4.71	12.33	17.33
	1				2.19	4.16
16	2				4.31	6.12
	3				1.79	5.17

Appendix IV A 1.3 . Oil Composition (%). LD x LNT.

(i) Harvest Date 1. (17/8/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.21	2.15	1.98	6.06	25.97	5.32	2.11	0.17	2.80	46.91	0.55
	2	0.97	1.42	2.31	4.45	11.02	8.72	1.95	1.32	3.23	61.12	1.64
	3	1.20	1.95	1.99	5.55	14.71	6.25	1.03	0.85	2.93	58.23	0.54
4	1	1.01	2.29	2.00	7.44	57.82	7.09	1.53	0.92	1.39	14.20	1.70
	2	1.07	1.88	2.32	7.74	30.16	6.08	1.87	0.17	1.89	39.87	1.50
	3	1.00	1.90	1.99	7.50	38.51	6.50	2.31	0.28	1.45	32.10	1.75
6	1	0.79	1.48	2.51	6.35	69.82	7.90	1.51	0.29	0.30	3.50	1.40
	2	1.07	2.66	2.11	8.60	56.91	4.98	1.02	0.21	1.03	15.10	2.88
	3	1.25	2.00	2.37	7.05	64.38	7.29	1.73	0.23	1.00	6.51	1.40
8	1	0.85	1.25	2.11	3.27	72.94	8.42	1.69	0.18	0.25	2.77	1.77
	2	0.70	1.30	2.07	3.79	72.48	8.90	2.11	0.20	0.79	2.50	1.50
	3	0.82	1.55	1.96	3.97	72.59	8.50	3.29	0.15	0.12	1.54	1.54

(ii) Harvest Date 2. (24/8/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	2.95	2.55	2.71	7.27	5.98	7.27	2.15	2.30	5.01	55.21	1.83
	2	1.52	2.05	2.01	4.65	5.32	7.52	2.17	2.25	3.65	60.29	2.91
	3	1.01	1.55	1.99	5.22	11.72	6.66	1.99	1.56	4.14	59.11	1.61
4	1	1.01	2.05	2.13	7.97	17.35	5.40	2.78	0.99	3.16	50.77	2.74
	2	0.67	1.20	3.17	4.51	20.92	4.05	2.00	1.21	2.41	55.62	1.34
	3	1.00	2.18	2.10	9.41	34.14	7.57	2.41	0.80	1.83	31.87	2.37
6	1	1.50	2.92	2.77	9.98	36.90	6.01	1.53	0.95	2.17	28.32	3.11
	2	0.95	1.92	3.29	6.62	36.26	5.42	2.49	0.81	2.30	34.31	2.55
	3	1.00	2.19	2.11	9.37	35.49	6.59	2.10	0.27	1.83	32.07	2.61
8	1	1.12	2.24	2.06	6.88	64.19	4.96	2.15	0.25	2.36	6.42	3.17
	2	1.01	1.51	2.91	4.21	64.80	5.50	1.78	0.12	2.17	8.47	2.99
	3	0.93	1.65	3.27	8.63	65.54	5.43	2.33	0.15	1.09	4.80	2.55
10	1	0.97	2.01	3.11	4.21	71.10	5.64	3.17	0.17	1.32	1.29	2.87
	2	0.92	1.24	2.71	2.97	72.72	6.29	2.99	0.29	1.36	1.48	3.20
	3	1.21	2.17	2.94	4.32	69.15	7.39	2.78	0.35	1.00	1.24	2.85

(iii) Harvest Date 3. (1/9/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.81	2.15	1.75	6.74	1.77	1.01	1.31	11.38	2.21	66.62	0.22
	2	1.01	1.33	1.10	4.91	9.41	2.32	1.13	4.33	1.54	68.89	1.92
	3	1.12	1.05	1.21	5.61	14.77	2.99	1.54	14.07	1.71	52.05	0.40
4	1	1.22	1.03	1.91	7.68	5.78	2.55	1.02	2.99	1.77	69.11	1.33
	2	1.36	1.18	1.07	7.87	10.56	3.84	1.15	7.33	1.81	58.99	0.90
	3	0.84	1.79	1.12	6.07	10.92	3.06	1.10	1.26	1.02	67.48	2.22
6	1	1.25	2.12	2.01	8.07	19.41	3.38	1.58	0.63	2.10	53.77	1.35
	2	0.94	2.52	1.53	5.70	31.76	3.38	1.92	0.36	2.91	42.53	3.32
	3	1.25	1.95	1.79	4.12	18.20	3.72	1.77	1.02	1.88	58.96	2.31
8	1	1.36	1.66	1.30	12.42	33.18	4.02	2.10	0.34	1.07	36.02	3.07
	2	0.97	2.31	1.75	7.29	50.46	5.88	1.05	0.30	1.29	20.91	4.55
	3	1.25	1.65	1.81	5.93	22.13	3.93	1.11	0.75	2.10	53.60	2.57
10	1	1.22	2.14	2.11	7.68	59.99	5.68	1.55	0.39	1.99	11.08	2.68
	2	0.90	1.10	1.02	5.23	73.38	5.27	1.09	0.43	1.07	3.72	4.65
	3	1.34	2.95	1.98	7.68	41.50	4.98	1.58	0.27	1.36	29.46	3.48
12	1	0.83	1.53	1.21	2.09	77.08	5.93	1.07	0.45	1.28	2.37	3.05
	2	0.70	1.02	1.01	2.28	81.79	5.18	0.81	0.32	1.31	1.24	3.23
	3	1.23	1.69	1.99	7.37	51.81	9.97	2.73	0.24	1.21	15.34	2.96
14	1	1.06	1.03	1.21	3.11	74.46	6.08	1.29	0.65	1.00	3.03	2.85
	2	0.98	0.74	1.11	1.33	75.63	9.94	1.53	0.18	1.20	2.18	2.05
	3	0.97	1.70	1.04	3.27	73.29	6.05	1.99	0.36	0.87	4.09	2.95

Harvest Date 4. (18/9/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.97	2.07	2.17	8.50	9.10	1.26	2.70	12.14	5.34	48.44	1.67
	2	1.65	1.54	2.59	9.50	5.41	1.29	2.21	27.37	6.12	36.28	2.15
	3	2.17	1.98	2.61	10.21	6.32	1.85	2.95	12.11	5.85	49.08	1.05
4	1	1.20	2.17	2.05	9.37	5.02	1.34	2.91	3.59	5.24	61.12	1.21
	2	1.29	2.33	2.45	8.46	6.36	2.19	1.65	3.02	5.78	59.79	2.31
	3	1.85	2.10	2.09	9.34	7.73	1.84	2.84	2.12	5.85	57.90	1.98
6	1	1.78	1.98	3.21	12.17	10.07	2.96	1.33	1.57	5.16	54.98	2.27
	2	2.01	3.21	2.97	10.21	10.74	3.54	2.91	1.12	5.92	51.76	2.49
	3	2.10	2.11	2.15	7.32	12.75	1.97	2.28	1.40	4.47	57.47	1.37
8	1	1.72	2.71	2.44	9.29	16.86	2.17	2.77	0.73	4.29	51.63	2.16
	2	2.11	2.22	2.73	8.35	18.21	2.29	3.34	0.62	5.17	46.73	2.34
	3	1.87	1.99	2.92	9.20	20.17	3.11	1.27	0.71	5.03	47.08	1.88
10	1	1.31	1.88	3.17	10.11	19.70	1.29	2.51	0.32	5.71	47.22	2.11
	2	2.02	2.43	1.98	11.29	34.02	2.55	2.49	0.41	4.28	31.54	3.22
	3	1.53	2.03	2.31	9.11	21.04	1.88	1.27	0.37	4.71	46.67	3.47
12	1	1.55	2.10	2.79	9.21	35.08	2.88	2.25	0.24	4.33	32.53	2.91
	2	1.89	2.71	3.12	8.73	47.87	2.91	2.23	0.34	4.54	19.14	2.87
	3	1.86	2.32	3.04	12.11	44.10	3.29	2.59	0.31	5.28	18.38	2.95
14	1	1.31	1.98	2.19	8.34	52.92	3.78	2.66	0.10	4.87	15.37	3.17
	2	1.08	1.75	2.73	10.11	48.61	3.92	2.18	0.15	4.99	17.89	2.80
	3	2.11	2.07	2.14	9.26	61.38	4.17	1.73	0.21	4.21	5.70	3.91
16	1	1.07	1.82	1.98	6.10	64.78	2.91	2.97	0.25	4.77	5.93	3.29
	2	1.29	1.95	2.05	7.23	68.55	3.09	3.62	0.31	4.15	1.27	3.61
	3	1.53	1.98	2.26	5.91	68.21	3.99	2.29	0.21	4.25	1.77	3.49

Harvest Date 5. (4/10/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
6	1	1.25	2.52	2.99	10.94	5.95	2.19	1.27	4.26	5.14	56.49	2.27
	2	1.07	2.12	3.21	10.56	2.51	1.92	2.11	5.28	4.25	61.38	1.94
	3	1.57	3.10	2.11	7.49	2.83	2.51	1.98	7.21	4.85	60.38	2.15
8	1	2.02	3.15	2.73	8.21	9.57	3.97	2.05	2.71	3.45	55.70	1.99
	2	1.74	3.25	1.92	12.17	7.36	3.51	2.01	3.21	5.13	53.62	2.19
	3	1.70	2.15	1.98	11.32	14.38	2.43	2.39	2.10	3.89	50.44	2.20
10	1	1.21	2.57	2.87	9.28	19.53	3.14	1.97	1.15	3.06	48.08	3.10
	2	1.25	2.07	2.43	10.15	18.35	3.62	2.85	1.10	4.29	47.32	1.95
	3	1.40	2.98	3.21	10.94	14.27	3.17	2.55	1.04	5.09	48.41	2.57
12	1	1.16	3.07	2.91	8.17	31.09	3.63	2.08	0.45	5.16	33.42	4.09
	2	1.39	2.58	2.87	9.25	32.10	4.73	2.77	0.39	5.10	29.77	3.88
	3	1.41	2.65	2.71	10.57	31.16	4.98	3.01	0.51	5.17	30.52	3.56
14	1	1.30	2.29	3.21	11.22	39.43	3.54	2.91	0.47	4.29	24.98	3.21
	2	2.31	2.17	3.11	10.51	37.57	4.58	2.56	0.35	5.07	24.11	4.32
	3	1.86	1.90	2.75	10.90	34.60	3.89	2.12	0.21	4.35	29.20	4.55
16	1	1.92	2.07	2.11	7.84	51.71	4.21	2.31	0.25	4.98	14.91	2.56
	2	1.97	2.57	2.68	9.21	50.50	3.75	3.29	0.17	4.15	13.56	3.54
	3	1.42	2.47	2.35	8.55	50.17	4.71	2.45	0.29	4.76	15.29	2.98

Appendix IV A 1.4. . Oil Composition (%). SD x HNT.

Harvest Date 1. (17/8/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.25	2.15	1.97	4.45	11.53	6.32	1.73	1.20	2.95	60.27	1.50
	2	1.07	2.51	2.10	5.39	16.00	5.90	2.11	1.00	2.80	55.71	1.95
	3	1.21	1.42	1.85	3.95	23.25	8.75	1.51	2.35	3.75	45.62	1.70
4	1	1.35	1.95	1.92	6.35	29.94	5.59	1.02	0.92	1.85	45.92	0.95
	2	1.51	2.10	1.74	6.45	33.90	7.87	1.54	0.78	1.89	37.04	1.29
	3	1.06	2.10	2.01	5.78	46.32	6.09	1.39	0.82	2.70	26.36	2.40
6	1	1.09	2.09	1.88	8.90	66.28	7.34	2.11	0.51	1.35	8.29	1.25
	2	0.95	1.90	2.00	5.50	67.25	6.95	1.07	0.31	1.08	7.31	2.27
	3	1.53	1.96	1.95	5.51	66.92	7.15	1.52	0.23	1.10	5.11	1.92
8	1	1.21	2.00	1.77	6.53	68.50	7.85	1.95	0.17	1.30	2.15	2.25
	2	1.09	2.10	1.91	4.53	70.32	7.89	1.76	0.21	1.50	2.33	2.29
	3	0.78	2.10	2.13	4.52	69.76	7.50	1.82	0.18	1.00	4.17	2.07

Harvest Date 2. (24/8/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.59	2.13	2.51	7.50	9.09	7.29	1.73	1.72	4.21	55.73	1.27
	2	1.01	1.98	2.00	4.29	10.06	4.24	2.10	1.33	4.24	60.81	2.73
	3	0.99	2.41	2.41	6.21	9.55	3.29	1.98	1.84	5.29	59.53	2.21
4	1	2.10	2.87	1.98	8.21	26.34	5.38	1.73	1.02	3.29	41.70	2.54
	2	2.25	2.57	1.72	4.38	22.04	3.29	1.57	1.11	5.12	51.43	1.07
	3	1.98	2.48	2.69	5.38	17.71	3.89	2.11	1.36	5.10	50.48	2.41
6	1	0.97	3.21	2.15	10.68	45.47	5.29	1.90	0.87	2.19	20.88	2.92
	2	0.98	2.19	2.91	5.61	47.11	4.28	2.17	0.65	3.09	24.39	2.90
	3	2.04	1.39	1.95	9.21	42.76	4.29	1.53	0.73	3.98	25.65	3.26
8	1	1.01	2.98	1.20	5.17	61.41	5.13	2.88	0.12	3.25	7.54	4.32
	2	1.29	2.28	2.31	7.29	59.69	4.59	3.11	0.31	2.13	8.29	3.25
	3	1.01	2.21	2.15	9.20	60.09	5.17	1.73	0.29	4.01	7.29	2.97
10	1	0.87	2.01	2.71	4.21	70.57	4.28	1.99	0.17	2.54	2.39	3.75
	2	1.21	2.17	3.10	3.87	70.75	5.25	2.00	0.23	1.57	1.54	2.62
	3	0.92	1.16	2.01	4.17	75.97	6.05	1.78	0.24	2.27	1.11	2.21

Harvest Date 3. (1/9/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.73	1.92	2.21	9.77	1.14	0.25	2.79	19.66	4.86	52.01	0.51
	2	1.92	2.10	1.27	6.81	2.10	0.98	1.98	15.21	3.80	57.50	0.98
	3	1.57	1.02	1.71	14.66	2.81	0.27	1.57	19.16	2.99	48.53	0.27
4	1	2.21	2.85	2.27	13.79	1.80	0.29	2.99	7.90	5.29	54.88	0.29
	2	2.10	2.19	1.98	7.79	2.15	0.25	2.75	6.19	4.21	63.89	1.73
	3	1.45	1.60	1.52	6.25	1.97	0.41	1.73	7.12	2.17	71.89	0.14
6	1	2.10	2.83	2.91	9.94	7.87	2.37	2.88	1.19	4.99	57.65	0.75
	2	1.92	2.10	1.79	11.23	7.80	2.58	2.07	0.89	5.44	58.63	1.21
	3	2.11	2.94	2.33	12.60	16.93	4.10	3.11	0.70	6.92	42.27	1.30
8	1	2.07	2.95	3.14	25.85	16.20	3.56	3.21	0.39	6.90	28.96	1.00
	2	2.31	2.50	3.10	10.50	18.29	3.17	4.21	0.42	7.10	39.65	3.10
	3	1.54	2.10	2.17	11.31	32.09	4.50	2.03	0.50	4.91	32.33	2.15
10	1	1.24	2.73	2.19	6.82	42.68	3.90	1.71	0.31	4.43	26.54	2.67
	2	1.29	1.91	1.73	8.31	41.28	2.98	2.73	0.39	2.91	30.74	1.41
	3	0.92	1.54	2.01	9.29	57.06	5.12	2.19	0.40	1.73	10.56	4.60
12	1	0.92	1.01	1.17	3.92	73.37	4.07	1.71	0.37	1.33	4.40	3.85
	2	1.53	2.10	1.77	6.35	68.92	4.32	1.88	0.10	1.17	6.65	2.10
	3	1.22	1.54	2.01	6.48	64.72	4.90	2.11	0.45	1.05	8.36	3.98
14	1	1.14	1.79	1.21	2.83	77.42	5.54	2.14	0.50	1.20	1.36	1.41
	2	1.02	1.51	1.29	2.92	72.56	5.98	1.17	0.31	1.53	7.11	1.15
	3	0.99	1.38	1.20	3.35	73.76	4.95	2.10	0.78	1.01	2.76	3.70

Harvest Date 4. (18/9/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
2	1	1.45	1.51	2.17	6.90	6.81	5.23	2.23	11.43	6.10	49.76	1.74
	2	1.60	1.89	2.10	9.20	3.33	2.58	3.15	22.32	6.22	42.52	1.44
	3	1.66	1.90	1.97	5.98	6.21	2.57	2.57	24.55	5.31	41.85	1.31
4	1	1.72	1.21	2.11	7.13	3.45	2.10	3.15	4.30	5.24	63.05	1.86
	2	2.44	3.21	3.29	12.51	2.99	1.07	2.11	12.69	6.40	47.08	1.32
	3	1.63	1.22	1.67	7.04	4.07	2.21	2.91	14.24	5.28	55.69	0.70
6	1	2.42	3.68	2.45	12.49	10.02	2.53	2.53	2.40	5.10	48.80	1.42
	2	1.36	2.39	2.90	9.67	5.43	1.90	2.17	2.99	5.80	59.99	1.65
	3	1.66	3.80	2.70	11.55	9.79	2.56	2.33	3.00	4.57	50.39	1.14
8	1	1.60	1.61	1.99	12.78	17.39	3.22	1.98	0.78	5.39	45.59	3.06
	2	1.29	2.45	2.17	8.29	10.29	2.53	2.51	1.02	5.29	56.10	3.29
	3	1.32	3.62	3.10	9.52	12.41	3.66	2.37	0.73	4.80	52.37	1.17
10	1	1.98	2.17	1.70	11.80	35.42	4.29	3.11	0.67	4.25	25.89	4.27
	2	2.41	1.95	1.95	9.23	29.28	4.28	3.20	0.54	4.87	34.10	2.87
	3	2.01	2.00	3.17	7.90	26.90	5.08	2.57	0.72	3.63	39.17	3.72
12	1	1.22	2.54	2.54	10.78	52.21	3.29	1.79	1.84	5.21	11.58	3.21
	2	2.17	2.67	2.71	8.93	44.21	3.78	2.04	0.42	4.71	21.18	2.95
	3	2.37	3.19	3.10	7.29	39.65	2.71	2.91	0.28	5.11	25.36	3.61
14	1	2.17	2.95	1.99	8.65	55.49	4.59	1.21	0.56	5.21	7.28	3.94
	2	1.99	1.84	2.00	7.29	58.73	5.32	2.19	0.73	4.98	6.30	3.72
	3	2.11	3.43	2.47	4.98	53.20	4.50	2.55	0.51	6.21	11.60	4.02
16	1	1.05	1.72	1.92	7.29	65.84	4.79	2.03	0.12	4.71	2.10	2.89
	2	1.09	1.84	2.41	8.11	63.32	4.97	2.91	0.17	5.01	2.98	4.07
	3	1.31	1.91	2.31	5.01	65.97	4.06	3.21	0.15	5.55	4.27	2.88

Harvest Date 5. (4/10/77).

Leaf Pair No.	Rep. No.	Component										
		α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
6	1	1.25	2.73	2.90	10.25	3.27	1.31	2.31	5.98	3.92	60.10	1.21
	2	1.12	2.92	2.87	10.74	3.17	1.41	2.17	5.97	6.21	58.89	1.42
	3	1.21	1.73	3.21	8.73	2.74	2.10	1.98	9.21	6.11	58.51	0.98
8	1	1.44	3.89	1.98	8.29	5.47	2.01	3.05	2.92	4.28	60.90	2.10
	2	1.40	2.98	2.51	9.23	6.49	0.98	2.17	2.91	5.29	57.98	2.94
	3	2.01	1.73	2.70	10.91	6.29	1.13	2.91	4.17	5.36	54.82	2.73
10	1	1.14	3.17	3.16	12.17	15.91	2.14	1.87	0.94	4.99	51.03	1.99
	2	1.07	3.87	3.20	11.29	14.97	1.74	3.29	0.95	4.86	47.71	2.71
	3	1.00	2.15	1.99	12.38	28.04	1.92	2.34	1.21	5.21	35.92	3.28
12	1	1.01	3.56	2.71	8.73	33.08	2.84	1.64	0.33	5.72	33.89	2.18
	2	1.73	3.95	2.32	10.29	31.98	1.98	2.56	0.73	5.29	32.19	3.28
	3	1.32	2.73	3.17	9.21	27.21	2.57	2.71	0.52	4.13	37.90	4.26
14	1	1.86	2.71	2.01	9.30	50.25	4.94	2.33	0.20	3.29	8.27	3.17
	2	2.16	2.54	2.91	7.26	55.66	2.50	2.77	0.41	3.78	12.90	2.90
	3	1.51	3.31	2.34	6.91	52.27	2.07	2.01	0.42	4.06	17.21	3.11
16	1	2.10	2.64	3.10	8.20	61.21	3.21	3.20	0.34	2.99	4.93	2.71
	2	1.95	2.10	2.91	9.11	57.39	2.81	4.21	0.15	3.75	8.29	4.32
	3	1.73	2.91	2.65	10.27	54.90	3.72	1.99	0.19	4.10	10.17	3.18

Appendix IV A 2.1. The effect of photoperiod on dry matter, oil and percentage oil yield.

Experiment 1

	Photoperiodic Treatment					
	13I			13H		
Rep. No.	1	2	3	1	2	3
Dry Herb Yield (g/plant)	4.52	4.87	3.57	2.29	2.29	1.91
Oil Yield (mg/plant)	81.30	79.08	70.43	27.29	25.73	28.93
% Yield (Dry Matter Basis)	1.80	1.62	1.97	1.19	1.12	1.52

Experiment 2

	Photoperiodic Treatment					
	12I			12H		
Rep. No.	1	2	3	1	2	3
Dry Herb Yield (g/plant)	3.78	3.92	4.11	2.17	2.21	1.87
Oil Yield (mg/plant)	69.21	75.13	73.29	25.11	24.27	23.11
% Yield (Dry Matter Basis)	1.83	1.92	1.78	1.16	1.10	1.24

Appendix IV A 2.2. The Effect of Photoperiod on Monoterpene Composition of Peppermint Oil.

Experiment 1.

Compound (% Total Peak Area)	Photoperiodic Treatment					
	13 I			13 H		
Rep. No.	1	2	3	1	2	3
1. α-Pinene	0.530	0.961	0.617	0.348	0.472	0.392
2. β-Pinene	1.308	1.968	1.428	0.705	0.798	0.808
3. Limonene	0.428	0.591	0.604	1.512	1.702	1.623
4. Cineole	6.235	6.916	5.962	0.523	1.302	0.807
5. Trans-Sabinene Hydrate	1.079	1.644	1.252	0.460	0.473	0.527
6. Menthone	42.556	42.848	45.906	7.280	7.134	9.991
7. Menthofuran	22.983	20.223	20.089	65.853	65.512	61.656
8. Menthyl Acetate	0.308	0.401	0.359	2.155	2.133	2.143
9. Neomenthol (+ Unknown)	2.416	1.922	1.893	1.622	1.050	1.408
10.Menthol	14.462	13.312	13.875	9.232	9.210	10.192
11.Pulegone	7.606	7.098	6.518	10.074	9.949	10.414
12.Unknown	1.060	1.245	1.499	0.235	0.265	0.224

Experiment 2.

Compound (% Total Peak Area)	Photoperiodic Treatment					
	12 I			12 H		
Rep. No.	1	2	3	1	2	3
1. α-Pinene	0.693	0.701	0.624	0.354	0.400	0.417
2. β-Pinene	1.421	1.298	1.375	0.921	0.837	0.713
3. Limonene	0.629	0.713	0.587	1.513	1.297	1.308
4. Cineole	6.421	5.873	6.017	1.291	1.397	1.091
5. Trans-Sabinene Hydrate	1.321	1.121	1.077	0.672	0.538	0.597
6. Menthone	40.770	42.712	41.050	9.283	8.719	7.222
7. Menthofuran	24.270	23.172	24.170	63.291	65.102	66.329
8. Menthyl Acetate	0.501	0.329	0.410	1.997	1.980	2.073
9. Neomenthol (+ Unknown)	1.986	1.735	1.298	1.076	0.923	0.801
10.Menthol	12.371	13.175	13.731	8.731	8.529	9.017
11.Pulegone	8.209	7.850	8.360	10.585	10.027	9.915
12.Unknown	1.417	1.327	1.297	0.286	0.311	0.417

Appendix IV A 3.1 The Effect of Night Temperature and Daylength on Oil Composition.

LD x LNT - Experiment 1.

Rep. No.	Leaf Pair No.	% Compound				
		Menthone	Menthofuran	Menthyl Acetate	Menthol	Pulegone
I	2	1.77	1.01	11.38	66.62	0.22
	4	5.78	2.55	2.99	69.11	1.33
	6	19.41	3.38	0.63	53.77	1.35
	8	33.18	4.02	0.34	36.02	3.07
	10	59.99	5.68	0.39	11.08	2.68
	12	77.08	5.93	0.45	2.37	3.05
	14	74.46	6.08	0.65	3.03	2.85
II	2	9.41	2.32	4.33	68.89	1.92
	4	10.56	3.84	7.33	58.99	0.90
	6	31.76	3.84	0.36	42.53	3.32
	8	50.46	5.88	0.30	20.91	4.55
	10	73.38	5.27	0.43	3.72	4.65
	12	81.79	5.18	0.32	1.24	3.23
	14	75.63	9.94	0.18	2.18	2.05
III	2	14.77	2.99	14.07	52.05	0.40
	4	10.92	3.06	1.26	67.48	2.22
	6	18.20	3.72	1.02	58.96	2.31
	8	22.13	3.93	0.75	53.60	2.57
	10	41.50	4.98	0.27	29.46	3.48
	12	51.81	9.97	0.24	15.34	2.96
	14	73.29	6.05	0.36	4.09	2.95

SD x HNT - Experiment 1.

Rep. No.	Leaf Pair No.	% Compound				
		Menthone	Menthofuran	Menthyl Acetate	Menthol	Pulegone
I	2	1.14	0.25	19.66	52.01	0.51
	4	1.80	0.29	7.90	54.88	0.29
	6	7.87	2.37	1.19	57.65	0.75
	8	16.20	3.56	0.39	28.96	1.00
	10	42.68	3.90	0.31	26.54	2.67
	12	73.37	4.07	0.37	4.40	3.85
	14	77.42	5.54	0.54	1.36	1.41
II	2	2.10	0.98	15.21	57.50	0.98
	4	2.15	0.25	6.19	63.89	1.73
	6	7.80	2.58	0.89	58.63	1.21
	8	18.29	3.17	0.42	39.65	3.10
	10	41.28	2.98	0.39	30.74	1.41
	12	68.92	4.32	0.10	6.65	2.10
	14	72.56	5.98	0.31	7.11	1.15
III	2	2.81	0.27	19.16	48.53	0.27
	4	1.97	0.41	7.12	71.89	0.14
	6	16.93	4.10	0.70	42.27	1.30
	8	32.09	4.50	0.50	32.33	2.15
	10	57.06	5.12	0.40	10.56	4.60
	12	64.77	4.90	0.45	8.36	3.98
	14	73.76	4.95	0.78	2.76	3.70

LD x LNT, SD x HNT - Steam Distilled Oil.

Growing Condition	Rep.*	Menthone	Menthofuran	Menthyl Acetate	Menthol	Pulegone
LD x LNT	1	30.29	4.30	1.29	50.70	2.10
	2	29.76	5.17	1.30	51.28	1.96
	3	29.83	5.09	1.02	51.29	1.71
SD x HNT	1	29.17	4.73	0.98	50.17	1.92
	2	31.29	5.28	1.29	49.89	1.83
	3	28.73	5.17	1.41	51.29	1.95

*5 plants/rep.

Appendix IV A 3.2. The Effect of Night Temperature, Light Intensity and Daylength on -

Dry Matter, Oil and Percentage Oil Yield - Experiment 2.

Yield Component / Treatment	Rep. No.	Dry Matter (g)	Oil Yield (g)	% Oil Yield (Dry Matter Basis)
LD x LNT	1	3.70	0.0925	2.50
(L1)	2	2.85	0.0750	2.63
	3	3.25	0.0810	2.49
LD x LNT	1	14.4	0.3538	2.44
(L2)	2	12.36	0.2954	2.39
	3	11.70	0.3054	2.61
LD x LNT	1	18.80	0.4662	2.48
(L3)	2	19.08	0.4942	2.59
	3	20.60	0.4965	2.41
LD x HNT	1	4.92	0.1205	2.45
(L1)	2	4.30	0.1062	2.47
	3	4.10	0.1029	2.51
LD x HNT	1	14.78	0.3577	2.42
(L2)	2	15.73	0.3760	2.39
	3	14.60	0.3650	2.50
LD x HNT	1	23.80	0.5736	2.41
(L3)	2	22.50	0.5355	2.38
	3	25.80	0.6218	2.41
SD x LNT	1	1.39	Trace	
(L1)	2	0.96	only	____
	3	0.99		
SD x LNT	1	4.10	0.0759	1.85
(L2)	2	3.65	0.0712	1.95
	3	4.92	0.0905	1.84
SD x LNT	1	9.02	0.1651	1.83
(L3)	2	8.65	0.1626	1.88
	3	8.55	0.1625	1.90
SD x HNT	1	1.64	Trace	
(L1)	2	1.06	only	____
	3	1.06		
SD x HNT	1	8.39	0.1535	1.83
(L2)	2	6.22	0.1176	1.89
	3	6.80	0.1210	1.78
SD x HNT	1	9.92	0.1805	1.82
(L3)	2	10.90	0.1886	1.73
	3	10.35	0.1967	1.90

Appendix IV A 3.3 The effect of night temperature, light intensity and daylength on oil composition.

Compound (% w/w) / Treatment	Rep. No.	α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
LD x LNT	1	0.83	1.04	1.49	2.52	48.38	20.74	4.18	0.45	0.73	7.78	7.69
(L1)	2	0.47	0.59	1.18	1.56	43.11	29.61	2.89	0.69	0.95	8.53	8.34
	3	0.49	0.68	1.72	1.72	44.29	29.84	2.55	0.32	0.40	6.22	7.02
LD x LNT	1	1.46	1.71	2.64	5.23	56.40	13.33	4.56	0.15	0.77	7.02	3.20
(L2)	2	1.69	1.98	3.27	5.28	49.28	15.42	5.53	0.18	1.00	7.12	5.10
	3	1.07	1.34	2.24	4.39	57.72	13.78	4.30	0.18	0.72	7.16	3.67
LD x LNT	1	1.93	2.16	3.63	7.21	52.79	9.90	5.48	0.20	1.14	8.76	2.59
(L3)	2	1.39	1.67	3.03	5.89	59.38	9.00	4.83	0.18	0.88	7.39	3.11
	3	2.00	2.16	3.73	6.60	51.37	11.35	5.52	0.22	1.19	8.65	3.41
LD x HNT	1	0.94	1.17	0.89	3.80	46.92	23.28	2.11	0.24	0.75	4.32	10.61
(L1)	2	0.50	0.78	0.83	2.08	46.62	24.47	3.08	0.50	0.96	6.70	11.71
	3	0.69	0.86	0.86	2.67	44.94	25.45	4.26	0.20	0.76	3.99	13.48
LD x HNT	1	1.41	1.71	2.11	6.62	51.02	16.89	4.08	0.16	0.88	5.84	5.91
(L2)	2	1.35	1.55	1.84	4.98	52.01	17.93	3.72	0.20	0.85	5.00	7.17
	3	1.27	1.57	1.82	5.35	48.58	19.80	4.39	0.20	1.00	5.41	6.86
LD x HNT	1	1.61	2.03	2.19	7.16	52.89	11.87	4.84	0.19	1.03	7.87	4.06
(L3)	2	1.35	1.66	2.63	6.24	50.07	14.57	5.75	0.29	1.44	7.29	4.97
	3	1.24	1.62	2.05	6.16	50.96	12.17	5.48	0.25	1.46	10.27	4.66
SD x LNT	1	0.52	0.89	2.48	2.48	39.53	27.04	1.45	1.89	0.58	15.66	6.09
(L1)	2	0.39	0.73	2.41	2.28	41.54	25.77	1.39	1.29	0.73	14.28	8.09
	3	0.62	0.95	2.61	2.58	40.14	25.69	1.50	2.31	0.61	17.20	4.15
SD x LNT	1	0.82	1.06	3.19	3.09	51.18	19.31	3.57	0.27	0.53	9.37	5.02
(L2)	2	0.63	0.95	3.01	3.11	52.11	15.21	2.98	0.32	0.59	12.21	5.12
	3	0.44	0.81	2.08	2.38	54.66	12.24	2.69	0.59	0.72	14.21	5.19
SD x LNT	1	0.86	1.02	3.85	3.88	48.95	15.91	4.71	0.28	0.70	12.24	6.06
(L3)	2	0.67	0.92	3.90	2.57	53.42	16.74	4.44	0.15	0.51	9.32	4.01
	3	0.84	1.03	3.30	3.35	53.29	14.74	4.38	0.22	0.68	11.01	4.33
SD x HNT	1	0.49	0.68	1.25	2.01	23.86	24.40	2.06	2.49	2.06	24.09	14.64
(L1)	2	0.52	0.73	1.32	2.12	29.21	24.42	2.11	2.45	2.11	18.27	15.03
	3	0.48	0.81	1.28	2.07	27.31	26.79	1.92	2.37	1.93	19.28	13.80
SD x HNT	1	1.16	1.45	2.59	4.79	40.40	24.19	4.45	0.27	0.63	8.35	9.14
(L2)	2	1.15	1.21	2.53	3.92	42.50	16.28	3.92	0.42	0.48	8.62	14.11
	3	0.81	1.03	1.83	3.01	39.27	27.30	3.37	0.27	0.56	7.85	12.03
SD x HNT	1	1.32	1.63	2.74	4.15	46.43	22.51	3.06	0.25	0.59	7.39	17.18
(L3)	2	0.77	0.96	2.96	3.11	41.12	28.03	2.85	0.10	0.19	7.86	10.54
	3	1.42	1.56	2.87	4.48	33.95	28.00	6.86	0.26	0.53	8.61	8.47

Appendix IV A 3.4. Analysis of Variance - Experiment 2.

(DL = Daylength; NT = Night Temperature; LI = Light Intensity)

Dry Matter Yield

S.O.V.	DF	SS	MS	F
DL	1	535.15	535.15	735.98***
NT	1	39.69	39.69	54.59***
LI	2	1041.04	520.52	715.86***
DL x NT	1	2.55	2.55	3.51 ns
DL x LI	2	137.56	68.78	94.60***
NT x LI	2	9.82	4.91	6.75*
DL x NT x LI	2	4.92	2.46	3.38 ns
Error	24	17.45	0.73	
Total	35	1788.18	51.09	

Oil Yield

S.O.V.	DF	SS	MS	F
DL	1	0.5239	0.5239	1328.43***
NT	1	0.0145	0.0145	36.63***
LI	2	0.6336	0.3168	803.24***
DL x NT	1	0.0021	0.0021	5.42*
DL x LI	1(1)	0.0754	0.0754	191.11***
NT x LI	2	0.0036	0.0018	4.58*
DL x NT x LI	1(1)	0.0018	0.0018	4.61*
Error	20(4)	0.0079	0.0004	
Total	29	1.2627	0.0435	

Percentage Oil Yield

S.O.V.	DF	SS	MS	F
DL	1	2.8606	2.8606	603.925***
NT	1	0.0270	0.0270	5.696*
LI	2	0.1048	0.0524	11.058***
DL x NT	1	0.0013	0.0013	0.270 ns
DL x LI	1(1)	0.0270	0.0270	5.699*
NT x LI	2	0.0016	0.0008	0.164 ns
DL x NT x LI	1(1)	0.0008	0.0008	0.177 ns
Error	20(4)	0.0947	0.0047	
Total	29	3.1177	0.1075	

α-Pinene

S.O.V.	DF	SS	MS	F
DL	1	1.68134	1.68134	35.820***
NT	1	0.05138	0.05138	1.095 ns
LI	2	3.22482	1.61241	34.351***
DL x NT	1	0.30250	0.30250	6.445*
DL x LI	2	0.36509	0.18254	3.889*
NT x LI	2	0.04629	0.02314	0.493 ns
DL x NT x LI	2	0.30320	0.15160	3.230 ns
Error	24	1.12653	0.04694	
Total	35	7.10116		

β-Pinene

S.O.V.	DF	SS	MS	F
DL	1	1.71610	1.71610	36.921***
NT	1	0.04840	0.04840	1.041 ns
LI	2	3.28762	1.64381	35.366***
DL x NT	1	0.12018	0.12018	2.586 ns
DL x LI	2	0.68422	0.34211	7.360**
NT x LI	2	0.01172	0.00586	0.126 ns
DL x NT x LI	2	0.32374	0.16187	3.483*
Error	24	1.11553	0.04648	
Total	35	7.30750		

Limonene

S.O.V.	DF	SS	MS	F
DL	1	1.8001	1.8001	16.966***
NT	1	6.3925	6.3925	60.251***
LI	2	14.4865	7.2433	68.270***
DL x NT	1	0.0017	0.0017	0.016 ns
DL x LI	2	0.4058	0.2029	1.912 ns
NT x LI	2	0.2388	0.1194	1.126 ns
DL x NT x LI	2	0.4624	0.2312	2.179 ns
Error	24	2.5463	0.1061	
Total	35	26.3342		

Cineole

S.O.V.	DF	SS	MS	F
DL	1	25.1335	25.1335	64.163***
NT	1	2.0544	2.0544	5.245*
LI	2	48.5122	24.2561	61.923***
DL x NT	1	0.0144	0.0144	0.037 ns
DL x LI	2	12.2046	6.0123	15.578***
NT x LI	2	0.6762	0.3381	0.863 ns
DL x NT x LI	2	1.7061	0.8531	2.178 ns
Error	24	9.4011	0.3917	
Total	35	99.7026		

Menthone

S.O.V.	DF	SS	MS	F
DL	1	607.294	607.294	64.543***
NT	1	465.696	465.696	49.494***
LI	2	787.064	393.532	41.824***
DL x NT	1	235.418	235.418	25.020***
DL x LI	2	69.567	34.784	3.697*
NT x LI	2	3.761	1.881	0.200 ns
DL x NT x LI	2	20.632	10.316	1.096 ns
Error	24	225.821	9.409	
Total	35	2415.252		

Menthofuran

S.O.V.	DF	SS	MS	F
DL	1	156.959	156.959	20.136***
NT	1	109.307	109.307	14.023***
LI	2	615.841	307.921	39.502***
DL x NT	1	35.621	35.621	4.570*
DL x LI	2	139.672	69.836	8.959**
NT x LI	2	120.215	60.108	7.711**
DL x NT x LI	2	15.692	7.846	1.007 ns
Error	24	187.081	7.795	
Total	35	1380.389		

Neomenthol

S.O.V.	DF	SS	MS	F
DL	1	0.13201	0.13201	5.401*
NT	1	0.63468	0.63468	25.967***
LI	2	0.61974	0.30987	12.678***
DL x NT	1	0.12018	0.12018	4.917*
DL x LI	2	2.40871	1.20435	49.275***
NT x LI	2	1.10777	0.55389	22.662***
DL x NT x LI	2	1.23167	0.61584	25.196***
Error	24	0.58660	0.02444	
Total	35	6.84136		

Isomenthone

S.O.V.	DF	SS	MS	F
DL	1	10.9340	10.9340	15.564***
NT	1	0.0514	0.0514	0.073 ns
LI	2	35.0851	17.5425	24.971***
DL x NT	1	0.8773	0.8773	1.249 ns
DL x LI	2	0.5134	0.2567	0.365 ns
NT x LI	2	0.1867	0.0933	0.133 ns
DL x NT x LI	2	1.3540	0.6770	0.964 ns
Error	24	16.8605	0.7025	
Total	35	65.8625		

Menthol

S.O.V.	DF	SS	MS	F
DL	1	280.562	280.562	123.628***
NT	1	8.142	8.142	3.587 ns
LI	2	110.126	55.063	24.263***
DL x NT	1	1.269	1.269	0.559 ns
DL x LI	2	189.883	94.941	41.835***
NT x LI	2	22.746	11.373	5.011*
DL x NT x LI	2	49.327	24.664	10.868***
Error	24	54.466	2.269	
Total	35	716.522	20.472	

Menthyl Acetate

S.O.V.	DF	SS	MS	F
DL	1	3.61000	3.61000	113.562***
NT	1	0.04134	0.04134	1.301 ns
LI	2	8.42102	4.21051	132.452***
DL x NT	1	0.10028	0.10028	3.154 ns
DL x LI	2	5.49915	2.74957	86.495***
NT x LI	2	0.10257	0.05129	1.613 ns
DL x NT x LI	2	0.36451	0.18225	5.733**
Error	24	0.76293	0.03179	
Total	35	18.90180		

Pulegone

S.O.V.	DF	SS	MS	F
DL	1	67.898	67.898	22.162***
NT	1	236.032	236.032	77.040***
LI	2	104.743	52.372	17.094***
DL x NT	1	48.025	48.025	15.675***
DL x LI	2	26.325	13.163	4.296*
NT x LI	2	6.514	3.257	1.063 ns
DL x NT x LI	2	1.410	0.705	0.230 ns
Error	24	73.530	3.064	
Total	35	564.477	16.128	

Appendix IV A 4.1.Net CO_2 Exchange Characteristics of Peppermint.

(a) Effect of Light Intensity

	Net CO_2 Exchange (mg CO_2 $dm^{-2}hr^{-1}$)					
Plant Type	High Light Intensity			Low Light Intensity		
Rep. No.	1	2	3	1	2	3
Light Intensity ($\mu\Sigma m^{-2}s^{-1}$)						
130	4.00	3.47	5.90	8.45	5.32	6.29
245	9.88	8.29	9.50	10.05	9.71	10.53
309	14.20	13.50	13.90	11.49	10.80	11.99
355	15.29	14.44	14.75	13.48	13.51	13.61
500	16.44	15.95	16.21	14.50	14.25	14.32
655	16.50	16.22	16.51	14.50	14.25	14.32
690	16.50	16.25	16.55	14.50	14.25	14.32
975	16.50	16.25	16.55	14.50	14.30	14.35
1100	16.50	16.25	16.55	14.50	14.30	14.35

(b) "Apparent" Photosynthesis

	Net CO_2 Exchange (mg CO_2 $dm^{-2}hr^{-1}$
Rep. No.	1	2	3
Temperature (^oC)			
5	4.90	3.29	4.50
10	7.34	6.18	6.70
15	11.03	10.73	10.95
20	15.04	14.20	14.66
25	12.86	11.22	11.24
30	10.12	9.74	10.07
35	7.95	6.52	7.33

(c) Dark Respiration

	Net CO_2 exchange (mg CO_2 $dm^{-2}hr^{-1}$)		
Rep. No.	1	2	3
Temperature (^oC)			
5	1.05	1.00	0.89
10	1.94	1.80	1.90
15	2.51	2.51	2.43
20	4.45	4.67	4.15
25	5.04	5.20	5.73
30	6.15	5.99	6.21
35	6.98	7.13	6.95

(d) Enhancement of Net CO_2 Exchange - Low O_2

	Net CO_2 exchange (mg CO_2 $dm^{-2}hr^{-1}$)		
Rep. No.	1	2	3
Temperature (^oC)			
5	6.10	5.20	5.88
10	9.11	8.73	8.95
15	14.75	13.29	14.50
20	21.25	21.74	20.98
25	24.80	23.75	24.05
30	23.40	22.88	23.55
35	21.32	20.29	20.54

(e) Photorespiration.

	Net CO_2 Exchange (mg CO_2 $dm^{-2}hr^{-1}$)		
Rep. No.	1	2	3
Temperature (^oC)			
5	1.20	1.91	1.38
10	1.77	2.55	2.25
15	3.72	2.56	3.55
20	6.21	7.54	6.32
25	11.94	12.53	12.81
30	13.28	13.14	13.48
35	13.37	13.77	13.21

(f) "True" Photosynthesis.

	Net CO_2 Exchange (mg CO_2 $dm^{-2}hr^{-1}$)		
Rep. No.	1	2	3
Temperature (^oC)			
5	7.15	6.20	6.77
10	11.05	10.53	10.85
15	17.26	15.80	16.93
20	25.70	26.41	25.13
25	29.84	28.95	29.78
30	29.55	28.79	29.76
35	28.30	27.42	27.49

Appendix IV A 5.1. Oil Composition - Short Day Pre-Treatment. (*Leaf pair no. 9 = youngest apical leaf pair).

Compound (%)	Rep. No.	Leaf Number* 1	2	3	4	5	6	7	8	9
Limonene	1	4.93	4.53	4.41	3.71	1.66	2.19	2.27	2.31	1.98
	2	4.72	4.21	3.92	3.90	2.19	2.07	2.00	2.11	2.05
	3	4.86	4.50	4.25	4.07	1.98	2.71	2.05	2.19	2.15
Cineole	1	1.58	1.97	2.40	4.92	6.13	9.64	6.51	5.74	3.25
	2	1.73	1.90	2.11	4.99	6.17	8.95	7.20	5.66	3.75
	3	1.62	1.87	2.51	4.52	5.99	9.51	7.00	4.99	4.00
Menthone	1	1.34	3.21	5.03	12.73	31.53	58.76	60.95	70.32	71.53
	2	1.29	3.25	4.71	11.51	25.71	60.11	62.95	72.11	73.78
	3	1.37	2.95	4.85	13.29	33.28	55.19	63.11	70.29	71.88
Menthofuran	1	60.64	53.97	54.76	38.09	15.09	7.48	5.21	3.56	5.86
	2	64.24	55.20	51.93	43.22	18.64	7.21	6.71	4.11	4.15
	3	61.36	55.56	53.94	37.64	15.17	6.91	5.52	5.13	5.99
Menthyl Acetate	1	9.72	6.29	1.52	1.21	0.38	0.25	0.20	0.11	0.04
	2	8.71	6.25	2.10	1.27	0.48	0.39	0.25	0.18	0.15
	3	7.95	6.01	0.95	0.90	0.73	0.49	0.32	0.21	0.17
Menthol	1	4.67	14.27	24.43	29.27	33.27	7.93	5.27	2.88	2.05
	2	4.53	13.99	26.29	27.21	35.73	6.17	5.43	5.11	2.11
	3	5.21	15.25	24.48	30.35	34.21	7.29	5.81	2.90	2.00
Pulegone	1	8.23	5.29	0.92	0.87	0.85	0.73	0.24	0.68	0.65
	2	7.29	5.41	0.90	0.91	0.73	0.52	0.73	0.61	0.55
	3	9.31	4.21	1.32	0.79	0.29	0.61	0.71	0.57	0.62

Appendix IV A 5.2. Oil Composition - Long Day Pre-Treatment. (*Leaf pair no. 9 = youngest apical leaf pair).

Compound (%)	Rep. No.	Leaf Number* 1	2	3	4	5	6	7	8	9
Limonene	1	2.11	2.15	2.30	2.51	1.87	2.11	2.35	2.49	1.92
	2	2.91	1.87	2.41	2.10	2.09	2.92	2.71	1.87	2.99
	3	2.07	2.05	1.79	1.97	2.51	2.71	2.50	2.91	2.40
Cineole	1	5.21	4.17	6.21	9.22	8.11	9.50	8.29	6.21	4.17
	2	4.89	5.17	6.00	7.31	10.29	9.78	7.53	5.98	4.29
	3	5.11	5.20	7.13	7.99	9.51	10.29	7.29	5.72	5.38
Menthone	1	2.91	4.95	8.99	13.15	29.50	50.28	62.91	70.29	71.86
	2	2.53	2.75	7.21	10.11	18.73	54.11	64.20	70.99	71.73
	3	1.17	3.31	4.37	13.21	35.11	51.07	59.29	68.70	70.29
Menthofuran	1	8.17	7.15	7.55	8.71	7.23	7.46	6.91	8.20	7.31
	2	5.19	6.31	7.29	7.11	6.52	7.38	6.50	6.02	6.92
	3	6.22	4.29	5.36	5.77	7.21	4.44	3.86	5.29	4.31
Menthyl Acetate	1	10.56	5.11	0.91	0.95	0.41	0.29	0.28	0.17	0.07
	2	12.11	7.32	1.77	1.05	0.37	0.25	0.12	0.21	0.13
	3	9.77	5.27	0.87	0.81	0.53	0.24	0.10	0.33	0.09
Menthol	1	58.83	62.60	62.20	52.33	45.27	19.02	7.24	1.82	1.06
	2	59.10	64.10	60.12	57.42	47.26	15.20	8.21	3.42	2.71
	3	64.76	65.33	64.30	55.86	30.34	19.54	14.51	7.93	5.05
Pulegone	1	2.75	1.86	1.17	1.49	1.54	1.55	1.71	0.61	1.29
	2	2.63	1.73	1.14	2.11	2.32	0.99	2.31	1.72	2.07
	3	2.51	1.45	2.29	1.95	1.77	1.70	2.10	0.95	2.13

Appendix IV B 2.1. Dry Matter, Oil and Percentage Oil Yield.

Yield Component			Dry Matter Yield (g/m^2)		Oil Yield (g/m^2)		Percentage Oil Yield	
Site →			Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
	Harvest Date	Block						
1	2/1/78	1	222.0	93.1	3.688	1.293	1.66	1.39
		2	297.8	49.8	4.052	0.637	1.36	1.37
		3	228.7	107.6	3.327	1.546	1.46	1.42
2	9/1/78	1	225.8	113.8	3.285	1.615	1.45	1.42
		2	375.1	151.1	7.098	2.142	1.89	1.42
		3	318.2	180.4	4.700	2.483	1.48	1.38
3	16/1/78	1	297.2	121.3	3.815	1.788	1.28	1.47
		2	543.8	215.1	7.423	2.780	1.37	1.29
		3	380.9	162.7	5.229	2.446	1.37	1.50
4	23/1/78	1	323.3	231.6	4.729	3.119	1.46	1.35
		2	402.9	220.0	6.143	3.019	1.53	1.37
		3	452.9	176.4	5.645	2.698	1.27	1.53
5	30/1/78	1	350.7	186.2	4.554	2.754	1.30	1.48
		2	568.9	222.7	8.140	3.804	1.43	1.71
		3	465.8	243.1	5.683	3.433	1.22	1.41
6	6/2/78	1	416.4	288.9	4.770	3.543	1.15	1.23
		2	548.4	266.2	6.999	3.943	1.28	1.48
		3	469.8	420.9	5.632	4.997	1.20	1.19
7	13/2/78	1	405.3	245.8	5.604	3.108	1.38	1.27
		2	568.4	338.2	6.607	4.858	1.16	1.44
		3	556.4	336.4	6.323	4.054	1.14	1.21
8	20/2/78	1	371.1	355.1	4.862	5.565	1.31	1.57
		2	870.7	269.3	6.229	3.753	0.72	1.39
		3	497.8	520.9	5.728	5.499	1.15	1.06
9	27/2/78	1	277.3	405.8	3.757	4.934	1.36	1.22
		2	343.6	421.8	3.625	4.896	1.05	1.16
		3	292.9	412.4	3.699	4.215	1.26	1.02

Appendix IV B 2.2 Oil Composition (%).

Compound (% w/w) →			Menthol		Menthone		Menthofuran		Menthyl Acetate	
Site →			Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
	Harvest Date	Block No.								
1	2/1/78	1	41.21	38.17	29.26	36.98	1.21	2.07	2.74	1.57
		2	41.34	36.45	30.49	40.86	1.38	1.93	2.33	1.27
		3	38.30	36.76	28.89	38.79	1.32	2.40	2.76	1.15
2	9/1/78	1	41.29	34.38	27.95	40.20	1.05	1.54	2.93	1.10
		2	40.68	31.26	22.08	39.87	1.57	1.96	1.02	0.68
		3	41.47	32.62	29.87	42.63	1.24	2.10	2.76	0.85
3	16/1/78	1	39.18	37.51	33.93	34.22	0.85	1.27	1.48	1.19
		2	38.80	40.90	33.57	46.18	1.16	1.32	2.08	0.85
		3	35.84	33.02	37.34	41.15	0.85	1.32	1.19	0.98
4	23/1/78	1	46.67	38.12	20.66	32.19	0.91	1.89	3.01	1.27
		2	39.75	43.19	29.22	29.47	0.72	1.91	2.08	0.89
		3	42.31	38.04	23.78	34.95	0.91	1.86	2.37	1.10
5	30/1/78	1	45.39	37.59	20.99	31.79	1.34	2.82	3.56	1.34
		2	47.99	37.96	25.92	32.31	1.69	2.85	2.44	1.11
		3	48.43	41.85	14.68	26.18	1.29	1.69	3.98	1.08
6	6/2/78	1	49.28	39.99	19.35	32.93	1.73	2.30	4.28	1.26
		2	44.39	44.59	22.34	29.60	1.91	3.09	3.07	1.48
		3	47.85	39.82	20.29	32.54	2.50	2.07	3.37	1.07
7	13/2/78	1	53.08	47.56	14.28	16.18	3.72	2.83	4.31	2.32
		2	46.29	45.59	19.36	19.73	1.27	2.52	2.96	1.34
		3	48.23	44.57	19.67	19.62	2.96	1.54	4.16	2.10
8	20/2/78	1	53.81	45.17	13.94	21.44	5.28	2.47	4.29	2.01
		2	53.17	44.02	16.20	13.03	4.40	1.85	3.80	2.33
		3	48.24	46.85	13.71	15.02	7.09	3.22	3.65	2.00
9	27/2/78	1	56.39	45.52	20.71	18.75	7.16	2.08	3.80	2.08
		2	55.40	52.82	19.04	10.88	3.81	2.38	4.24	2.38
		3	52.47	53.91	14.83	20.71	4.50	2.56	3.71	2.56

Appendix IV B 2.3. Analysis of Variance.

Dry Matter (g/m^2)

Source of Variation	DF	SS	MS	F
Site	1	344880	344880	78.185***
Harvest Date	8	449571	56196	12.740***
Site x Harvest Date	8	134814	16852	3.820**
Site x Block	4	164397	41099	9.317***
Error	32	141154	4411	
Total	53	1234816		

Oil Yield (g/m^2)

Source of Variation	DF	SS	MS	F
Site	1	50.8940	50.8940	103.874***
Harvest Date	8	38.7190	4.8399	9.878***
Site x Harvest Date	8	21.4254	2.6782	5.466***
Site x Block	4	17.5023	4.3756	8.930***
Error	32	15.6788	0.4900	
Total	53	144.2194		

Oil Yield (Dry Matter Basis)

Source of Variation	DF	SS	MS	F
Site	1	0.02081	0.02081	0.883 ns
Harvest Date	8	0.67041	0.08380	3.555**
Site x Harvest Date	8	0.28573	0.03572	1.515 ns
Site x Block	4	0.08721	0.02180	0.925 ns
Error	32	0.75433	0.02357	
Total	53	1.81848		

% Menthone

Source of Variation	DF	SS	MS	F
Site	1	572.65	572.65	46.512***
Harvest Date	8	3084.12	385.51	31.312***
Site x Harvest Date	8	326.35	40.79	3.313**
Site x Block	4	25.06	6.26	0.509 ns
Error	32	393.98	12.31	
Total	53	4402.16		

% Menthol

Source of Variation	DF	SS	MS	F
Site	1	308.262	308.262	50.563***
Harvest Date	8	1405.286	175.661	28.813***
Site x Harvest Date	8	77.751	9.719	1.594 ns
Site x Block	4	43.043	10.761	1.765 ns
Error	32	195.091	6.097	
Total	53	2029.432		

% Menthofuran

Source of Variation	DF	SS	MS	F
Site	1	0.6622	0.6622	1.362 ns
Harvest Date	8	50.4453	6.3057	12.966***
Site x Harvest Date	8	30.7990	3.8499	7.916***
Site x Block	4	1.9659	0.4915	1.011 ns
Error	32	15.5626	0.4863	
Total	53	99.4351		

% Menthyl Acetate

Source of Variation	DF	SS	MS	F
Site	1	34.2567	34.2567	202.177***
Harvest Date	8	20.6850	2.5856	15.260***
Site x Harvest Date	8	3.2529	0.4066	2.400*
Site x Block	4	2.4927	0.6232	3.670*
Error	32	5.4221	0.1694	
Total	53	66.1093		

Appendix IV B 3.1 (a). Irrigation x Nitrogen. Harvest 1. Yield Components.

Treatment	Dry Matter Yield (g/m^2)			Oil Yield (g/m^2)			Percentage Oil Yield (Dry Matter Basis		
	Block			Block			Block		
	1	2	3	1	2	3	1	2	3
I (L) N1	240.8	243.2	217.2	2.890	2.917	2.910	1.20	1.20	1.34
I (L) N2	268.0	213.2	234.0	2.989	2.432	2.637	1.12	1.14	1.13
I (L) N3	251.6	237.6	227.2	3.292	2.987	3.721	1.31	1.26	1.64
I (L) N4	230.8	240.8	266.8	3.031	2.534	2.815	1.31	1.05	1.06
I (H → L) N1	212.8	251.6	227.2	2.738	2.873	2.500	1.29	1.14	1.10
I (H → L) N2	276.8	285.2	250.0	2.342	2.017	2.475	0.85	0.71	0.99
I (H → L) N3	272.0	211.2	253.6	3.700	2.710	3.291	1.36	1.28	1.30
I (H → L) N4	260.4	280.4	220.0	3.030	3.176	3.015	1.17	1.13	1.37
I (L → H) N1	343.6	321.2	307.6	3.651	3.450	3.702	1.06	1.07	1.20
I (L → H) N2	350.4	343.6	346.8	3.876	3.928	4.015	1.10	1.14	1.16
I (L → H) N3	398.8	323.2	326.4	4.984	4.732	4.829	1.25	1.46	1.48
I (L → H) N4	331.6	368.0	354.4	4.975	5.014	4.807	1.50	1.36	1.36
I (H) N1	325.0	310.0	325.6	2.963	2.735	2.990	0.91	0.88	0.92
I (H) N2	411.2	358.4	334.0	3.913	3.875	3.705	0.95	1.08	1.11
I (H) N3	402.8	419.2	351.6	4.247	4.521	4.591	1.05	1.08	1.31
I (H) N4	395.2	386.8	363.2	4.629	5.102	4.927	1.17	1.32	1.36

Appendix IV B 3.1 (b). Irrigation x Nitrogen. Harvest 2. Yield Components.

Yield Component	Dry Matter Yield (g/m^2)			Oil Yield (g/m^2)			Percentage Oil Yield (Dry Matter Basis)		
Treatment	Block			Block			Block		
	1	2	3	1	2	3	1	2	3
I (L) N1	170.22	163.73	147.72	1.5835	1.2626	1.2297	0.93	0.77	0.83
I (L) N2	112.70	124.88	160.06	1.0448	1.0074	1.7392	0.93	0.81	1.09
I (L) N3	181.10	179.71	208.61	1.8909	2.0110	2.0838	1.04	1.12	1.00
I (L) N4	199.06	155.09	220.91	2.0757	1.9873	1.9650	1.04	1.28	0.89
I (H → L) N1	182.23	155.86	186.33	1.7973	1.7217	1.7433	0.99	1.11	0.94
I (H → L) N2	172.44	154.73	178.03	1.7195	1.9201	2.0142	1.00	1.24	1.13
I (H → L) N3	229.66	174.83	182.50	1.8180	1.9329	2.0051	0.79	1.11	1.10
I (H → L) N4	213.34	194.58	191.16	1.9835	2.0710	1.3557	0.93	1.06	0.71
I (L → H) N1	200.17	160.43	209.29	1.5590	1.7400	1.9281	0.78	1.09	0.92
I (L → H) N2	239.98	215.88	230.99	2.0461	2.0793	2.0570	0.85	0.96	0.89
I (L → H) N3	264.27	244.22	266.25	2.4034	2.3873	2.6066	0.91	0.98	0.98
I (L → H) N4	341.66	318.27	331.41	3.1460	3.0049	2.9873	0.92	0.94	0.90
I (H) N1	185.24	202.71	213.56	1.7315	1.7555	2.0701	0.94	0.87	0.97
I (H) N2	234.80	172.88	222.17	2.0135	2.0145	2.1870	0.86	1.17	0.98
I (H) N3	254.36	252.04	273.01	2.4172	2.4472	2.3650	0.95	0.97	0.87
I (H) N4	296.85	274.86	352.85	3.0150	3.0241	3.0610	1.01	1.10	0.87

Appendix IV B 3.1 (c). Nitrogen x Irrigation. Harvest 1. Oil Composition (%).

Compound	α-Pinene			β-Pinene			Limonene			Cineole			Menthone			Menthofuran			Isomenthone			Menthyl Acetate			Neomenthol			Menthol			Pulegone		
Treatment	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
I (L) N	0.52	0.62	0.69	1.16	1.20	1.32	1.08	1.31	1.31	4.77	5.11	4.28	30.01	27.29	28.13	2.24	1.31	1.51	2.17	2.71	2.95	3.07	2.71	3.17	3.29	3.71	3.41	42.52	43.71	45.20	1.66	1.01	1.32
I (L) N2	0.67	0.58	0.54	1.16	1.19	1.18	1.29	1.30	1.29	5.43	5.29	5.19	27.57	25.17	26.20	1.45	1.23	1.47	2.72	2.03	3.01	3.07	2.93	3.02	3.94	3.89	3.29	48.36	45.29	44.17	1.03	1.29	1.41
I (L) N3	0.59	0.63	0.53	1.22	1.25	1.22	1.35	1.27	1.01	5.11	5.17	4.37	23.35	26.15	23.29	1.83	1.45	1.93	2.92	2.91	3.21	4.07	2.07	2.71	3.66	4.10	3.75	43.93	44.31	42.19	1.06	1.31	1.53
I (L) N4	0.64	0.60	0.51	1.32	1.31	1.29	1.30	1.02	0.91	5.15	5.03	4.98	27.15	29.90	27.10	1.47	1.77	1.07	2.60	2.75	2.67	3.65	3.25	2.75	3.38	3.27	3.10	45.10	43.72	43.27	1.24	1.02	1.71
I (H → L) N1	0.63	0.71	0.70	1.15	1.14	1.49	1.26	1.07	1.21	5.06	5.00	5.41	26.13	30.17	24.20	1.21	1.61	1.56	1.05	2.63	2.07	3.99	2.98	2.91	3.75	2.97	3.21	43.41	44.10	43.11	1.04	1.71	1.29
I (H → L) N2	0.67	0.58	0.59	1.37	1.17	1.31	1.20	1.27	1.37	4.62	5.17	5.00	28.20	29.02	31.21	1.14	1.39	1.43	3.09	2.91	2.91	3.09	3.42	3.10	3.47	3.33	3.53	42.87	45.17	44.20	1.05	1.02	1.53
I (H → L) N3	0.64	0.62	0.65	1.35	1.15	1.21	1.14	1.18	1.17	5.10	4.83	5.13	29.70	31.10	30.01	1.32	1.13	1.29	2.65	2.85	3.01	3.19	2.75	3.27	3.25	3.29	3.47	44.70	44.70	41.73	1.60	1.91	1.47
I (H → L) N4	0.58	0.67	0.67	1.28	1.27	1.24	1.18	1.23	1.12	4.94	4.29	5.29	27.82	26.29	32.10	1.28	1.92	1.33	2.58	3.01	2.93	3.06	3.98	3.51	3.53	4.17	4.21	45.28	42.91	43.27	1.61	1.06	1.99
I (L → H) N1	0.62	0.72	0.51	1.28	1.18	1.31	1.02	1.31	1.31	4.45	5.41	4.97	30.03	28.30	30.70	1.89	1.76	1.67	2.53	2.47	3.21	2.72	2.35	3.29	3.76	3.00	3.29	40.64	41.30	41.32	1.63	1.73	1.53
I (L → H) N2	0.57	0.58	0.61	1.18	1.19	1.25	1.12	1.30	1.27	4.47	5.21	5.63	31.72	29.17	26.71	1.79	1.31	1.71	2.83	2.58	3.01	2.62	3.71	3.01	3.24	3.31	3.71	40.76	45.61	41.70	1.91	1.09	1.42
I (L → H) N3	0.53	0.61	0.57	1.16	1.27	1.23	0.88	1.09	1.00	4.54	5.01	5.00	34.80	28.18	27.29	1.07	1.58	1.09	2.69	2.93	2.53	2.11	3.01	2.97	3.32	3.78	3.99	41.23	43.72	44.20	1.51	1.21	1.23
I (L → H) N4	0.63	0.69	0.63	1.19	1.20	1.31	1.29	1.31	1.11	4.99	5.27	5.18	29.70	30.21	28.11	1.09	1.43	1.07	2.71	2.81	2.73	2.99	2.54	3.11	2.99	3.54	3.71	42.90	44.10	43.71	1.53	1.33	1.11
I (H) N1	0.56	0.62	0.65	1.14	1.10	1.13	0.81	1.32	1.37	4.69	5.33	4.28	29.82	27.29	25.11	1.87	1.75	1.31	2.93	2.89	2.30	2.67	2.91	2.71	3.59	3.11	3.45	41.79	40.89	45.17	1.03	1.07	1.63
I (H) N2	0.56	0.64	0.53	1.19	1.29	1.29	1.14	1.17	1.29	4.58	4.29	5.71	28.66	29.31	24.29	1.34	1.20	1.29	2.19	2.00	2.91	2.89	2.87	2.83	3.40	3.31	3.71	42.83	44.27	42.30	1.16	1.12	1.02
I (H) N3	0.45	0.53	0.60	1.08	1.25	1.31	1.00	1.10	1.34	4.45	5.73	5.01	31.22	27.08	25.80	1.91	1.00	1.92	2.70	2.75	2.51	2.39	2.91	2.91	3.23	3.72	3.80	43.64	43.11	44.13	1.57	1.49	1.00
I (H) N4	0.51	0.55	0.71	1.10	1.15	1.04	0.96	1.61	1.28	4.41	5.09	5.17	32.94	26.29	29.30	1.04	1.85	1.78	2.76	2.36	2.37	2.10	3.82	3.10	3.20	3.79	3.43	42.99	44.80	43.27	1.28	1.68	0.99

Appendix IV B 3.1 (d). Nitrogen x Irrigation. Harvest 2. Oil Composition (%).

Compound	α-Pinene			β-Pinene			Limonene			Cineole			Menthone			Menthofuran			Isomenthone			Menthyl Acetate			Neomenthol			Menthol			Pulegone		
Treatment	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
I (L) N1	0.44	0.37	0.34	0.84	0.71	0.64	1.37	1.26	1.54	2.48	2.25	1.97	8.82	10.74	11.32	9.97	9.74	10.39	2.25	1.85	2.04	7.94	7.50	7.57	2.95	3.84	3.02	60.14	57.34	57.34	1.75	1.63	1.90
I (L) N2	0.38	0.47	0.37	0.74	0.79	0.70	1.64	1.41	1.81	2.20	2.56	2.19	8.04	11.73	11.17	13.56	12.64	9.61	1.89	2.10	1.95	8.39	7.24	6.76	2.70	3.35	3.26	58.16	56.56	55.92	1.87	1.34	1.84
I (L) N3	0.44	0.41	0.42	0.84	0.81	0.80	1.70	1.65	1.64	2.25	2.50	2.54	8.41	10.29	12.47	12.61	11.75	9.90	2.10	2.00	2.16	7.62	7.58	7.24	3.04	3.11	3.17	55.65	56.79	56.84	1.34	1.64	1.89
I (L) N4	0.55	0.50	0.48	0.78	0.79	0.87	1.88	1.73	2.11	2.23	2.49	1.92	12.57	11.53	10.64	10.07	11.29	13.24	2.72	2.17	1.75	7.94	8.10	8.63	3.92	3.17	3.68	56.27	57.63	56.89	1.82	1.71	1.91
I (H → L) N1	0.39	0.40	0.40	0.74	0.78	0.76	1.47	1.54	1.34	2.39	2.37	2.51	12.89	9.39	13.06	10.38	11.99	10.49	2.01	1.91	2.31	6.58	7.57	6.69	2.84	2.97	3.64	55.33	57.95	57.55	1.70	1.60	1.83
I (H → L) N2	0.41	0.43	0.46	0.75	0.80	0.89	1.50	1.52	1.54	2.47	2.85	2.77	11.20	10.54	12.77	10.65	10.29	9.09	2.10	1.85	1.93	6.42	7.10	5.94	2.89	3.10	3.79	55.11	57.88	58.32	1.81	1.73	1.92
I (H → L) N3	0.44	0.63	0.43	0.83	0.95	0.84	1.56	1.38	1.57	2.63	3.40	2.58	11.02	10.65	10.58	10.09	9.84	9.98	1.96	2.19	1.79	6.73	7.67	6.97	3.53	3.88	3.74	56.13	58.08	54.47	1.42	1.09	1.81
I (H → L) N4	0.43	0.42	0.47	0.84	0.80	0.79	1.70	1.73	1.81	2.81	2.81	2.38	12.07	11.29	11.51	9.83	10.53	11.93	1.93	1.79	2.10	6.66	6.99	6.95	3.57	2.85	2.86	54.58	55.29	54.72	1.63	1.92	1.74
I (L → H) N1	0.58	0.45	0.41	0.93	0.81	0.80	1.43	1.42	1.41	2.92	2.85	2.73	15.13	14.78	13.29	10.02	9.10	9.99	2.26	2.11	2.10	6.09	5.78	5.37	2.48	2.95	3.91	53.75	50.10	51.29	1.34	1.97	1.07
I (L → H) N2	0.40	0.55	0.50	0.79	0.83	0.92	1.52	1.53	1.69	2.69	2.71	2.59	13.44	14.97	14.58	9.20	9.52	9.50	1.92	2.80	1.97	5.82	6.54	5.68	3.54	3.17	4.07	53.51	53.51	51.89	1.72	1.84	1.92
I (L → H) N3	0.40	0.44	0.31	0.83	0.71	0.61	1.70	1.27	1.45	2.42	2.51	1.95	14.29	16.19	15.99	8.27	9.41	8.49	1.97	2.08	2.29	5.92	6.15	6.43	3.10	3.25	3.80	52.11	50.22	52.95	1.73	2.09	1.87
I (L → H) N4	0.44	0.47	0.44	0.81	0.88	0.80	1.56	1.52	1.80	2.73	2.86	2.23	16.79	17.04	18.74	8.27	8.45	8.73	2.44	2.26	2.33	5.27	5.58	5.63	3.45	3.39	3.82	52.29	52.33	49.08	1.89	2.00	1.90
I (H) N1	0.41	0.42	0.40	0.81	0.57	0.73	1.72	1.45	1.61	2.63	2.72	2.25	17.29	18.40	16.52	8.21	8.71	10.24	2.11	2.40	2.23	4.28	4.93	5.45	2.97	2.60	3.24	52.51	52.10	52.89	1.62	1.62	1.37
I (H) N2	0.45	0.41	0.49	0.73	0.85	0.90	1.69	1.53	1.94	2.59	2.61	2.58	15.35	17.28	16.77	7.37	8.91	8.24	2.00	1.75	2.39	5.01	4.29	5.12	2.85	3.00	2.93	50.71	49.87	50.22	1.53	1.71	1.77
I (H) N3	0.43	0.47	0.50	0.75	0.82	0.93	1.43	1.71	1.90	2.89	2.61	2.79	16.91	18.17	18.97	6.99	7.28	8.79	1.85	1.93	2.54	4.71	4.11	4.98	3.11	2.05	3.19	50.11	49.99	50.78	1.92	1.85	1.80
I (H) N4	0.44	0.44	0.51	0.81	0.85	0.94	1.57	1.48	1.82	2.64	2.87	2.62	19.29	19.71	19.16	6.46	7.93	8.34	2.10	2.24	2.62	4.29	4.85	4.12	3.51	3.62	2.89	50.27	50.30	50.27	1.05	1.54	1.01

Appendix IV B 3.2. Split Plot in Time and Space.
Analysis of Variance: Nitrogen x Irrigation x Harvest Date.

Dry Matter Yield (g/m^2)

Source of Variation	DF	SS	MS	F
Blocks	2	4093.2	2046.6	5.033**
Irrigation	3	199477.8	66492.6	163.517***
Nitrogen	3	39507.7	13169.2	32.385***
I x N	9	14231.0	1581.2	3.889***
Time	1	181427.3	181427.3	446.162***
I x T	3	11283.0	3761.0	9.249***
N x T	3	15561.3	5187.1	12.756***
I x N x T	9	6987.8	776.4	1.909 ns
Blocks x Time	2	6463.9	3231.9	7.948***
Residual	60	24398.5	406.64	
Total	95	503431.5		

Oil Yield (g/m^2)

Source of Variation	DF	SS	MS	F
Blocks	2	0.14562	0.07281	1.834 ns
Irrigation	3	21.56712	7.18904	181.034***
Nitrogen	3	12.63372	4.21124	106.047***
I x N	9	4.63414	0.51490	12.966***
Time	1	52.77730	52.77730	1329.035***
I x T	3	3.59153	1.19718	30.147***
N x T	3	0.69276	0.23092	5.815**
I x N x T	9	1.78049	0.19783	4.982***
Block x Time	2	0.08998	0.04499	
Residual	60	2.38266	0.039711	
Total	95	100.29532		

% Oil Yield (Dry Matter Basis)

Source of Variation	DF	SS	MS	F
Blocks	2	0.04201	0.02101	1.978 ns
Irrigation	3	0.07904	0.02635	2.481 ns
Nitrogen	3	0.33625	0.11208	10.566***
I x N	9	0.18379	0.02042	1.923 ns
Time	1	1.08588	1.08588	102.249***
I x T	3	0.17804	0.05935	5.589**
N x T	3	0.29060	0.09687	9.122**
I x N x T	9	0.36516	0.04057	3.820***
Blocks x Time	2	0.14940	0.07470	7.034**
Residual	60	0.63718	0.01062	
Total	95	3.34735		

% α-Pinene

Source of Variation	DF	SS	MS	F
Blocks	2	0.011556	0.005778	1.744 ns
Irrigation	3	0.015536	0.005179	1.563 ns
Nitrogen	3	0.011453	0.003818	1.152 ns
I x N	9	0.028826	0.003203	0.967 ns
Time	1	0.628884	0.628884	189.817***
I x T	3	0.015636	0.005212	1.573 ns
N x T	3	0.020636	0.006872	2.074 ns
I x N x T	9	0.039393	0.004377	1.321 ns
Block x Time	2	0.003056	0.001528	0.461 ns
Residual	60	0.198787	0.003313	
Total	95	0.973763		

% β-Pinene

Source of Variation	DF	SS	MS	F
Blocks	2	0.018731	0.009366	1.614 ns
Irrigation	3	0.028951	0.009840	1.696 ns
Nitrogen	3	0.021046	0.007015	1.209 ns
I x N	9	0.104096	0.011566	1.993 ns
Time	1	4.284150	4.284150	738.216***
I x T	3	0.031475	0.010492	1.808 ns
N x T	3	0.011883	0.003961	0.683 ns
I x N x T	9	0.066625	0.007403	1.276 ns
Blocks x Time	2	0.009531	0.004766	0.821 ns
Residual	60	0.348203	0.005803	
Total	95	4.926261		

% Limonene

Source of Variation	DF	SS	MS	F
Blocks	2	0.14685	0.07343	3.828 ns
Irrigation	3	0.10869	0.03623	1.889 ns
Nitrogen	3	0.26969	0.08990	4.686**
I x N	9	0.09513	0.01057	0.551 ns
Time	1	3.88413	3.88413	202.457***
I x T	3	0.05143	0.01714	0.893 ns
N x T	3	0.24110	0.08037	4.189**
I x N x T	9	0.32346	0.03594	1.873 ns
Blocks x Time	2	0.22068	0.11034	5.751**
Residual	60	1.15107	0.019185	
Total	95	6.49223		

% Cineole

Source of Variation	DF	SS	MS	F
Blocks	2	0.7190	0.3595	3.488*
Irrigation	3	0.4768	0.1589	1.542 ns
Nitrogen	3	0.1427	0.0476	0.462 ns
I x N	9	0.9798	0.1089	1.057 ns
Time	1	140.2875	140.2875	1361.420***
I x T	3	0.6839	0.2280	2.213 ns
N x T	3	0.0470	0.0157	0.152 ns
I x N x T	9	0.8136	0.0904	0.877 ns
Block x Time	2	0.6110	0.3055	2.965 ns
Residual	60	6.1827	0.10345	
Total	95	150.9440		

% Menthone

Source of Variation	DF	SS	MS	F
Blocks	2	3.654	1.827	0.580 ns
Irrigation	3	290.076	96.692	30.686***
Nitrogen	3	26.634	8.878	2.818*
I x N	9	33.173	3.686	1.169 ns
Time	1	5039.332	5039.332	1599.280***
I x T	3	174.875	58.292	18.500***
N x T	3	3.609	1.203	0.382 ns
I x N x T	9	38.257	4.251	1.349 ns
Blocks x Time	2	29.912	14.956	
Residual	60	189.065	3.151	
Total	95	5828.600		

% Menthofuran

Source of Variation	DF	SS	MS	F
Blocks	2	0.4945	0.2472	0.444 ns
Irrigation	3	34.6038	11.5346	20.725***
Nitrogen	3	1.5984	0.5328	0.957 ns
I x N	9	8.0669	0.8963	1.610 ns
Time	1	1627.0713	1627.0713	2923.443***
I x T	3	34.5052	11.5017	20.666***
N x T	3	0.7893	0.2631	0.473 ns
I x N x T	9	6.4155	0.7128	1.281 ns
Blocks x Time	2	0.6526	0.3263	0.587 ns
Residual	60	33.3934	0.55656	
Total	95	1747.5909		

% Isomenthone

Source of Variation	DF	SS	MS	F
Blocks	2	0.2747	0.1373	1.508 ns
Irrigation	3	0.3512	0.1171	1.286 ns
Nitrogen	3	0.2910	0.097	1.066 ns
I x N	9	1.1018	0.1224	1.345 ns
Time	1	7.3151	7.3151	80.355***
I x T	3	0.2920	0.0973	1.069 ns
N x T	3	0.4778	0.1593	1.750 ns
I x N x T	9	1.1966	0.1330	1.461 ns
Block x Time	2	0.0891	0.0446	0.490 ns
Residual	60	5.4621	0.09104	
Total	95	16.8514		

% Menthyl Acetate

Source of Variation	DF	SS	MS	F
Blocks	2	0.0558	0.0279	0.126 ns
Irrigation	3	36.8434	12.2811	55.520***
Nitrogen	3	0.0441	0.0147	0.067 ns
I x N	9	1.8682	0.2076	1.248 ns
Time	1	259.7784	259.7784	1174.405***
I x T	3	25.5279	8.5093	38.469***
N x T	3	0.4391	0.1464	0.662 ns
I x N x T	9	1.2564	0.1396	0.631 ns
Blocks x Time	2	0.2234	0.1117	0.505 ns
Residual	60	13.2746	0.2212	
Total	95	339.3113		

% Neomenthol

Source of Variation	DF	SS	MS	F
Blocks	2	0.7829	0.3914	3.311*
Irrigation	3	0.6274	0.2091	1.769 ns
Nitrogen	3	0.6383	0.2128	1.800 ns
I x N	9	0.3712	0.0412	0.349 ns
Time	1	1.6511	1.6511	13.967***
I x T	3	0.5605	0.1868	1.580 ns
N x T	3	0.1700	0.0567	0.480 ns
I x N x T	9	2.6953	0.2995	2.533*
Blocks x Time	2	0.2484	0.1242	1.051
Residual	60	7.093	0.1182	
Total	95	14.8381		

% Menthol

Source of Variation	DF	SS	MS	F
Blocks	2	2.802	1.401	0.788 ns
Irrigation	3	255.543	85.181	47.892***
Nitrogen	3	5.291	1.764	0.992 ns
I x N	9	16.095	1.788	1.01 ns
Time	1	2671.155	2671.155	1501.83***
I x T	3	115.863	38.621	21.714***
N x T	3	21.251	7.084	3.983*
I x N x T	9	12.541	1.393	0.783 ns
Block x Time	2	1.400	0.700	0.394 ns
Residual	60	106.714	1.7786	
Total	95	3208.655		

% Pulegone

Source of Variation	DF	SS	MS	F
Blocks	2	0.05185	0.02593	0.380 ns
Irrigation	3	0.51007	0.17002	2.491 ns
Nitrogen	3	0.04942	0.01647	0.241 ns
I x N	9	0.50030	0.05559	0.814 ns
Time	1	2.59384	2.59384	37.997***
I x T	3	0.09679	0.03226	0.473 ns
N x T	3	0.24412	0.08137	1.192 ns
I x N x T	9	1.37639	0.15293	2.240*
Blocks x Time	2	0.05998	0.02999	0.439 ns
Residual	60	4.09584	0.0683	
Total	95	9.5786		

Appendix IV B 3.3 Leaf Diffusive Resistance Measurements (s cm^{-1}).

Date	Block No.	Irrigation Treatment			
		$I_{(H)}$	$I_{(L\rightarrow H)}$	$I_{(H\rightarrow L)}$	$I_{(L)}$
Abaxial Surface					
31/12/78	1	1.27	1.47	1.69	1.05
	2	1.89	1.58	1.34	1.82
	3	1.20	1.01	1.17	1.41
7/1/79	1	1.80	1.92	1.32	1.83
	2	1.48	1.72	1.41	1.29
	3	1.63	1.10	1.76	1.64
28/1/79	1	1.97	1.71	7.29	7.21
	2	1.43	1.53	6.53	8.10
	3	1.56	1.62	5.19	8.29
11/2/79	1	2.03	1.84	7.32	6.34
	2	1.53	1.73	6.98	7.29
	3	1.79	1.92	8.29	6.53
Adaxial Surface					
31/12/78	1	65.29	61.29	68.10	61.23
	2	66.73	67.30	60.27	70.18
	3	62.91	59.28	70.18	62.95
7/1/79	1	58.21	65.85	69.20	68.27
	2	70.21	68.27	70.39	78.32
	3	73.09	70.10	60.73	65.21
28/1/79	1	78.21	70.11	95.20	100.71
	2	68.57	72.90	103.73	96.27
	3	69.20	61.34	89.27	85.10
11/2/79	1	65.98	70.22	104.73	97.33
	2	73.21	63.97	98.95	100.50
	3	68.24	79.28	93.27	95.76

Appendix IV B 4.1. Change in yield components with time - postharvest regrowth.

Yield Component \ Harvest Date	Block No.	25th April 1979	15th May 1979	19th June 1979
Dry Matter Yield (g/m^2)	1	296.85	285.80	253.50
	2	274.86	279.81	259.20
	3	352.85	299.35	268.15
Oil Yield (g/m^2)	1	3.0150	2.7723	1.7997
	2	3.0240	2.3783	1.8922
	3	3.0610	2.1853	2.0916
Percentage Oil Yield (Dry Matter Basis)	1	1.01	0.97	0.71
	2	1.10	0.85	0.73
	3	0.87	0.73	0.78

Appendix IV B 4.2. Changes in oil composition with time - postharvest regrowth.

Compound (% w/w) / Harvest Date	Block No.	α-Pinene	β-Pinene	Limonene	Cineole	Menthone	Menthofuran	Isomenthone	Menthyl Acetate	Neomenthol	Menthol	Pulegone
25th April 1979	1	0.44	0.81	1.57	2.64	19.29	6.46	2.10	4.29	3.51	50.12	2.05
	2	0.44	0.85	1.48	2.87	19.71	7.93	2.24	4.85	3.62	50.30	1.54
	3	0.51	0.94	1.82	2.62	19.16	8.34	2.62	4.12	2.89	50.27	1.02
15th May 1979	1	0.48	0.89	1.55	2.88	8.50	7.76	1.57	8.67	3.82	56.88	1.70
	2	0.39	0.76	1.46	2.84	10.28	5.96	1.97	8.45	3.03	61.02	0.95
	3	0.40	0.76	1.50	2.59	10.36	5.92	2.22	8.32	3.11	59.86	1.17
19th June 1979	1	0.42	0.78	1.74	2.12	1.95	5.67	0.69	23.47	5.40	50.50	2.03
	2	0.52	0.91	2.10	2.22	1.92	7.23	0.72	24.37	5.07	49.54	2.53
	3	0.50	0.87	2.19	2.06	2.15	9.33	0.76	22.93	4.83	48.02	2.45

Appendix IV B 4.3. Analysis of variance.

Dry Matter Yield (g/m^2)

S.O.V.	df	SS	MS	F
Blocks	2	3475.4685	1737.7343	2.919 ns
Harvest Date	2	2102.6699	1051.3350	4.824 ns
Error	4	1440.8332	360.2083	
Total	8	7018.9716		

Oil Yield (g/m^2)

S.O.V.	df	SS	MS	F
Blocks	2	0.0160	0.0080	0.1533 ns
Harvest Date	2	1.8300	0.9150	17.5287 *
Error	4	0.2088	0.0522	
Total	8	2.0548		

Percentage Oil Yield (Dry Matter Basis)

S.O.V.	df	SS	MS	F
Blocks	2	0.0207	0.0104	1.1064
Harvest Date	2	0.0968	0.0484	5.1489 ns
Error	4	0.0376	0.0094	
Total	8	0.1551		

% α-Pinene

S.O.V.	df	SS	MS	F
Blocks	2	0.00096	0.00478	0.14960 ns
Harvest Date	2	0.00509	0.00254	0.79652 ns
Error	4	0.01278	0.00319	
Total	8	0.01883		

% β-Pinene

S.O.V.	df	SS	MS	F
Blocks	2	0.00136	0.00067	0.09807 ns
Harvest Date	2	0.00669	0.00334	0.48392 ns
Error	4	0.02764	0.00691	
Total	8	0.03569		

% Limonene

S.O.V.	df	SS	MS	F
Blocks	2	0.07509	0.03754	1.43787 ns
Harvest Date	2	0.042062	0.21031	8.05447 *
Error	4	0.10444	0.02611	
Total	8	0.60015		

% Cineole

S.O.V.	df	SS	MS	F
Blocks	2	0.07296	0.03648	5.19051 ns
Harvest Date	2	0.74149	0.37074	52.75415 **
Error	4	0.02811	0.00703	
Total	8	0.84256		

% Menthone

S.O.V.	df	SS	MS	F
Blocks	2	0.94349	0.47175	1.28847 ns
Harvest Date	2	445.03049	227.51524	621.40941 ***
Error	4	1.46451	0.36613	
Total	8	457.43849		

% Menthofuran

S.O.V.	df	SS	MS	F
Blocks	2	2.36709	1.18355	0.55417 ns
Harvest Date	2	1.83400	0.91701	0.42937 ns
Error	4	8.54285	2.13571	
Total	8	12.74394		

% Isomenthone

S.O.V.	df	SS	MS	F
Blocks	2	0.25682	0.12841	4.87123 ns
Harvest Date	2	4.14136	2.07068	78.55047 ***
Error	4	0.10544	0,02636	
Total	8	4.50362		

% Menthyl Acetate

S.O.V.	df	SS	MS	F
Blocks	2	0.88347	0.44173	3.33804 ns
Harvest Date	2	612.28460	306.14230	2313.41788 ***
Error	4	0.52933	0.13233	
Total	8	613.69740		

% Neomenthol

S.O.V.	df	SS	MS	F
Blocks	2	0.60247	0.30123	4.83262 ns
Harvest Date	2	6.26640	3.13320	50.26524 **
Error	4	0.24933	0.06233	
Total	8	7.11820		

% Menthol

S.O.V.	df	SS	MS	F
Blocks	2	2.11736	1.05868	0.41719 ns
Harvest Date	2	180.19909	90.09955	35.50519 **
Error	4	10.15058	2.53764	
Total	8	192.46703		

% Pulegone

S.O.V.	df	SS	MS	F
Blocks	2	0.22462	0.11231	0.60109 ns
Harvest Date	2	1.84002	0.92001	4.92394 ns
Error	4	0.74738	0.18684	
Total	8	2.81202		

ns Not significant
* Significant at 5%
** Significant at 1%
*** Significant at 0.1%

Appendix IV B 5.1. The effect of harvest date and number, irrigation and nitrogen on -

(i) Oil Yield (g/m^2).

Treatment	Rep.No.	Harvest Date											
		19/12/79	7/1/80	14/1/80	21/1/80	29/1/80	4/2/80	11/2/80	18/2/80	25/2/80	7/3/80	16/3/80	23/3/80	31/3/80
$I_{(L)}\ N_{0.5}$	1	2.2645	4.5088	4.8768	7.3673	8.0530	5.5648	4.9453	5.8829	7.4079				
	2	3.0860	4.2457	5.4746	6.4179	8.4514	6.9100	6.0129	8.3344	5.3287				
	3	4.4359	5.7602	4.0884	4.2348	6.7586	6.7731	8.0116	5.2173	6.4400				
$I_{(L\rightarrow H)}\ N_2$	1	4.7613	5.9770	6.8639	6.8639	7.4416	5.3637	7.1414	7.2670	8.0028				
	2	3.2063	6.1499	5.9022	6.5718	7.1431	7.1608	8.2646	6.9204	7.7608				
	3	4.0838	5.8039	6.5606	6.9781	7.7400	7.0053	9.0899	7.3665	7.3309				
$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$	1	3.6136	5.3757	6.7204	9.5400					1.4042	3.1387	4.5096	3.4293	3.1387
	2	3.4066	6.5427	6.5512	6.5923					1.9271	3.3144	3.9873	3.7335	3.3144
	3	4.0759	5.5149	6.9217	7.3169					2.0081	4.0626	4.4280	3.8608	4.0626

(ii) Menthone (%)

Treatment	Rep.No.	Harvest Date												
		19/12/79	7/1/80	14/1/80	21/1/80	29/1/80	4/2/80	11/2/80	18/2/80	25/2/80	7/3/80	16/3/80	23/3/80	31/1/80
$I_{(L)}\ N_{0.5}$	1	39.2	27.4	23.5	29.3	31.6	36.8	29.6	23.4	27.3				
	2	41.6	32.4	22.2	28.7	30.7	33.7	31.8	25.9	18.8				
	3	35.4	27.3	29.4	25.6	29.1	36.1	32.6	26.9	22.8				
$I_{(L\rightarrow H)}\ N_2$	1	53.6	35.2	35.4	36.9	42.9	43.3	38.3	31.6	26.9				
	2	53.1	33.9	34.6	33.3	41.5	40.2	35.4	26.2	25.6				
	3	52.0	39.5	39.5	37.3	42.4	41.2	38.9	36.4	25.2				
$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$	1	50.6	38.9	32.4	33.3					56.6	50.9	37.5	26.4	15.8
	2	49.2	36.1	31.8	33.6					57.5	47.9	39.2	25.1	19.3
	3	50.5	39.3	30.3	34.1					57.0	42.6	36.3	23.6	20.8

(iii) Menthol (%)

Treatment	Rep.No.	Harvest Date												
		19/12/79	7/1/80	14/1/80	21/1/80	29/1/80	4/2/80	11/2/80	18/2/80	25/2/80	7/3/80	16/3/80	23/3/80	31/3/80
$I_{(L)}\ N_{0.5}$	1	32.1	41.3	41.6	37.7	34.2	29.8	36.5	44.8	43.7				
	2	31.6	38.5	44.0	39.3	37.0	33.7	35.3	38.8	45.4				
	3	35.7	38.9	40.0	38.8	38.7	33.1	37.0	39.3	48.2				
$I_{(L\rightarrow H)}\ N_2$	1	26.8	35.8	32.9	34.8	27.4	31.3	34.0	38.4	45.8				
	2	23.6	37.2	35.1	34.6	26.8	31.0	34.6	44.8	45.4				
	3	24.4	34.8	32.9	33.6	28.2	29.0	32.7	38.6	45.5				
$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$	1	25.0	34.1	36.2	33.4					19.2	31.8	36.2	47.7	57.4
	2	24.4	36.5	38.4	34.3					18.3	28.8	34.5	49.2	53.5
	3	25.4	33.9	40.6	33.4					20.6	31.7	36.8	51.4	54.9

Apendice IV B 5.2

Appendix IV B 5.2.

The effect of harvest date and number, irrigation and nitrogen on the commercial yield and composition of peppermint oil.

(1) Oil Yield(kg/ha)	Treatment Rep. No.	$I_{(L)}\ N_{0.5}$	$I_{(L\rightarrow H)}\ N_2$	$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ Harvest 1 (H_1)	$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ Harvest 2 (H_2)	$I_{(L\rightarrow H)}\ N_{2+1}$ Combined ($H_1 + H_2$)
	1	48.92	61.00	60.10	19.50	79.6
	2	47.40	58.50	58.80	24.55	83.35
	3	53.21	63.80	56.20	26.95	83.15
(2) Composition (5) Compound	Treatment Rep. No.	$I_{(L)}\ N_{0.5}$	$I_{(L\rightarrow H)}\ N_2$	$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ Harvest 1 (H_1)	$I_{(L\rightarrow H)}\ N_{2+1}\ 2H$ Harvest 2 (H_2)	$I_{(L\rightarrow H)}\ N_{2+1}$ Combined ($H_1 + H_2$)
α-Pinene	1	0.67	0.64	0.70	0.63	0.68
	2	0.76	0.66	0.74	0.69	0.73
	3	0.71	0.68	0.67	0.65	0.66
β-Pinene	1	1.47	1.42	1.59	1.37	1.54
	2	1.68	1.46	1.69	1.44	1.62
	3	1.52	1.48	1.57	1.28	1.48
Limonene	1	1.85	1.77	2.21	2.07	2.18
	2	1.91	1.83	2.37	1.52	2.12
	3	1.92	1.99	3.17	1.98	2.78
Cineole	1	5.51	5.53	6.78	4.21	6.15
	2	5.41	5.49	6.52	4.17	5.83
	3	5.22	5.62	6.29	3.23	5.30
Menthone	1	19.32	18.51	34.80	17.11	30.47
	2	18.95	18.74	32.51	17.25	28.02
	3	17.29	19.37	31.73	15.66	26.52
Menthofuran	1	2.84	1.56	0.75	3.92	1.53
	2	2.73	1.62	0.82	5.02	2.06
	3	2.59	1.51	0.64	5.46	2.20
Isomenthone	1	2.05	3.18	2.66	3.14	2.78
	2	2.42	2.11	2.62	3.00	2.73
	3	2.06	2.87	3.08	2.99	3.05
Menthyl Acetate	1	2.87	3.31	1.44	4.97	2.30
	2	3.24	3.07	1.59	4.72	2.52
	3	3.25	3.07	2.22	3.83	2.74
Neomenthol	1	5.08	5.45	3.95	3.98	3.96
	2	5.25	5.03	4.21	4.29	4.23
	3	5.46	5.17	3.57	3.72	3.62
Menthol	1	51.34	52.31	38.69	53.02	42.02
	2	48.37	52.49	39.74	54.65	44.13
	3	52.79	49.21	38.22	56.35	44.10

Appendix IV B 5.3

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Analysis of Variance.

The effect of harvest date and number, irrigation and nitrogen on the commercial yield and composition of peppermint oil.

1. Oil Yield (g/m^2)

S.O.V.	df	SS	MS	F
Blocks	2	25.8123	12.9062	3.3728 ns
Treatments	2	1601.1131	800.5566	209.2083***
Error	4	15.3062	3.8266	
Total	8	1642.2316		

2. Composition (%)

α-Pinene

S.O.V.	df	SS	MS	F
Blocks	2	0.0044	0.0022	2.75 ns
Treatments	2	0.0043	0.0022	2.75 ns
Error	4	0.0031	0.0008	
Total	8	0.0118		

β-Pinene

S.O.V.	df	SS	MS	F
Blocks	2	0.0211	0.0106	2.8649 ns
Treatments	2	0.0195	0.0098	2.6486 ns
Error	4	0.0147	0.0037	
Total	8	0.0553		

Limonene

S.O.V.	df	SS	MS	F
Blocks	2	0.1650	0.0825	2.5385 ns
Treatments	2	0.4654	0.2327	7.1600 *
Error	4	0.1301	0.3250	
Total	8	0.7605		

Cineole

S.O.V.	df	SS	MS	F
Blocks	2	0.1847	0.0924	1.5635 ns
Treatments	2	0.2177	0.1089	1.8426 ns
Error	4	0.2362	0.0591	
Total	8	0.6386		

Menthone

S.O.V.	df	SS	MS	F
Blocks	2	4.3693	2.1847	1.3834 ns
Treatments	2	186.0465	93.0233	58.9053 **
Error	4	6.3166	1.5792	
Total	8	196.7324		

Menthofuran

S.O.V.	df	SS	MS	F
Blocks	2	0.0422	0.0211	0.3442 ns
Treatments	2	2.0964	1.0492	17.0995 *
Error	4	0.2451	0.0613	1.0482
Total	8	2.3837		

Isomenthone

S.O.V.	df	SS	MS	F
Blocks	2	0.1202	0.0601	0.3792 ns
Treatments	2	0.7709	0.3855	2.4322 ns
Error	4	0.6341	0.1585	
Total	8	1.5252		

Menthol

S.O.V.	df	SS	MS	F
Blocks	2	0.2088	0.1044	0.0212 ns
Treatments	2	117.9867	58.9934	11.9959 *
Error	4	19.6712	4.9178	
Total	8	137.8667		

Menthyl Acetate

S.O.V.	df	SS	MS	F
Blocks	2	0.0569	0.0285	0.6628 ns
Treatments	2	0.7578	0.3789	8.8116 *
Error	4	0.1721	0.0430	
Total	8	0.9868		

Neomenthol

S.O.V.	df	SS	MS	F
Blocks	2	0.0139	0.0070	0.0831 ns
Treatments	2	3.4006	1.7003	20.1936 **
Error	4	0.3369	0.0842	
Total	8	3.7514		

VIII PUBLICATIONS

Publications

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3. Clark, R.J. and Menary, R.C. (1980). The effect of irrigation and nitrogen on the yield and composition of peppermint oil (*Mentha piperita* L.). *Aust. J. Agric. Res.* 31, 489-498.
4. Clark, R.J. and Menary, R.C. (1980). Environmental effects on peppermint (*Mentha piperita* L.). I. The effect of daylength, light intensity, night temperature and day temperature on the yield and composition of peppermint oil. *Aust. J. Plant Physiol.*
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Manuscript Submitted for Publication

Clark, R.J. and Menary, R.C. (1980). A study of variations in composition of peppermint oil in relation to production areas. *Econ. Bot.*