A study of some factors affecting the yield and composition of fennel oil. (*Foeniculum vulgare* Mill.)

by

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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 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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LIST OF ABBREVIATIONS

ABA	Abscisic acid
a.i.	Active ingredient
BAP	6-Benzylaminopurine
CCC	2-Chloroethyltrimethylammonium chloride (Cycocel)
CoA	Coenzyme A
EL500	alpha-(1-methylethyl)-alpha-(4-(trifluromethoxy) phenyl)-5-pyrimidine-methanol (Flurprimidol)
FID	Flame ionization detector
GA	Gibberellic acid
GC-MS	Gas chromatography-mass spectrometry
IAA	Indole-3-ylacetic acid
IRGA	Infra red gas analysis
LD	Long day(s)
LSD	Least significant difference
LSR	Least significant range
MH	Maleic hydrazide
NAA	Naph-1-ylacetic acid
PFD	Photon flux density
PP333	(+/-)-beta-((4-chlorophenyl)methyl)-alpha-(1,1-di methyl)-1H-1,2,4-triazole-1-ethanol (Paclobutrazol)
SD	Short day(s)
SEM	Scanning electron microscopy

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SUMMARY

The aim of this research programme was to investigate factors influencing the production of essential oil from fennel (Foeniculum vulgare Mill.). Fennel is a perennial plant belonging to the family Apiaceae, formerly referred to as the Umbelliferae. This plant is cultivated in Europe, and more recently in Tasmania, for its essential oil. The essential oil is comprised of anethole (65 to 70 percent), fenchone (15 to 17 percent), estragole (2 to 3 percent) and terpenes, mainly limonene and beta-phellandrene. The oil is present within secretory canals in most parts of the plant. However, in mature plants approximately 95 percent of the total oil is present in the seed. Therefore, an understanding of the physiological factors controlling umbel initiation and seed development are of utmost importance, when considering the production of essential oil from fennel.

The components of oil yield in fennel include: the number of umbels, the number of rays per umbel, the number of seeds per ray and the oil yield per seed. The anethole content of the oil largely determines the commercial value of fennel oil. In order to manipulate these components, it is necessary to understand the physiological factors which control these components. Detailed studies on the reproductive physiology of fennel have not been previously reported.

Examination of differentiating meristems by scanning electron microscopy revealed that floral initiation occurred when the daylength exceeded 13 hours. Within 5 days of primary umbel initiation, secondary and tertiary umbel initiation was observed.

Glasshouse trials using night break treatments indicated that the daylength response in fennel was a phytochrome mediated response rather than merely a response to longer photosynthetic periods. A minimum of 10 long day inductive cycles resulted in initiation of primary and secondary umbels.

Many plants are not responsive when exposed to inductive conditions during early growth stages. This juvenile phase may extend from days to several months. In fennel, it was apparent that such a juvenile phase exists, and continues until the main stem has differentiated 12 nodes and has produced 8 fully expanded leaves. Under field conditions, perennial fennel plants reach this "ripe to

flower" stage prior to the onset of inductive photoperiods.

Once induced to flower, fennel plants commence a period of rapid stem elongation. No reversion of floral primordia was observed in any of the experiments conducted.

In commercial fennel fields it is often difficult to control excessive vegetative vigour. This results in very tall plants which are low yielding and difficult to harvest. The plant response to application of exogenous gibberellins indicated that this group of phytohormones was a major regulator of this rapid stem elongation. To investigate whether such gibberellin induced stem elongation could be suppressed without adversely affecting initiation, a gibberellin biosynthesis inhibitor (flurprymidol) was applied. When applied at the appropriate concentration and timing, this growth retardant suppressed stem elongation without adversely affecting flowering and oil yield.

Approximately 90 to 95 percent of fennel oil is contained within the umbels. Of the total oil yield from umbels, 84 percent is contributed by the secondary and tertiary umbels. Harvesting is timed to coincide with the maximum oil yield of these umbels.

The supply of photosynthate is known to be important in the biosynthesis and interconversion of essential oil components. Maximum net CO_2 fixation occurred at high light intensities (800 µmole $m^{-2}s^{-1}$) and between 20 to 25°C. From data collected on net CO_2 exchange characteristics of umbels and plant defoliation studies, it was apparent that maturing umbels were capable of supplying a significant proportion of their required photosynthate. Studies utilizing $^{14}CO_2$ and autoradiographic techniques clearly indicated that the umbels are photosynthetically active units and that during seed development the leaves play a minor role in photosynthate supply to the maturing seed. Competition for assimilates between the various umbel orders was observed, the lower the umbel order the lower the sink strength.

I

INTRODUCTION

I. Introduction

Fennel (Foeniculum vulgare Mill.) belongs to the family Apiacaea which was formerly known as Umbelliferae. Throughout this study, Umbellifera will be used as a general term for this plant family, as many references exist using this earlier terminology. Approximately twelve different varieties have been recorded (Guenther 1949). These varieties differ in both their morphology and composition of essential oils.

Although fennel has been cultivated for centuries as a spice crop and also for its essential oil, little is known of the floral initiation and elongation processes controlling the plants habit.

The volatile oil of fennel (Foeniculum vulgare Mill.) is obtained by steam distillation of part or all of the above ground portion of the plant. The oil is present in secretory canals which are located in all parts of the plant (Kadry et al 1978), but occur most frequently within the seed coat.

The compound trans-anethole is the major commercially important compound obtained from the volatile oil. This compound is widely used as a flavouring in numerous food products especially confectionary, apperitifs and liqueurs.

The cultivation of fennel for essential oil is well established in Europe, especially France. At the commencement of this study, the first commercial plantings of fennel for essential oil were undertaken in Tasmania. Approximately 20 ha was planted in three areas of the state in 1984. At the completion of this study the commercial area totalled 450 ha. Throughout the expansion production difficulties were encountered resulting in inconsistent average yield of anethole per unit area. During its establishment phase, the Tasmanian industry adopted overseas techniques of production. For example weed control, harvesting techniques and harvest date prediction based on French experience. Since this time it has been apparent that modifications to these techniques were necessary to optimize anethole production from fennel plants grown under Tasmanian economic and environmental conditions. To enable implementation of changes to crop management, a basic understanding of the physiology of the plant is necessary.

Aims of the present study:

(1) Compare the oil yield and oil composition between the first and second year of production and examine the contribution of each umbel order to the overall oil yield and oil compostion.

(2) Examine the morphological changes associated with flowering and to determine the environmental conditions necessary for floral initiation in fennel.

(3) Investigate the effects of various plant growth regulators on initiation and stem elongation and to select potential regulator(s) for commercial evaluation.

(4) Measurement of the effects of temperature and PFD on photosynthesis of various plant organs.

(5) Examine the source/sink relationships within the above ground portion of the plant to more fully interpret the responses observed in (1).

II

LITERATURE REVIEW

II. LITERATURE REVIEW

1. Introduction

1.1 The Umbelliferae (Apiaceae)

The Umbelliferae family is characterised by the monotelic type of inflorescence where the main axis, as well as side branches, are terminated by flowers (Foebe, 1971). The central main stem terminates in the primary, or first order umbel. Secondary lateral branches form in successive order from the apex and are terminated by second order umbels, Figure II.1. Sub-laterals are terminated by third order umbels and so on. The primary umbel is generally the largest with successive umbel orders decreasing in size (Hiller and Kelly, 1985). Tsvetkov (1970) refers to the primary umbel as the central umbel. Successive orders are then designated 1, 2 and so on. Such a classification system does not appear to be widely accepted in the literature. Consequently the terminology of primary, secondary etc. as shown in Figure II.1 will be utilized throughout this study.

The flowers are pollinated by unspecialized pollinators, hence fennel is referred to as a promiscuous plant. Self fertility is the norm. Although Bell (1954) stated that self sterile plants are rare, examples of male sterile plants have been discovered in more recent years (Desmarest pers. comms.).

The flowers are perfect, generally protandrous exhibiting exposed nectary and prominent stylpodium. Nectar secretion can be copious for a long period of time, even after stamens and petals shed, possibly to attract unspecialized pollinators to other orders.

The stylpodium often has a sticky surface. Varying quantities of pollen may adhere to its surface. Through this mechanism, there is a remote possibility that the stylpodium serve as a pollen reservoir. This is most likely to occurr in the genus Foeniculum, were flowers are strongly protandrous and stigmas are not developed until well after anthers are shed. The developing stigma then grows from, or through, the smooth surface of the stylpodium and the nectar film containing viable pollen (Bell, 1971).

1.2 Inflorescence structure

The Umbelliferae family contains a large number of herbaceous plants: annuals, biennials, and perennials. Many are of economic importance, such as carrots, parsnips, celery and parsley.

Umbelliferae is characterised by the type of structure of the inflorescence, a compound design with secondary divisions called umbellets. The fruit is schizocarpous, composed of two mericarps which cohere by their inner faces during maturation. These are borne on slender branched carpophores (Hayward, 1938).

The umbellets of the compound umbel develop centripetally hence the central umbellets mature last. Within each umbellet, flowers develop similarly with the peripheral inflorescences developing first (Jurica, 1922; Beghtel, 1925; Gupta, 1969), Figure II.2.

The different phases of reproductive development, flowering, seed set, and maturation, may all be represented at one time on a single plant. Such variation will ultimately affect the yield of essential oil from different umbel orders at any one time.

1.3 Seed Production

The nature of the growth habit of Umbelliferae presents a number of problems when grown for production of seed. Differences in the rate of development of the umbel orders described above results in uneven maturation of seed.

A study of the flowering and fruiting of carrots by Borthwick (1931), indicated that in any one plant anthesis proceeds as waves. Each wave corresponded to one umbel order. The succession of the development of the umbel orders resulted in anthesis occurring over a period of a month.

The nature of this anthesis pattern has important ramifications on fertilisation. As flowers of a primary umbel, and its compound umbellets, open in sequence from the outer whorl to the center, pollen from the protandrous outer flowers is supplied by the protandrous inner flowers. Pollen for the inner flowers is supplied by the outer flowers of the secondary umbel and so on (Bell, 1971). The flowers of the highest umbel order present are likely to remain unpollinated. This is usually the quarternary umbels, but depending on the rate of development this may be the inner tertiary flowers. The unpollinated flowers will abort as a result of a lack of pollen. In carrots, for

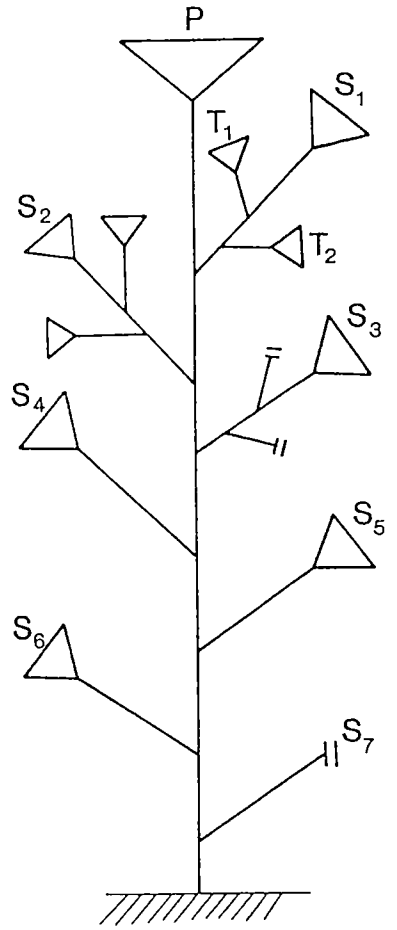


Figure II.1.
Schematic diagram of a carrot plant showing the general arrangement of umbels; P = primary order, $S_1 \ldots S_7$ = secondary order, and $T_{1\ etc}$ = tertiary order.

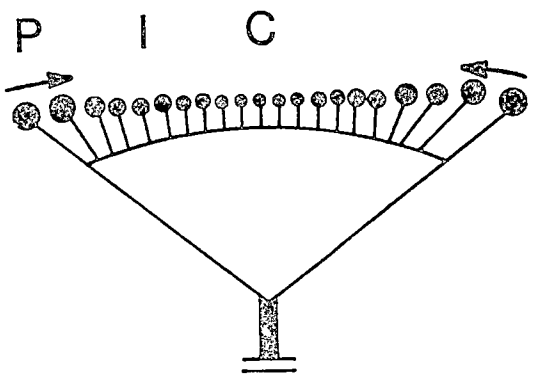


Figure II.2.
Schematic diagram of carrot umbel showing the flowering sequence and the category of umbellets; P = peripheral umbellets, I = intermediate umbellets and C = central umbellets.
(Taken from Hiller and Kelly, 1985)

example, only a few quaternary umbels are formed and the first three orders normally account for approximately 90 percent of the total seed (Hiller and Kelly, 1985).

As discussed previously there is also a spread of flowering within an umbel order due to the nature of the development of a compound umbel. This time of flowering may increase further within an umbel order. For example increased density of sowing of carrots results in a further spread of seed maturation rates (Gray and Steckel, 1985).

Hawthorn et al. (1962) examined the seed yield from the first three umbel orders. The results, as shown below, indicate the effect that the nature of the Umbelliferous growth habit has on the origins of the seed within the canopy. As the number of umbels increase with increasing umbel order, the weight of seed from each umbel decreases. The final contribution to overall seed yield from a plant is dependent on the number of umbels present and the seed weight per umbel. The results of this study indicated that the secondary umbels were the major seed producing units followed by the tertiary and then primary umbels.

	Number of Umbels	Yield/umbel (gm)	% of total Yield
Primary	1	3.4	13
Secondary	8	1.9	53
Tertiary	15	0.6	34

Shading or removing umbels before anthesis is one technique which is used to alter the supply of assimilates to the developing embryo-sacs. Such studies confirm the importance of assimilates in determining mature seed weight (Gray et al. 1986 a).

It is not clear how differences in ovule and embryo-sac volume influence the development of endosperm. The close correlation between endosperm cell number and final seed weight found in carrot (Gray and Ward, 1985) suggests that ovaries with large embryo-sacs and ovules provide the potential for a longer period of cell division. The importance of which is not the production of endosperm but the increase in oil yield due to concomitant development within the seed coat of secretory canals.

1.4 Varieties

Fennel was first classified in the genus Anethum, as Anethum foeniculum. Fennel was separated from this genus by Miller (1768). The basis for Millers separation is described by Jansen (1981). The major distinction involves the structure of the seeds: fennel seeds are thick, oblong and channelled whilst dill seeds are flat and bordered.

Three species of Foeniculum were distinguished:

1. F. vulgare - decompounded leaves, leaves small and short seeds.
2. F. dulce - decompounded leaves, small leaves and longer seeds.
3. F. azoricum- dwarf fennel with fleshy stalk and an annual root.

Later Thellung (1925) distinguished only one species (F.vulgare Mill.) with two sub-species:

1. piperitum - perennial, 0.5-2m height, leaf to be shorter than 20mm, leaf-sheaths 1-3 cm long, primary rays 4-12, fruit not sweet.
2. capillaceum - short living, leaf lobes 20-50 mm, leaf sheaths, 2-5 cm long, primary rays 12-25 mm.

A study of fennel grown in Turkey did not make any subdivision of F. vulgare (Hedge and Lamond, 1972). In Ethiopia, Jansen (1981) further stated that the variability observed was due to the environmental circumstances only. Finally Jansen suggested that the original division of Foeniculum and Anethum was not justifiable and that a return to Anethum foeniculum was required.

Even based on a limited morphological basis such declassification must be questionable. Consideration of the variation in the composition of the essential oil produced by fennel must also be taken into account. A chemotaxonomic examination of fennel and dill indicates two different species of plants. Differing genetic makeup between the two plants can be supported by the differences in production of secondary metabolites. The major chemical compounds of commercial significance in dill seed oil are carvone, followed by

limonene (Guenther, 1949; Chubbey and Dorrel, 1976; Embong et al. 1977a; Porter et al. 1983). Whilst in fennel, anethole and fenchone are the major compounds (Guenther, 1949; Toth, 1967;Betts, 1968; Embong et al. 1977b). Alpha-phellandrene and limonene are the only two other compounds present in the essential oil of fennel in large amounts. Some of the terpenes present in dill and fennel are similar but major differences in metabolic pathways must exist for the production of major secondary metabolites.

Most recently Demarest (1978) and Badoc (1988) recognized two sub-species, piperitum and capillaceum, in agreement with Thellung (1925). The major essential oil yielding varieties were classified under the sub-species capillaceum. The only major exception to this was the variety commonly referred to as bitter fennel.

Separation of bitter and sweet varieties on the basis of chemical composition has been suggested by Lawrence (1980). Sweet fennel is higher in anethole whist bitter fennel is higher in fenchone. The separation by composition may not be clear cut as variation may occur. For example, fenchone contents of sweet and bitter oils may be low and anethole high (Clark and Menary, 1983). Therefore, such a basis of varietal distinction does not appear conclusive as these components may vary within any one population for a number of reasons. The composition of an oil may be affected by a number of factors. For example, harvest date as well as extraction techniques, where temperature and duration of distillation are important factors, may affect the balance of the components present in a resultant oil (Peterson, 1984; Koedam et al. 1979).

A further point in regard to fennel taxonomy was made by Betts (1976), who found an anethole free variety which was high in estragole (methylchavicol). Such a variety has been reported in Tasmania which also exhibits a dwarf habit (Clark pers comms). The mixture of features exhibited by this particular variety further complicates any clear distinction between the species.

In addition to these naturally occurring varieties, a recurrent selection programme has been undertaken by Demarest (1978) to produce a number of commercial varieties. Two of the resultant phenotypes were used in this study. Similar programmes have been undertaken in India on a range of Umbelliferous plants (Hore, 1979). The aim of these breeding programmes has been to increase seed yield, essential oil content and quality, pest and disease resistance.

2. Fennel as an essential oil crop

2.1 Oil yield

A direct comparison of oil yields from fennel cultivated in different parts of the world is difficult due to the method of expression of results. It is not always clear whether oil extracted is expressed as percent of dry or fresh weight of material extracted.

Embong et al. (1977b) obtained values of 1.2 percent from an extraction of herb, leaf and stem material of fennel. Whilst only 1.5 percent was obtained from seeds on a dry weight basis. Ashraf and Bhatty (1975) analysed an Indian bitter variety and obtained 2 to 2.5 percent from seed and 0.3 to 0.5 percent from leaf and stem. In Bulgeria, Karlsen et al. (1969), obtained yields varying from 2.9 to 5.8 percent from seed of bitter fennel. These results demonstrate the great variability that may be encountered within the commercial varieties. One major factor contributing to the oil yield obtained is the duration of the steam extraction. Very few workers indicate the length of their extraction procedures.

The major parameters which alter commercial oil yield are oil content of seed and herb and the amount of plant material per unit area. Some yield determinations have indicated very low yields. Randhawa et al. (1981) recorded a highest yield of 13.9 kg of oil per ha and a lowest of 0.57 kg of oil per ha. The low yields were explained by low yield of seed per unit area. Raev et al. (1980) claimed yields of 700 to 800 kg of oil per ha from all the above ground portions of the plant, such an exceptionally high result must be questionable.

Dill grown for essential oil production in New Zealand produced 30.4 to 84 kg/ha depending on variety (Chubbey and Dorrell, 1976). The size of the umbels was the major factor producing the variation in yield of oil per unit area. Porter et al. (1983) examined dill oil production in more detail. They demonstrated the importance of the contribution of the umbels to the total oil yield. From all aerial parts of the plant, the primary and secondary umbels were the major oil producing structures throughout the development of the crop.

	Percentage of total oil yield	
	At flowering	At harvest
Primary	36.3	60.9
Secondary	25.0	33.5
Stems	19.8	4.1
Leaves	18.8	1.7

The total oil yield from this work varied between 60 and 80 kg/ha.

Other essential oil bearing plants in the family Labiatae, for example peppermint, have oil evenly distributed throughout the plant. Optimal yields are associated with maximum leaf development (Clark and Menary, 1979). For the Umbelliferae, for example caraway, dill, parsley and fennel, oil accumulation appears mostly in the umbels. Caraway seed yields on average 4 percent oil. This represented a total yield of 40 kg/ha (Guenther, 1949). Again only a small percent is obtained from the vegetative parts of the plant. This yield distribution may be explained by the distibution and morphology of the oil bearing structures within the plant. In the family Labiatae glands are carried externally on the surface of leaves while the Umbelliferae has internal glands or ducts. These structures are present throughout the plant but are most frequent in the seed. These will be discussed in more detail in following sections. Demarest (1978) indicated that the distribution of glands determined the distribution of oil within the plant. Approximately 60 percent of the total oil yield originated in the seed, 15 percent from the rays, whilst the remaining 25 percent originated from the herbaceous parts of the plant. No data was presented to circumstantiate such distribution figures in the fennel plant, nor was there any indication of the likely changes in oil yield distribution during maturation of the fennel plant.

The maximum oil yield for Umbelliferous essential oil crops is difficult to determine due to the sequential nature of inflorescence development. This means that different umbel orders mature at different rates with resultant effects on dry matter yield and oil content.

The percent oil yields obtained by Embong et al. (1977b) were recognized as low. The major contibuting factor was the stage of maturity at harvest. Harvesting was performed when the majority of the umbels were green. This stage was much earlier than the stages

of maximum oil yield indicated by other workers. For instance the stage of 'wax ripeness' of the primary umbel referred to by Kapelev. (1980) Raev et al. (1980) is one example of a simple visual assessment technique utilized to determine the period of maximum yield. The stage of optimal yield was shown by Tvetkov (1970) to be dependent on the variety. The determination of the period of maximum oil yield from annual fennel was based on primary umbel maturity. Whilst maximum oil yield from perennial fennel was based on maturity of 70 to 75 percent of all plant umbels.

Such methods for maximum oil yield determination may not be reliable. Changes in the exterior appearance of the seeds may result from other environmental stimuli. These may not necessarily affect the oil production, but may provide a false indication of the oil yield. To achieve optimum oil yields in fennel further investigation into the factors affecting seed production and maturation as well as changes in oil yield are necessary.

Tsvetkov (1970) demonstrated that the percent oil yield from the first three orders of umbels from annual fennel were similar. A range of 2.53 to 2.12 percent was detected, whilst the oil yield from perennial fennel decreased from 3.69 percent in the primary umbel to 2.26 percent in the tertiary umbel. These results were obtained from seed which was analysed 2 to 2.5 months after harvest. Consequently the extractions do not indicate the actual oil yields at time of harvest. In addition, as no statistical data was presented, these results may only be used as a rough indication of the percent oil yield expectations from the various umbel orders.

Tsvetkov (1970) also stated that the period of maximum oil yield was related to a particular developmental stage of the plant. Such a statement was made without any determination of the change in oil yield in relation to plant development.

2.2 Oil Composition

The main compound of commercial interest produced by fennel is trans-anethole.

This compound is commonly associated with the taste of aniseed due to the high proportions found in oil from star anise (Illicium verum), the original source of aniseed flavouring. Anethole is also found in Pimpinella anisum oil, ranging from a level of 70 percent

(Nofal, 1982) to as high as 90 percent of the total oil yield (Clark and Hart, pers. comms.).

Both fennel and anise are reputed to have estrogenic activity (Albert-Puleo, 1980), possibly due to formation of polymers of anethole acting as pharmacologically active agents; di-anethole and photo-anethole. These two compounds have been described by Guenther (1949) as decomposition products of anethole and are hence readily formed.

Oxidation products and other artefacts are often present in oil samples. Autoxidation of anethole produces mainly anisaldehyde and acetylaldehyde which arise from cleavage of the double bond. The presence of these compounds can be used to indicate the age of the oil. High temperatures during storage, especially for long durations, may result in increased cis-anethole content (Kraus and Hammerschmidt, 1980), a very undesirable compound. Typical levels are 0.2 to 0.3 percent but may be as high as 8.5 percent.

The oil yield and anethole content are generally combined to determine the amount of anethole produced per unit area. Such determinations have been used by Desmarest (1978) to select for high anethole yielding varieties of fennel. Initial experiments obtained variations from 21 to 121.7 kg of anethole per hectare. The highest yielding variety originated from Germany and has since formed the basis of a breeding programme which has yielded the commercial varieties utilizied in this study.

Although anethole is the major compound of interest in fennel it is necessary to give consideration to other oil components. In particular, commercial quality oil requires a low fenchone content. This compound is a ketone of intense bitter camphor-like flavour (Guenther, 1949).

Anethole and fenchone plus estragol, limonene and alpha-phellandrene have been used as the elements for chemotaxonomy of fennel varieties. Desmarest (1978) stated that the difference between sweet and bitter fennel was a lower content of anethole and higher content of fenchone and estragole in the former. Clark and Menary (1983) differentiated sweet from bitter fennel by examination of the levels of alpha-phellandrene and limonene in the resultant oil from extractions of whole plants. Alpha-phellandrene was present as 13 percent of the total oil from bitter fennel and less than 1 percent of the oil from sweet fennel. Limonene content was minimal in oil from

bitter fennel whilst oil from sweet fennel contained as much as 30 percent.

Such absolute levels need only be used as a guide for determination of a variety of fennel. The origin of the oil from various parts of a plant may produce variations in oil composition. For example the composition of the oil extracted from seed alone may vary markedly in composition to oil from herbaceous portions of the plant canopy. Embong et al. (1977b) examined oil from both the seed and herbaceaous portions of sweet fennel. Anethole content was 30 percent higher in oil obtained from the seed whilst the herb oil was higher in both limonene and fenchone.

Variation in oil composition may also occur within the various umbel orders. Tsvetkov (1970) determined the composition of oil from primary, secondary and tertiary umbels in annual and perennial fennel grown in Bulgaria. The oil from the higher order umbels of annual fennel was found to contain less anethole than the lower order umbels. The levels of estragole and fenchone increased in the higher order umbels. In perennial fennel the reverse situation existed. Such variation possibly reflects the change in composition of the oil during the maturation of any single umbel order in any particular variety. No detailed study has been made of the change in oil composition of the various umbel orders. Examination of the changes may indicate biosynthetic pathways involved in the synthesis of the oil components by suggesting which compounds are precursors.

Some examination of the change in oil composition has been performed in dill. These observations may be utilized to model the possible changes in oil composition from fennel. The terpenes alpha-pinene, limonene and phellandrene decrease with maturity in dill seed oil whilst carvone, the major compound, increases with maturation (El-Gengaihi and Hornok, 1978). Porter et al. (1983) detected a decline in alpha-phellandrene in all parts of the plant except the leaves whilst limonene levels remained reasonably stable. The carvone content increased mainly in the umbels and coincided with the onset of senescence. This is in agreement with the observations of Hornok (1973) who suggested carvone levels in dill herb oil are related to developmental stage rather than crop age.

Carvone is an oxygenated compound, similar in structure to anethole. Both compounds are members of the phenyl-propanoid group. These compounds appear to be produced at the expense of the terpenes.

Such changes are in accordance with those observed in Eucalyptus (Penfold, 1950) and Ajowa (Balba et al. 1973). A detailed study of the compositional changes of the oil from the plant as a whole as well as from the various umbel orders would enable further understanding of the processes of anethole production. In addition, the yield of anethole per unit area may be increased through the determination of the anethole rich yielding umbel orders in the canopy of the crop.

2.3 Gland Morphology and Distribution

The volatile oil components are accumulated within glandular structures (Desmarest 1978). These take the form of canals present in all parts of the plant. Metcalf and Chalk (1950) reported that the secretory canals are present to varying degrees in all Umbelliferae. Secretory canals differ from secretory cells, such as in the Labiatae, in that they are intercellular spaces resulting from either dissolution of cells (lysigenous spaces) or by a separation of cells (shizogenous spaces). Lysigenous spaces are found in Citrus, Eucalyptus and Gossypium whilst shizogenous spaces are common in the Compositae and are the most common form in the Umbelliferae (Esau, 1965).

Shizogenous splitting results in the formation of a duct. This process commences as three cells which, after successive divisions, produce several epithelial cells which are densely cytoplasmic with large nuclei. These cells secrete ethereal oil which accumulates in the glandular canal (Hayward, 1938). The glandular canals are continuous in most parts of the plant.

Kadry et al. (1978) examined the distribution and morphology of glands in fennel. Secretory canals were present in the roots, similar canals but larger in diameter were found in the stems. Leaf petioles contained wide glandular canals on the epidermal side of each vascular bundle. In the leaf blade the canals were associated with the phloem side of the vascular bundles.

Within the stem, glandular canals were centrally located in the medullary bundles, with the phloem arranged in concentric layers around the duct. The glandular canals in the leaf blade were surrounded by three vascular bundles as were the canals in the peduncle. The only region devoid of glandular canals is the medullary regions of lateral branches of the peduncle.

The morphology and distribution of glandular canals in the

inflorescence depends on their location. For example, in the basal region of the pedicel, three glandular canals are present in the outer parenchyma. Whilst in the apical sections of the pedicel five glands are present. Within the ovary wall twelve glandular canals are present, six per carpel, on the internal side of each vascular bundle. Importantly, no connection was observed between the pedicel and the glandular canals of the ovary wall at any stage of development (Peterson, 1984). Thus no transfer of oil could occur between these structures. The production of secondary metabolites must occur *in situ*.

On top of the ovary exists a glandular disc, a mass of glandular tissue covered by a glandular epidermal layer. This layer has been observed to be covered with stomata which are believed to be responsible for the nectar secretions referred to in Section II 1.1. These stomata are refered to as nectar-stoma by Fritsch and Salisbury (1955).

The size and distribution of glandular canals described by Kadry *et al*. (1978) correlates well with the distribution of oil within the various plant structures. The ovary walls contain the largest number of canals as well as the largest in diameter, and as already indicated the seed is the best oil yielding portion on the plant. The question arises as to the importance of photosynthates to the developing seed as metabolites for oil synthesis. If the oil is accumulated *in situ* in canals and these are most frequent in the seed then the availability of assimilates to the developing umbel will control the potential oil yield.

2.4 Oil Synthesis

The diversity of compounds produced as secondary plant products and the complexity of the biosynthetic pathways for the derivation of such compounds is beyond the scope of this review. Only information available on the possible biosynthesis of anethole will be presented.

Anethole is classified as a phenylpropene, a member of the phenylpropenoid group. This group of phenolic compounds are divided into 5 subgroups dependent on the hydroxylation or methylation pattern of the benzene ring (Harborne, 1979). Anethole is the simplest member of this group, and was first reported in *Pimpinella anisum* by Dumas as long ago as 1833. This compound was originally referred to as Aniskamfer.

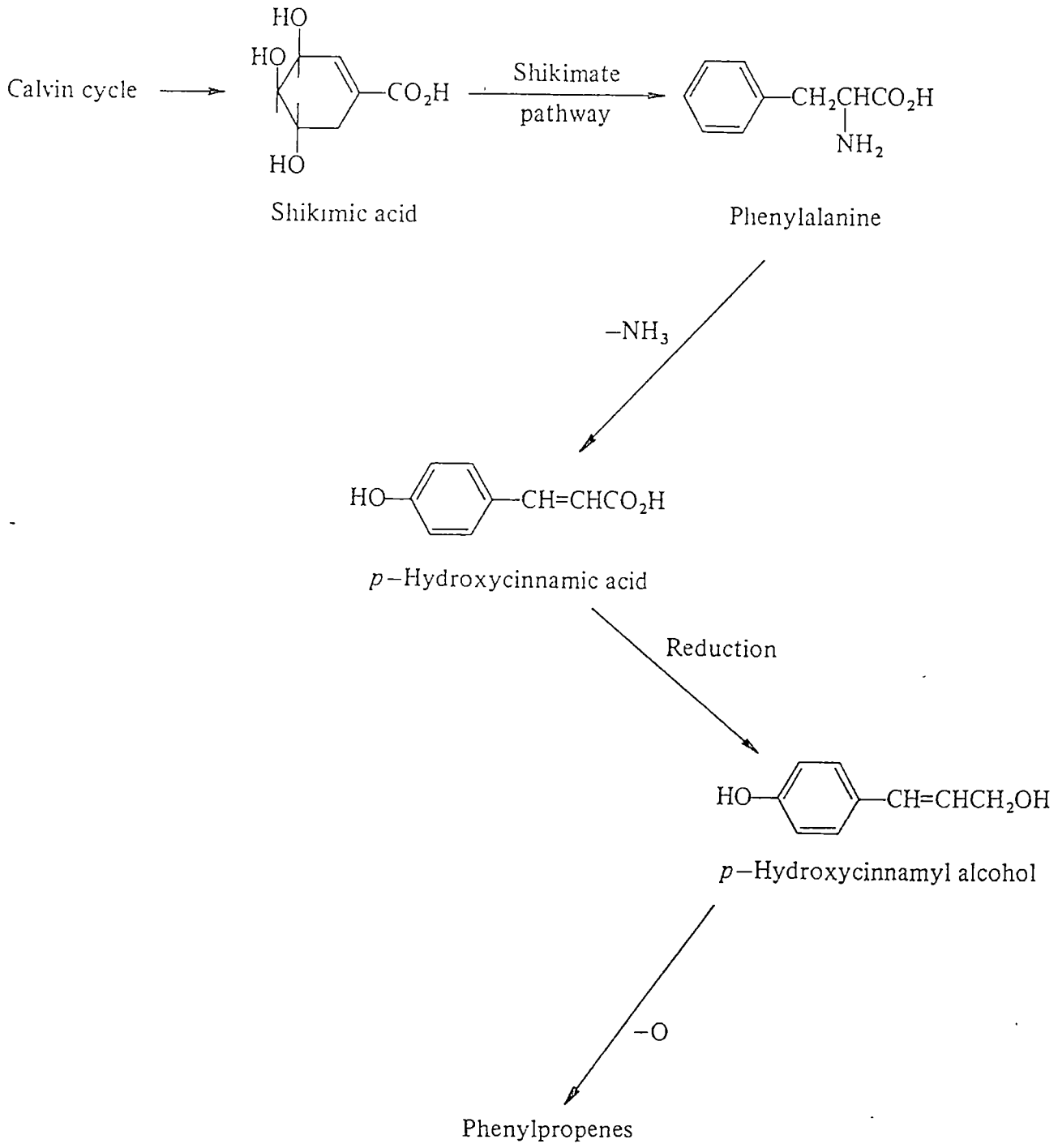


Figure II.3.

Biosynthetic origin of the phenylpropenes from shikimate and phenylalanine.

Taken from Harbourne (1979).

The biosynthetic origins of phenylpropenes is indicated in Figure II.3. All plant phenolics originate through shikimate and phenylalanine from the Calvin Cycle. The biosynthesis of these compounds is very closely related to the production of lignins. By o-hydroxylation and subsequent cyclisation, p-hydroxycinnamic acid (p-coumaric acid) can give rise to hydroxycoumarins. On reduction, it yields p-coumaryl alcohol, which is one of the monomeric 'building blocks' of the secondary cell wall polymer lignin. Dehydrogenation of this alcohol produces a polypropene (Harborne, 1980).

The other major group of compounds present in fennel are the terpenes. The biosynthesis of the terpenoid compounds are well documented. The major building block for terpenes, either mono-, hemi-, sesqui-terpenes etc. is active isoprene units. These are produced from sucrose through mevalonic acid (Croteau and Loomis, 1980).

As a result, the factors affecting essential oil biosynthesis will be the availability of sucrose, acetyl-CoA, ATP and NADH. The physiological source of these compounds is presumably sugar phosphate metabolism hence the overall limiting factor will be the availability of carbohydrate. For the production of secondary metabolites *in situ*, the question arises, what are the major limitations to oil synthesis ? Such limitations may be either, availability of precursors from other parts of the plant or, a basic relation in respect to the availability of photosynthate.

Clark and Menary (1980) indicated that the balance between the production and utilization of photosynthates was important in the determination of the composition of peppermint oil. The balance between the daytime accumulation and the night time utilization of photosynthate determines the composition of terpenes. The factors affecting the levels of photosynthate are the daylength, photon flux density and temperature, particularly night temperatures. That is the factors which affect the carbon dioxide fixation and respiration components within the plant. Any examination of oil composition and explanation of changes in oil composition should then be accompanied by a detailed study of the photosynthesis and respiration levels present in the particular plant part. Such determinations have not been performed on fennel.

2.5 Importance of Some Cultural Requirements

2.5.1 Plant Density

Plant spacing may affect oil yield by changing the floral canopy size and number of inflorescences present. Generally as the population density increases plants have to share the resources available. The yield increases at a slower rate than the plant density, until a point is reached at which there is little or no increase in yield. This is referred to as an asymptotic relationship and has been readily demonstrated in carrots, radishes (Bleasdale, 1967) and onions (Frappell, 1973). In some other crops, such as red beet (Frappell, 1968) and parsnips (Bleasdale and Thompson, 1966) the yield rises to a maximum with increasing plant density and then declines at higher densities. This is referred to as the parabolic relationship.

Although a plant may exhibit an overall asymptotic yield density relationship, within the yielding structures of the canopy, parabolic relationships may occur, and vice versa.

El-Genghaihi and Abdallah (1978) promoted taller fennel plants when experimenting with various plant densities. A density of 4 plants per m^2 for a bitter variety increased plant height as well as increased seed yield per plant. Thereby indicating that plant height may also be controlled by plant spacing. In contrast, Hawthorn (1952) examined row spacing effects on carrot seed production and no consistent over-all effect on the plant height or lodging was determined. As a result of competition between plants due to increased plant density, plant height was affected by competition for soil moisture. Another effect of interest was a marked change in the foliage colour to a lighter green as density increased. Increased competition for nutrients was proposed as the main cause of this effect. Other factors not discussed which could also have produced this effect are increased competition for incident radiation and the effects on the amount of chlorophyll present in the plant.

In carrot seed production increased plant density increased the spread of flowering time of the primary umbels (Gray and Steckel, 1985). Such an effect in fennel may result in a possible decrease in maximum oil yield by further spread of maturation time.

Studies of various population densities of both bitter and sweet fennel in France (Demarest, 1978) has indicated that a range of 6 to

24 plants per m^2 had no significant effect on oil yield per hectare or anethole content. A density of 10 plants per m^2 has been adopted in France for commercial production of fennel (Demarest, 1978) and also for commercial areas cultivated in Tasmania (Clark pers comms). The ability of fennel to compensate yield over a range of plant densities has proved useful in commercial applications. For example irregularities in seed germination have been experienced in the field in Tasmania. This has produced variations to predicted densities.

2.5.2 Seed Germination

In general, seed of the Umbelliferae require a long germination time due to the relatively small embryo present within a large seed. This embryo grows initially within the seed utilizing the stored endosperm. Root emergence occurs when the embryo is about 0.5 to 0.75 times the length of the seed (Jacobsen, 1983).

Germination is affected by a variety of factors: temperature, seed size, leaching and drying techniques. Putievsky (1980) examined these factors in coriander, caraway and dill seeds. Optimum temperatures varied with each species, caraway preferring lower temperatures. Pre-treatment by leaching improved caraway seed germination by 50 percent as well as decreasing the time to germination. Such results indicate the presence of some dormancy factor which may be removed by leaching.

Similarly parsley seeds are reputed to germinate slowly. Chaturvedi and Muralia (1976) indicated this may be due to a dormancy mechanism such as an inhibitor substance present in the seed. Osmotic pre-treatment, priming, by Ely and Heydecker (1981) using poly-ethylene glycol solutions reduced the time to radicle emergence at 15°C from one week to one or two days. Care had to be taken not to over-prime the seeds, any treatment beyond one week was likely to remove the inhibitor resulting in germination during priming.

Borthwick (1931) noted that the time to germination in carrots appeared to be related to embryo size, the smaller the embryo the longer the germination. More recently, larger seed has been shown to give better crop establishment and higher yields (Austin and Longden, 1967).

The nature of floral development in fennel where anthesis occurs at different times for each order, as well as within each umbel order, has profound effects on the age of seeds at harvest and hence the

final germination. Hawthorn et al. (1962) examined the viability of carrot seeds as affected by the position of umbel and time of harvest. Generally the lower the umbel order, the lower the germination. However, for maximum yield of seed secondary umbel maturity should be the prime guide to determining harvest as 50 percent of the seed consistently results from this order.

2.5.3 Sowing Date

Randhawa et al. (1981) examined sowing dates for fennel. Delayed sowing decreased the number of branches and umbels per plant. A delay of two months decreased the number of umbels per plant from 19.9 to 10.1. These results were suggested to be mainly due to increased time of vegetative growth by earlier sowing. Similarly El-Genghaihi and Hornok (1978) produced taller plants and a greater number of compound umbels with earlier sowing whilst there was no effect on percent oil yield. Consequently any changes in oil yield per unit area may result from change in the number of umbels.

The effect of sowing date varies greatly depending on the variety in question. In France bitter fennel has a vegetative cycle of 30 to 35 weeks in the first year of growth. After the first year and in all subsequent years the vegetative cycle is approximately 10 weeks shorter. In sweet fennel, umbel formation intervenes after 20 to 22 weeks of growth (Demarest, 1978). The correct time to sow will mainly be determined by the expected weather conditions at the time of harvest. Earlier spring sowing will lessen the likelihood of inclement weather at harvest in the following autumn. The prevailing weather may affect harvest and subsequently result in an inability to harvest at the time of maximum oil yield for the crop. Certainly when sowing fennel with a view to perennial production, the importance of sowing date on plant performance needs only be taken into account in the first year. The more rapid time to umbel development in the second year means that the fennel crop will achieve maximum oil yield at an earlier date than a first year crop.

3. Growth and Differentiation

3.1 Juvenility

During early stage of development most plants are totally insensitive to inductive conditions, growing vegetatively for some time. This juvenile phase has been observed in some herbaceous annuals and biennials where the duration may vary from days to several months. In many plants, sensitivity to inductive conditions increases with increasing age. The completion of this early vegetative growth is the attainment of the condition of ripeness-to-flower.

Holdsworth (1955) proposed the concept of minimum leaf number to describe the measurement of the juvenile phase. The minimum leaf number in many plants has been shown not to be an irreducible minima. Rather the minimum leaf number is the lowest number of leaves produced under optimal conditions for flowering. To determine more exactly the minimum leaf number, treatments involving other factors such as nutritional stress have been utilized (Bernier et al. 1981)

Juvenility has been shown to be almost inseparable from 'earliness', a genetically controlled characteristic. Reid and Murfet (1977) have shown that the node at which flowering occurs in peas appears to be a gene controlled effect. The control is mediated through the ratio of a floral promoter to a floral inhibitor. Flowering requires this ratio to rise above a certain base threshold level.

Juvenility has been observed in cold requiring Umbelliferae such as carrots. Atherton et al. (1983) observed two cultivars of carrots to be insensitive to vernalization until the 7.5 leaf stage. No literature was available to indicate the presence or absence of such a juvenile response in fennel.

Other explanations for juvenility have been based on the physiology of the leaves, (Bernier et al. 1981). Insufficient leaf area has been proposed as the mediator of this response. However the only information available for the Umbelliferae family is for dill where only one fully expanded leaf is sufficient to allow induction (Naylor, 1941). Another explanation for the juvenile phase is an unfavourable ratio of immature to mature leaves. Immature leaves may act by producing floral inhibitors or by interfering with assimilate

translocation and floral stimuli from the lower mature leaves to the meristem (Bernier et al. 1981). Insensitivity of the leaves to daylength is yet another proposal whereby in many plants the cotyledons and the first-formed leaves are incapable of responding to photoperiod. For example, flowering results from the application of GA_3 to the juvenile leaves of the long day plant, Bryophyllum when held in short day conditions. Such a result suggests that juvenile leaves produce insufficient GA following the change from long day to short day conditions (Zeevart, 1969).

Consequently many internal factors may be involved prior to the plant reaching a receptive stage. Before any major photoperiodic determinations are carried out on fennel, first a study of the juvenility exhibited by the plant must be undertaken.

3.2 Stem Elongation

Extension of internodes is considered by many workers as the earliest sign of generative growth. Rapid stem elongation or bolting, is most obvious in plants possessing a rosette habit of growth at the vegetative stage. In most cases stem elongation begins before the formation of any reproductive structure (Bernier et al. 1981)

Although stem elongation and flower initiation are usually associated processes, they can be separated in many plants. This may be achieved in several cold requiring biennials such as brussel sprouts where two different optimal temperatures exist for each process. Separation of elongation and flowering has been achieved in Rudbeckia, a long day plant. When grown in short days after an inductive long day photoperiod, flowers result with the absence of bolting (Murneek, 1940). Seedstalk height in carrots can be reduced without affecting flowering by increasing post vernalization temperature (Hiller and Kelly, 1979). The effect of sowing date on flowerstalk length and flower bud differentiation in carrots was examined by Kurata (1982). Early sowing produced a greater number of flower stalks than late sowing but their development was less rapid. Vernilization appears obligatory for stem elongation in celery but not for differentiation of the inflorescence (Hanisova and Krekule, 1975). It is possible that the elongation and flowering responses in fennel may also be separated. Limited literature is available on the environmental conditions which affect flowering in fennel. Once the

conditions which induce these responses are known then more detailed investigation into the individual responses of stem elongation and flowering may be made. The most promising method of examination is the application of plant growth regulating substances.

Stem elongation has been widely studied and all five major catagories of growth hormones have been implicated. Unfortunately most of the literature relies on individual hormonal effects without sufficient consideration to the interaction of hormones in respect of stem elongation. By far the most studied are the gibberellins (GA's).

The use of growth retardents which suppress vegetative growth do not prevent flower initiation in numerous species. Conversely application of GA_3 can cause stem elongation in a variety of plants without resultant flower initiation. At this stage it is not clear whether fennel could be catagorized into this group. Literature involving the application of GA's on fennel is covered in the later sections on growth retardants, but most studies examine only the affect on seed production without any detailed examination of the elongation and flowering responses.

3.3 Transition to floral development

Differentiation of the meristematic zone into floral primordia occurs after some stem elongation. This observation is typical of the Umbelliferae eg. carrots (Borthwick et al. 1931; Hiller et al. 1979).

The literature on Umbelliferae does not contain a detailed description of the transition of the vegetative apex into a floral apex. The best account is given by Hayward (1938) who examined floral development in celery. Haywood observed floral primordia arising from the meristem as club-shaped stuctures followed by differentiation of petals, stamens and carpels which occurred in an acropetal succession. Borthwick (1931) observed that carrots showed a flattening of the apex and around the periphery and primordia of involucral bracts appeared as small protuberances. These enlarged and were followed centripetally by umbellet primordia. Sepals, petals and stamen primordia appeared almost simultaneously, only their rates of differentiation varied. These were then followed by carpel primordia. The division of the floral apex in fennel requires elucidation. The technology available today through the scanning

electron microscope, enables the determination of such processes in great detail.

The scanning electron microscope is now frequently used to study floral ontogeny as it provides a three-dimensional image which allows a more detailed view of developmental patterns. For example in Brassica, Polowick and Sawhney (1986) determined the initiation and developmental patterns of the floral organs in great detail, as well as detailed studies of each floral structure. Such detailed study would not be possible using a light microscopy.

Scanning electron microscopy is not without its problems, many artifacts can be produced by both freeze drying and critical point drying techniques. As well, changes due to the effect of high voltages can result in misinterpretation of the surface structure (Eveling, 1984).

The time at which the transition from vegetative to floral occurs in caraway has been studied by Chladek (1972) in Czechoslovakia, where 80 to 90 percent of the plants showed transition on the 10th of October for one cultivar and the 29th of October for another. At these times the heights of the apices were 359 mm and 438 mm respectively. The duration of differentiation of the inflorescences in the field lasted 7 to 15 days.

The change from vegetative to floral primordia in caraway was observed by Novak (1974) to occur when the thirteenth or fourteenth node was initiated at the meristem. Such data is not available for fennel but because of the similarities observed in growth habit between this plant and caraway, comparable results may be expected from any determination of the floral transition.

4. Environmental effects on flowering and photosynthesis

4.1 Photoperiod

Daylength is one of the major environmental factors controlling flower initiation in many plants. Response to daylength is divided into two main groups, (i) short-day plants which flower if given light periods alternating with dark periods in which the light period is shorter than a critical length, and (ii) long-day plants which flower in light periods greater than a critical daylength. Short and long-day plants are not separated on the basis of their requirements of different absolute length of illumination but rather on whether

these light exposures are longer or shorter than a critical period (Wareing and Phillips, 1981). Species in which daylength does not markedly affect flowering are known as day neutral plants.

In addition to these rather distinct types of responses, there are plants which show still different behaviour to photoperiods. These do not necessarily follow broad taxonomic lines. The behaviour may not be the same even within a given species, but rather may occur differently among different varieties or lines.

The photoperiodic response of plants may also be altered profoundly by temperature, photon flux density or atmospheric conditions. Thus classification of a plant in one or other response group is only valid under a given set of conditions. In many plants response is dependent on both the length of the light application and the total energy delivered. Photon flux density (PFD) may or may not play an important role in the photoperiodic responses of a plant. The literature available detailing the photoperiodic classification of a plant is sometimes lacking in the specification of these parameters under which the experimentation has been made.

Photoperiodic sensitivity is a property of all plant parts, but as a rule, only leaves need to be exposed to a favourable daylength to achieve floral induction (Hamner and Naylor, 1939). Generally once flowering has been initiated the floral primordium will be formed regardless of whether or not the plant is kept under promotive conditions, hence exhibiting a marked all or none character (Bernier et al. 1981). However continued inductive conditions may result in acceleration of the rate of development of the primordium and initiation of more inflorescences (Lang, 1965).

Kinet et al. (1985) further segregated plants into five groups based on the photoperiodic requirements for the complete flowering process.

Group 1. Plants with the same photoperiodic requirement for initiation and flower development.

Group 2. Plants with a photoperiodic requirement for initiation but day neutral for flower development.

Group 3. Plants which are day neutral for initiation but with photoperiodic requirement for development.

Group 4. Plants with different photoperiodic requirements for initiation and flower development.

Group 5. Plants which are day neutral for both initiation and development.

Plants in group five are usually influenced solely by temperature or PFD.

Classification of fennel into the groupings above has not been performed. Shimada (1959) indicated that fennel was a long day plant, but did not determine the conditions required for floral development.

The sensitivity of leaves to photo-induction varies with physiological age, generally maximum sensitivity coincides with fully expanded leaves. Also position of the leaf on the stem may affect the inductive capacity. Variation in sensitivity to daylength can be found in the number of photoperiodic cycles required to induce flowering. Xanthium requires only one short day cycle to initiate flowering, however the majority of photoperiodic plants require more than one cycle (Bernier et al. 1981).

Some plants will remain permanently vegetative if kept under non-inductive conditions and are generally refered to as obligate photoperiodic plants. The short day plant Xanthium pennsylvanii and the long day plant Hyoscyamus niger respond in such a manner (Lang, 1965). Bernier et al. (1981) disagree with the concept that some plants may be maintained in a vegetative state for years, unless steps are taken to remove older mature shoots continuously. These workers suggest that many plants will ultimately flower under non-inductive conditions. Thereby indicating that they are not in a complete vegetative state, but are progressing slowly towards the reproductive state.

In plants where flowering is under strict photoperiodic control there is abundant evidence that a flowering stimulus is formed in the leaves under photoinductive conditions and exported to stem apices triggering the process of evocation, leading to flower formation (Vince-Prue, 1975). In some cases factors coming from other parts of the plant such as the roots, may also influence evocation (Miginiac, 1978). The area of such hormonal regulation of flowering will be discussed in later sections.

4.2 Photoperiodism in Umbelliferae

Knowledge of the floral initiation and elongation processes controlling the growth habit of the Umbelliferae is limited. The majority of economically important plants within this family respond to vernilization. Consequently photoperiodic information on other members of this family is limited particularly when compared with other families which contain a large number of economically important photoperiodic plants.

Studies of other Umbelliferae have been instrumental in the determination of vernilisation and photoperiod phenomenon in plants, particularly dill and carrots, but the variety of responses observed within this family do not allow any possible prediction of how fennel may respond to varying environmental conditions.

The long day response in dill is easily quantified in terms of both stem elongation and flower initiation. Floral transition and stem elongation occurs at a critical photoperiod between 10 and 14 hours of light (Naylor 1941). Under 18 to 19 hours plants flower even when only 3 to 4 expanded leaves are present (Hamner and Naylor, 1939).

Putievsky (1983) examined responses of caraway, dill and coriander to variations in daylength and temperature. In all three species the time to flowering was reduced by long-day treatments. But plant weight and most other morphological characters measured, except plant height, were reduced as a result of earlier flowering. To achieve high seed yields caraway required short-days, whilst dill required long-days. Coriander on the other hand was not affected by daylength.

Hiller and Kelly (1979) examined seedstalk elongation and flowering in carrots, they showed that photoperiod did not affect the percentage of plants that flowered. Dickson and Peterson (1958) obtained flowering under short-days of winter in the greenhouse whilst Imden (1950) reported that long day treatments accelerated seedstalk elongation but were not essential for complete floral expression, rather vernilisation temperatures and post vernilisation temperatures control elongation and flowering.

Celery is a short-day-long-day plant with a vernilisation requirement. Long-days or night break during vernilisation reduces, while after vernilisation long-days promotes, elongation and flowering (Pressman and Negbi, 1980).

Mol (1981) subjected Florence fennel seedlings to different daylengths of 16.5, 13.5 and 12 hours. A decrease in the percentage of 'bolters' as daylength decreased was achieved, with no clear critical daylength period. The 16.5 hour daylength resulted in 77 percent elongating whilst the 12 hour photoperiod produced 57 percent. Certainly it would have been expected that at least one of the treatments should have produced virtually 100 percent bolting, but this was not observed or explained fully. One possible explanation is that some of the plants required daylenghts in excess of 16.5 hours.

In addition Mol (1981) also included treatments which examined the effect of varying inductive cycles at these particular daylengths. Treaments of 5, 10 and 15 cycles were applied but did not appreciably affect elongation and flowering responses, stating that 5 to 7 days was considered suitable induction. In dill, only 4 days of inductive conditions are necessary for induction, (Hamner and Naylor, 1939).

From the literature it is not possible to determine the photoperiod and number of inductive cycles necessary for the initiation of flowers in fennel. Determination of the photoperiodic conditions required for stem elongation and flowering is necessary for the variety of fennel under cultivation in Tasmania.

4.3 Light interception and utilization

The dry matter yields of many crops appear to be proportional to their interception of radiant energy. Examples well documented are, cereals (Gallagher and Briscoe, 1978), sugar beet (Monteith 1977) and oil seed rape (Mendham et al. 1984). These studies all involved annual plants. Although fennel is a perennial plant the nature of its growth pattern and habit may be considered on an annual basis. Consequently comparisons to annual plants may be justified.

Such crops as oil seed rape and sugar beet are generally slow to attain maximum light interception because of delayed leaf emergence and slow leaf growth in spring. Fennel certainly follows similar growth patterns in the first year of growth but in subsequent years leaf growth is already well advanced in spring. Maximum light interception would then be expected to occur at an earlier stage in the season.

In annual crops the measurement of the leaf area index is used to determine the photosynthetic efficiency by using it in conjunction

with measurement of incident radiation (Monteith 1977). A general equation has been developed describing the penetration of light through a canopy:

$$\frac{\text{Light penetrating}}{\text{Incident light energy}} = e^{-KL}$$

where l = The leaf area index of the canopy
k = The extinction coefficient for visible radiation

Light interception is therefore a logarithmic function of the leaf area index as the light intensity declines logarithmically from the top of the canopy to the ground. This classical light interception model may be inappropriate if the leaves are not the sole photosynthetic tissue supplying developing seed. Allen et al. (1971) examined the relative importance of leaves of rapeseed. They suggested that the pods produce the photosynthates required for the growth of the seeds and that leaves had little direct contribution to the photosynthate requirements of developing seed.

Determination of light utilization in fennel by leaf area index would be virtually impossible at any stage of development due to the exceptionally fine structure of the leaves. Other techniques such as infra red photography of the canopy may provide an insight into the light inteception by the fennel canopy. Such a technique has been successfully used in determination of light interception by blackcurrants at various plant densities by Kerslake (1984). Such a technique may only provide a visual assesment as quantification may be difficult, again due to the very fine structure of the leaves.

Additionally, the stems of many of the Umbelliferae appear to contain chlorophyll, and may also be photosynthetically active units as well. Such structures also present problems for surface area measurment. In order to determine the importance of the various plant structures to seed growth and finally oil yield other methodology must be utilized. In recent years the major photosynthetic areas within the plant canopy of many crops have been examined by the use of defoliation and autoradiography experiments.

Freyman et al. (1973) further examined the role of leaves in the development of seed yield of rape using both these methods. Plants were defoliated following late anthesis. Although the degree of

defoliation was not stated, removal of 100 percent of the leaves was assumed. Seed yield was decreased indicating a contribution by the leaves. $^{14}CO_2$ was used to detect the translocation of photosynthates by application to the leaves, again at late anthesis, and examined by autoradiography. Their results indicated that translocation occurred from the leaves to the seed at this time. Major et al. (1978) demonstrated that lower leaves in rape export assimilates to the roots whilst the upper stems and leaves exported primarily to the seeds and pods in rape. Pods did not export labelled assimilates to other pods or plant parts.

Chapman et al. (1984) demonstrated that at early flowering in Brassica the leaves were the most important photosynthetic organs. Midway between flowering and maturity the stems became the major supplier of photosynthates, the role eventually taken over by the developing pods.

More recent work, again using $^{14}CO_2$, has indicated the importance of the topmost, or flag leaf, in oil seed rape seed development. Addo-Quaye et al. (1986) demonstrated that this leaf contributed 58 percent of its labelled assimilates to reproductive organs, but only during the period of rapid pod filling.

No experimentation using autoradiographic techniques to study the seed development in the Umbelliferae have been reported. Similarities in the habit of some of the Brassica and the canopy of the Umbelliferae exist. This allows speculation that such determination of assimilate partitioning would yield similar results. That is the contribution by the leaves is minimal and that the umbels are possible self supporting photosynthetic units behaving similar to the pods of the developing seed in oil seed rape.

5. Role of growth regulator substances in elongation and flowering

There appears to be only one general agreement between all workers examining physiological and morphological changes associated with flowering and stem elongation. This is, that all these processes are controlled at the molecular level involving a great number of specific compounds all involved in particular biochemical reactions which eventually lead to macromorpholgical changes. The most studied of these are those considered as plant growth regulating, or growth regulators.

Plant physiologists refer to five types of chemical growth regulator systems:

The auxins, substances which generally resemble indole acetic acid.

The gibberellins, diterpenes which stimulate cell elongation.

The cytokinins, usually substituted adenines, which stimulate cell division.

Ethylene gas, stimulating isodiametric growth of stems and roots.

The inhibitors, particularly abscisic acid which ordinarily suppresses growth.

Leopold and Kriedeman (1979) stress that while each group is distinctive, both in chemical characteristics and being able to bring about characteristic growth responses, each type of growth regulator is capable of altering most aspects of growth. These include cell division, cell enlargement, differentiation and differential growth phenomena as well as the various stages of plant development.

Consequently investigations into promotive or inhibitory effects take the form of exogenous application, but caution must be taken when concluding that similar responses by a plant are mediated by endogenous levels of these chemicals. The important principles to apply when engaging such research are outlined by Bernier et al. (1981). The quantity of the compound applied is important, wide concentration ranges should be used, in many cases concentrations below and above a particular value are stimulatory and inhibitory respectively. Interaction between substances may occur when two or more compounds are applied, also pure preparations may pose problems

as the active principle may not be the major component, but an impurity in low amounts. The mode and site of application are important, generally applications should be localized. The effectiveness of an application may not necessarily reveal the site of action but only reflect the differences in uptake of a compound. Timing and duration of treatments are important, reactivity of plant tissues to applied growth regulators varies with physiological age as well as developmental stage of a particular process such as leaf induction, apex evocation etc.

The present review is intended to facilitate a rapid acquisition of general knowledge of plant hormones, only work in relation to Umbelliferous crops will be discussed in detail. For more comprehensive descriptions see Letham et al. (1978) and Moore (1979).

5.1 Auxins

The amino acid tryptophan is a logical pre-cursor for indole acetic acid (IAA) because of the close chemical similarity. Pisum seedlings and intact tomato shoots are two plants which show the highest activity in conversion of tryptophan to IAA in terminal buds and young leaves (Goodwin, 1978).

Application of auxin generally does not affect stem elongation. Promotive responses appear specific to the gibberellins. Auxin application effects on flowering are strongly dependant on dose. Generally inhibition of flowering at high rates, whilst promotion at low doses occurs, particularly when photoinductive conditions are close to threshold levels (Bernier et al. 1981).

Commercial applications of auxins have been limited, only two usages are noteworthy. Firstly, to control fruit set in China field tomato, flowers are dipped in 2,4 dichlorophenoxyacetic acid which increases yields markedly. Secondly, sex expression in flowers has been modified in curcubitaceous crops, particularly cucumbers. In this case an increased proportion of female flowers is induced in the presence of auxin (Wittwer, 1983).

5.2 Cytokinins

A cytokinin is defined by Letham (1978) as a compound which in the presence of optimal auxin, induces cell division in tobacco pith, or similar tissue cultures. As a result of such a classification, numerous compounds both naturally occurring and synthetic are classed

as cytokinins. Within higher plants root tips, xylem sap, developing fruits, tumour tissue and germinating seeds are particularly rich sources of cytokinins.

Inhibition or promotion by exogenous application appears to be dependent on the amount of cytokinin applied and/or timing. Hence the optimal dose rates as well as the optimum period of sensitivity to cytokinins has been shown to be the major considerations for exogenos cytokinin work.

In some species, cytokinins can promote flower initiation under non-inductive conditions. For example flower formation in duck weed, a short day plant, is induced by cytokinin application when grown under long days. There is no evidence at present that endogenous cytokinins individually play an important role in the flowering process, rather their role may be to control early mitotic stimulation and associated cell synchronization, splitting vacuoles, precocious initiation of axillary meristems and increased rate of appendage production by the meristem (Zeevart, 1978).

Commercial usage of cytokinins has been limited. The most readily available compound of this group is benzyladenine. Some potential usages are described by Wittwer (1983). Very low concentration sprays and dips are useful in delaying senescence in lettuce, asparagus, broccoli and celery.

A more recent commercial usage for benzyladenine has been as a mixture with Gibberellins, marketed as Cytolin. This is registered for use in apple production for increasing the 'typiness' of the fruit, particularly in the variety Red Delicious.

5.3 Gibberellins

The Gibberellins (GA) have been implicated in all phases of plant development. The effect of exogenous GA on stem elongation is well documented. One of the more dramatic effects is the transformation of dwarf plants into tall plants by greatly increased stem elongation. Such results has implicated the involvement of this group of the plant hormones in the process of natural stem extension.

In a number of long-day plants, eg. Rudbeckia, vigorous elongation results when placed under inductive conditions. Prior to this elongation phase of growth a sharp increase in GA activity occurs (Goodwin, 1978) (Figure II.4).

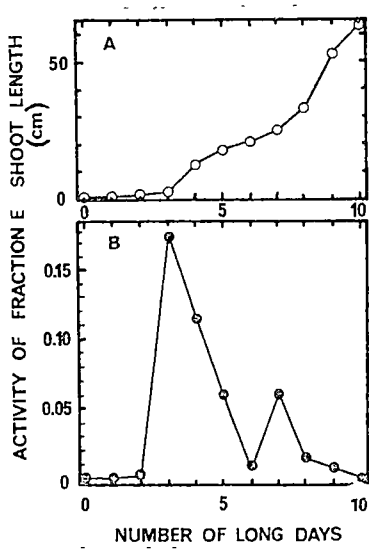


Figure II.4. Stem elongation (A) and extractable GA (B) in Rudbeckia speciosa after transfer to long days. From Goodwin (1978)

The increase in endogenous activity is probably of significance in respect to stem elongation. The effects of such environmental stimuli can be substituted in many long-day and cold requiring biennials by exogenous application of GA's (Krishnamoorthy, 1975).

Further examples of the importance of GA's in stem elongation, include a decrease in GA activity when long-day plants are transferred to short-days.

The use of growth retardants on rosette long-day plants such as Rudbeckia spp. and Samolus spp. can suppress both the elongation and flowering response. The application of GA can reverse these effects. Such results imply that GA's are controlling both these developmental processes. But in other rosette long-day plants such as spinach and Silene spp., growth retardants only suppress elongation. Despite reduced endogenous GA levels, flowering is unaffected (Zeevart, 1983).

Chailakhyan (1976) has postulated that flowering is controlled by the interaction of GA's and anthesins. Anthesins are unknown flower-inducing substances. Chailakhyan suggests that in long-day plants anthesins are present in non-limiting levels under all daylengths but GA's are limited for flowering under short-days. While in short-day plants GA's are present in non-limiting amounts under all daylengths with anthesins limited under long-days. In many long-day plants that exhibit the rosette growth pattern, application of GA stimulates flowering under short-days (Zeevart, 1978).

However, GA treatment does not induce flowering in some long-day rosette plants, for example Beta vulgaris, Centaurea calcitrap, Hieraceum pratense and others (Krishnamoorthy, 1975). These reactions are only to one particular GA, in this case GA_3. Different GA's differ in their affects on any one plant species, but generally there is evidence that the main function of GA's is to stimulate elongation and not flowering (Cleland and Zeevart, 1970; Jones and Zeevart, 1980).

Application of GA's after floral initiation produce an acceleration of development and a decrease in the time to anthesis in numerous species. The success of the application is relative to the stage of development of the flower bud. The particular GA applied will also alter the response, for example GA_1, GA_3 and GA_{4+7} are very active in Chrysanthenum whereas GA_{13} is almost without effect (Menhenett, 1981).

5.4 Ethylene

Ethylene being a small molecule is able to move readily through plant tissues by diffusive processes. Ethylene is produced by all living tissue but the highest levels are reported in ripening fruits, flowers, seeds, leaves, and roots (Bearder, 1980). The production of ethylene is often enhanced by high levels of auxin. The most readily agreed upon precursor of ethylene in plants appears to be methionine. Conversion to ethylene requires oxygen and is susceptible to several inhibitors (Leopold and Kriedeman, 1975). Foremost among functions which may be regulated by ethylene are the inhibitory effects on growth, and the stimulation of ripening of fruit in many species.

Plant tissues have the ability to increase their ethylene production under stress, or in response to high auxin concentrations, and this is generally correlated with an inhibition of stem elongation. All these responses are reviewed by Abeles (1972).

Application of exogenous ethylene inhibits flowering in many short-day plants (Bernier et al. 1981). No general conclusions can be made on the importance or the level of participation of ethylene in flowering. The only possible exception is the bromeliads which are induced to flower in the presence of ethylene. This response orginally led to the discovery of the hormonal action of ethylene.

5.5 Abscisic Acid

In general abscisic acid is inhibitory to growth and seed germination. Regulatory mechanisms involving abscisic acid are generally long-term seasonal responses such as seed dormancy, vegetative dormancy, abscission, senescence as well as responses related to stress.

The two suspected pathways for the biosynthesis of abscisic acid are, through the isoprenoid pathway from melavonic acid or through the oxidation of xanthophylls which have similar basic structures (Leopold and Kriedeman, 1975). Movement of abscisic acid appears to lack the consistent or polar activities observed by other phytohormones.

Bernier (1988) suggests that in photoperiodic species, abscisic acid is a general "background" inhibitor of flowering. Abscisic acid is produced more or less constantly irrespective of daylength. Generally the effects of abscisic acid may be overcome by increasing the amount of promoters under inductive conditions.

5.6 Exogenous applications of Growth Regulators

By the early 1980's some 10^6 hectares of cropland were treated annually with growth regulatory chemicals of which six; chloremquat, daminozide, maleic hydrazide, ethephon, gibberellic acid and glyphosine account for more than 90 percent of the total (Garrod, 1982).

The growth regulatory properties of maleic hydrazide (MH) were first discovered in 1949. MH is a potent suppressor of growth and was used initially as a herbicide and to retard the growth of turf. Soon after some quarternary ammonium compounds were shown to have growth retarding activities, one in particular was chloremquat chloride (CCC). CCC and MH have been used on ryegrass by Hebblethwaite and Burbridge (1976) in an attempt to control lodging. While MH significantly decreased straw length and lodging, the application decreased seed yield and germination. CCC increased seed yield but failed to control lodging. The difference in mode of action of these chemicals was such that CCC acted as a growth retardant while MH as a growth inhibitor. Such a distinction between the actions of growth affecting chemicals is important and it is in the field of growth retardants that additional chemicals have been sort.

5.7 Further Growth Retarding Chemicals

Following the early investigations with CCC, quaternary ammonium compounds were reported to have plant growth retarding activity.

The growth retardant Daminozide, a compound which lacks a quaternary group, is currently used in fruit crops where it has been observed to be very effective in prevention of pre-harvest fruit drop. Other uses include promoting firmness and quality and the inducement of early cropping in closely spaced orchards (Luckwell, 1976).

Retardants are among the most readily translocated organic compounds applied to plants and Daminozide can be transported in both the phloem and xylem of a number of herbaceous species (Dicks, 1976). Quantitative studies suggest the extent that stem growth is restricted by Daminozide is related to the concentration of this compound in the stem tissue (Dicks, 1977; Dicks and Charles-Edwards, 1973). The tissue concentration will be affected by the dose rate, formulation, timing and site of application. In addition the time elapsed since treatment appears important. Some growth retardants, particularly Daminozide, have a limited effectiveness especially when applied to foliage. This phenomenon of 'growing-out' probably occurs by dilution of the compound through growth or by storage at inert sites rather than by degradation since Daminozide appears relatively stable within plant tissue (Dicks, 1972).

5.7.1 Ethephon

Following the introduction of Daminozide, perhaps the most significant introduction into the world of commercially viable plant growth regulators has been that of ethephon, an ethylene-releasing compound. Below pH 4.1 ethephon is chemically stable but on entering plant tissues which are less acid it undergoes a base catalysed elimination reaction to liberate ethylene into the tissues. Most work with this compound is associated with ripening, senescence and abscission phenomena and has been used for one or more of these purposes on apples, blackberries, blueberries, cantaloupes, cherries, coffee, cranberries, figs, lemons, tomatoes and walnuts. Other uses include the promotion of flower initiation in pineapples and other bromeliads and the modification of sex expression in cucumbers (Wareing and Phillips, 1983). Ethephon has been registered for use, alone and in conjunction with mepiquat chloride as an anti-lodging agent in barley, but relatively few uses have been developed for major

crops. The failure to find applications in crops may be due to the inability to control the extent of ethylene release.

5.7.2 Naturally Occurring Regulators

In considering the practical exploitation of naturally occurring plant growth regulators, the gibberellins follow the auxins both in order of discovery and usage. In spite of extensive studies on gibberellins since the mid 1950's the number of practical uses to arise have been small. Most relate to specific horticultural situations, such as increasing yield and quality of seedless grapes and delaying ripening of citrus fruits on the tree (Garrod, 1982). The major factor contributing to the lack of use of gibberellins is their high cost of production.

The use of mixtures of gibberellins with other plant hormones has been investigated but only appear applicable to minor high value crops. Considerable experimentation with well balanced mixtures is required for each specific crop and variety of crop (Thomas, 1982). A commercial example is Cytolin, a mixture of GA_4 and GA_7 with BAP, utilized especially on Red Delicious apples to improve colour and shape.

Other natural occurring growth regulators, abscisic acid and cytokinins have found only extremely specialized use but again high cost of synthetic production has discouraged work directed towards discovering practical usages.

5.7.3 Triazoles and Pyrimidines

More recently, potent growth retardants have become available, notably the pyrimidines. For example ancymidol released as A-rest by Elanco Products. Ancymidol acts against gibberellin stimulated growth systems, possibly inhibiting gibberellin biosynthesis by blocking the conversion of ent-kaurene to ent-kaurenol, compared to CCC which inhibits the conversion of trans-geranylgeranyl -pyrophosphate to copalylpyro-phosphate (Leopold, 1971). Ancymidol is considerably more active than CCC because of this difference in the mode of action between the two chemicals. But to date, ancymidol has only been registered for use on ornamentals as a dwarfing agent. Elanco trials indicate that rates ranging from 0.25mg to 0.5mg per 150 mm pot produce optimal results when used on lilies and chrysanthemums. Application was recommended at an early vegetative stage of growth.

The degree of height reduction could be controlled by application date as well as concentration.

Paclobutrazol, or PP333, is another potent growth retardant which is representative of the triazoles, and active on a broad spectrum of plants (Lever et al. 1982). PP333 also appears to inhibit gibberellin biosynthesis, the exact method of inhibition has yet to be elucidated. The general effects of application are a prolonged retardation of growth and internode compression. Effects are dose related and are not accompanied by any phytotoxicity. This compound is taken up by roots, stems and foliage. Consequently activity can be achieved from foliar, or ground applications. More rapid effects are obtained by foliar applications whilst ground sprays take longer to act. Due to the residual nature of this chemical, some retardant effects frequently occur in the year after application with either foliar or ground sprays. As a result, lower rates may therefore be needed in subsequent years (Fua pers. comms.). As PP333 is translocated almost entirely in the xylem elements, foliar sprays accumulate in the leaves and little may reach the meristematic area of cell division which is the site of action (Lever et al. 1982). Hence as growth increases the growth regulator is diluted further lessening its effect on meristematic tissue.

Another member of the pyrimidine family which demonstates very similar mode of action to PP333 is flurprimidol, coded by Elanco as EL500. This retardant has been released for use on high quality turf grasses such as golf courses, home lawns and roadside turf under the product name of Cutless. The activity of both PP333 and EL500 when applied either as a soil or foliage spray has not been observed with CCC or Daminozide. Growth retardation effects from these chemicals will only result by application directly to the foliage. Consequently, no residual activity in the following season would be expected when CCC or Daminozide are applied to a perennial crop. The phenomenon of 'growing out', a decreased concentration of growth regulator in plant tissue as the plant grows, becomes more pronounced with the usage of CCC and Daminozide when examining effects on crops that produce high amounts of dry matter per unit area in a short time. This may be particularly prevalent in Umbelliferous crops where large amounts of dry matter are produced above the meristematic regions of the plant (Peterson, 1984).

Further benefits observed through the application of CCC on

grasses have been reported and these may also apply to EL500 and PP333 due to the similar modes of action.

These include:

1. Reduction in apical dominance.
2. Changes in response to plant density due to the growth regulator interfering with the phytochrome system.
   The gibberellin mediated response of smaller and fewer ears as plant density increases (Batch, 1981) may be inhibited allowing higher plant densities whilst retaining high yields per plant (Sampson et al. 1980).
3. Changes in photosynthetic efficiency. Early spring applications are claimed to produce more erect plants with a consequent increase in photosynthetic efficiency (Humphreys, 1968; Batch, 1981; Sampson et al. 1980).

McDaniel (1986) compared the effects of PP333 and EL500 application in poinsettia, both compounds achieved favourable control of height at the following rates, 0.5 mg per plant for PP333 and 0.03 to 0.06 mg per plant for EL500, but delays in time to anthesis were produced by both chemicals. EL500 delayed up to 5 days whilst PP333 extended time to anthesis by 7 days. The effect of application of these chemicals on herbaceous plants is difficult to determine from the work indicated, which mainly involves small flowering or woody perennial plants. Due to the more rapid growth rates exhibited by herbaceous plants, higher rates of application may be necessary to control stem elongation.

Additional advantages other than growth regulation may be afforded by the application of PP333. While the principal activity is as a growth regulator, PP333 also has useful fungicidal properties. Low rate sequential sprays have given good control of powdery mildew (Podosphaera leucotricha) and scab (Venturia inequalis) on apples, reduced incidence of fireblight on pears (Lever et al. 1982) and controlled rust (Puccinia spp.) and mildews (Erysiphe gramminis) in ryegrass (Chilcote et al. 1981). Frost protection in apples also has been recorded by Lever et al. (1982). The application of PP333 improved resistance to frost in flower buds and flowers in the year

following treatment. A major disease problem in fennel grown commercially in Tasmania is a fungal infection of the leaves which, if left unchecked will infect the umbels. It is possible that this fungus, Cercosporidium, may be controlled by the application of PP333.

5.8 Growth Regulators in Umbelliferous Crops

Literature detailing growth regulator applications on Umbellifera crops is limited. Few workers have examined effects on fennel. Some of the studies undertaken on members of this family include the application of gibberellins and other growth regulators to celery to increase the internode length (Aloni and Pressman, 1980). Other studies have examined the application of ethephon to carrots to increase the size of the roots (Jacobsohn, 1978) and Daminozide and chloremquat on carrots to reduce seed stalk height without affecting root size and delaying bolting (Jacobsohn et al. 1980).

Application of Daminozide to both fennel and caraway resulted in a significant decrease in stem height of both species especially at high rates (Abou-Zied, 1974). The seed yield increased in both species with increasing Daminozide application. This was interpreted as an effect on the umbel development increasing the number of umbels per plant. In fennel, an application of a 4000 ppm solution of Daminozide increased the mean number of umbels per plant from 5.5 to 8.6. Such low numbers of umbels present on the control plants possibly indicates the presence of another factor other than the growth regulator under scrutiny. The nutrient or moisture status may have been limiting or the environmental conditions could have been sub-optimal. Under such circumstances, the application of a growth regulator may be seen to have a significant effect.

The percentage oil yield from the seeds of both caraway and fennel was observed to increase when an application of a 4000 ppm solution was utilized. The explanation of this oil increase was an assumption that the compound increased the activity of biosynthetic pathways for oil production. No evidence for such an assumption was presented and a more realistic explanation would be that the control of vegetative growth by this compound resulted in the cycling of secondary metabolites into reproductive tissues, thereby increasing the percentage oil yield of the seeds.

Cycocel was applied to caraway, anise and fennel by Ahmed and Eid

(1975). The result was a decrease in plant height of all three species. At high concentrations, 4000 ppm, a slight increase in percentage oil yield of the seeds was shown for anise, while the percentage oil yields in fennel remained the same. Caraway percentage oil yield decreased slightly after cycocel application.

More recently Elbella (1986) applied ancymidol to carrots. Stem height was reduced by 72 percent in respect to the control plants, but unfortunately a reduction in the seed yield also resulted.

Under non-inductive conditions, most members of this family exhibit a rosette form of growth followed by a marked change to a rapidly elongating plant once conditions are inductive. Consequently the gibberellins and retardants that affect endogenous gibberellins have been the major aspects studied in the Umbelliferae.

Amrutavalli (1979) decreased the time to anthesis of the primary inflorescence and improved flower yield in coriander by applying GA_3. The assumption that this was a result of an increased carbohydrate metabolism was not supported by any conclusive evidence, and can only be considered as mere speculation. Hanisova and Krekule (1975) attempted to replace the cold induction period in celery with GA_3 but only increased the percentage of bolting plants and also hastened the onset of bolting. Vernilization could not be replaced.

Another effect of GA_3 that has been reported is enhanced flowering in some Umbelliferae. Flowering following application of GA_3 to carrots and parsley (Lang, 1957) and dill (Wittwer and Bukovac, 1957) has been reported. But, in all cases the environmental conditions at and prior to the applications were not precisely defined. More recently these findings have been questioned. In carrot the replacement of the low temperature requirement has only occurred when very large amounts of GA_3 was applied or after prolonged treatment or when temperatures were near threshold levels for induction. Nieuwhof (1984) found the GA_3 effect on flowering in carrots to be inconsistent, and the application of this phytohormone did not promote flowering irrespective of the growth stage and physiological condition of the plant.

Specificity to particular gibberellins also exists. In celery GA_3 and GA_{4+7} induce bolting, however GA_{4+7} will promote bolting at much lower levels of application (Pressman and Sachs, 1985a).

Umbelliferae which respond to GA_3 under non-inductive conditions through increased stem elongation and not flowering are chervil and

parsnips (Pressman and Sachs, 1985b). Fennel is also reported to be affected similarly (Shimada 1959). Two GA_3 treatments were carried out in an attempt to induce flowering. GA_3 was applied to plants under short day conditions, 50 ppm solutions weekly for 4 to 8 weeks and also to plants near threshold long day treatments. The first treatment increased stem elongation but did not result in flowering. The stage of development of the plants at time of application was not detailed in these experiments. The possible existence of a juvenile phase in fennel may confound such experiments. At sub optimal environmental conditions, the application of GA_3 did hasten the onset of flowering.

To date, this and the affore mentioned workers studies, represents the only investigations on fennel in respect to growth regulators and their possible uses in production.

Investigation into the possible affects of the more recently released growth regulatory chemicals has not been widely studied in the Umbelliferae in general. No such studies of the use of these chemicals on fennel was observed. The use of retardants such as flurprimidol and paclobutrazol on such strongly elongating plants may allow the division of the flowering response from the elongation response. As already indicated, bolting without flowering is readily achievable in many Umbelliferae, including fennel (Shimada, 1959) but can these plants be induced to flower without bolting? Certainly this is observed in some varieties of celery where the stalk of the inflorescence grows directly from axillary buds in the bulb (Hanisova and Krekule, 1975). In this case the vernalization requirement for celery appears obligatory for stem formation but not for differentiation of the inflorescence.

The application of retardants such as flurprymidol and paclobutrazol may allow further manipulation and separation of these factors. At the same time, other benefits may be afforded by their usage in relation to seed and oil yields, disease reistance and many other factors which are as yet unknown.

III

MATERIALS AND METHODS

III. General Materials and Methods

In this section the techniques and experimental materials common to most experiments will be detailed.

1 Plant Material

The varieties of fennel (Foeniculum vulgare Mill.) used in all glasshouse and field trials were supplied by Pernod Ricard Pty. Ltd., France. These particular varieties were derived from an ongoing selection programme and referred to as C22 and C25. C22 was used for the initial field work while C25 was used in later field trials and initial glasshouse trials. The majority of the glasshouse experimentation used either seedling or clonal material of C25. The clonal plants of C25 were obtained by tissue culture methods.

2 Oil Extraction and Compositional Determination

2.1 Material Preparation

In all field experiments the whole plants were harvested at ground level. For determination of oil yields from whole plants subsamples were chopped with secateurs and distilled as outlined below. Where experiments investigated individual plant parts (eg. primary or secondary umbels), these were removed, counted, weighed and distilled as outlined below. Such samples were treated separately from the whole plant subsample. To determine percentage yields on a dry weight basis subsamples were oven dried at 70^{o}C for 48 hours.

2.2 Distillation

All fennel samples, either whole or specific parts of the plant were steam distilled to extract the essential oil. To enable the large number of distillations to be processed within 2 to 3 days of harvest, four distillation units were used (Plate III.1). The units consisted of a 45 litre aluminium distillation vessel fitted with a glass condenser to the top of the lid. The condensers were designed to retain the condensed oil and return the distllation water to the distillation vessel. Within each vessel, stainless steel screens

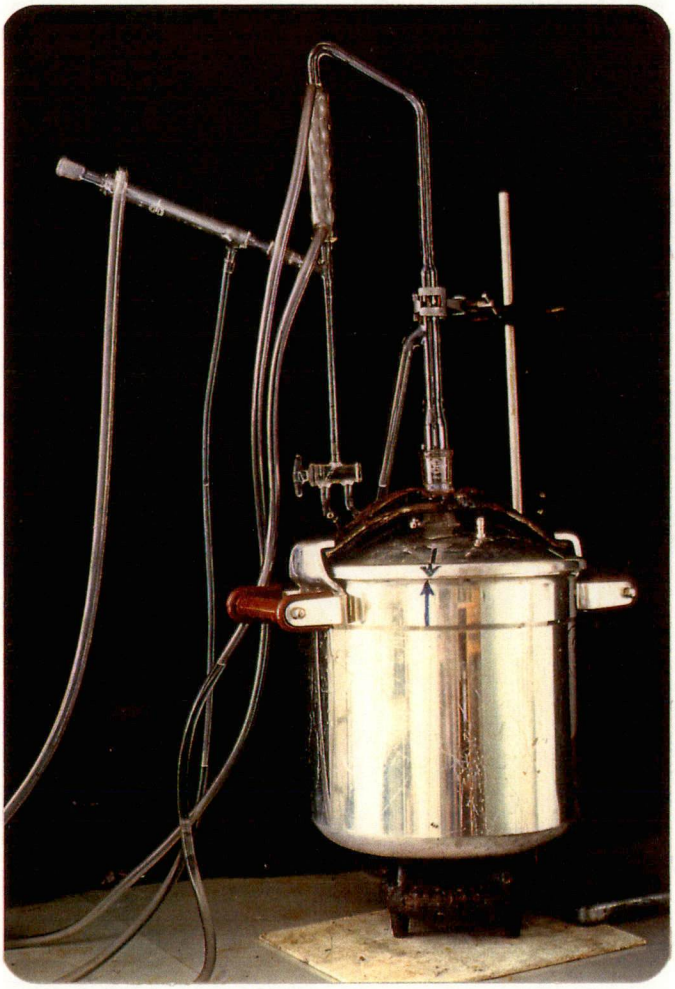


Plate III.I.

Laboratory steam distillation unit. Initially, this unit was 20 l internal capacity, but was enlarged to 45 l for later experiments. No variations in oil recovery was observed.

supported the plant material approximately 5 cm above the surface of the boiling water. To each vessel, approximately 1.5 litres of water was added before distillation and the maximum capacity of the units was approximately 4.5 kg of fresh material. A distillation rate of 8 to 10 ml per minute was maintained for the duration of the distillation. The time required for complete extraction of fennel oil varied depending on the maturity of the material. To ensure complete extraction all samples were distilled for 2.5 hours. After distillation all oil samples were removed from the condensers, weighed and stored in glass vials with anhydrous sodium sulphate.

2.3 Compositional analysis of oil samples

Gas chromatographic analyses of oil samples were conducted using a Hewlett-Packard 5880A series gas chromatograph. The column used for analysis was a 50m BP20, 0.32mm internal diameter bore with a 0.5µ coating. Operating conditions were: carrier gas Helium 20psi, column oven temperature was programmed from 50°C to 220°C at 6°C per min. Peaks eluting from the column were detected by a flame ionization detector interfaced to a Hewlett-Packard 5880A integrator.

Prior to injection, samples were prepared as follows: 5µl of the oil sample was taken up in a Hamilton 5µl syringe and injected into 1ml of glass distilled hexane in a 2ml glass vial. The vials were capped and placed in a Hewlett-Packard 7671A automatic sampler. The automatic sampler had a maximum capacity of 35 samples and allowed accurate sample injection as well as continuous operation of the Gas Chromatograph. Identification of oil components eluting from the column was achieved using a UG 7070F mass spectrometer interfaced to the gas chromatograph.

Nine major peaks were identified by GC-MS. An example of the chromatogram eluting from this GC technique is shown (Figure III.2.3). In all experiments the percent FID response of the following nine compounds were determined: Beta-Phellandrene plus Cineole, Cis-Beta-Ocimene, Myrcene, Alpha-Pinene, Alpha-Phellandrene, Limonene, Fenchone, Estragole and Anethole.

Only six major peaks were identified for samples in experiment A.1. This was due to insufficient GC-MS data available at the time of initial experiments.

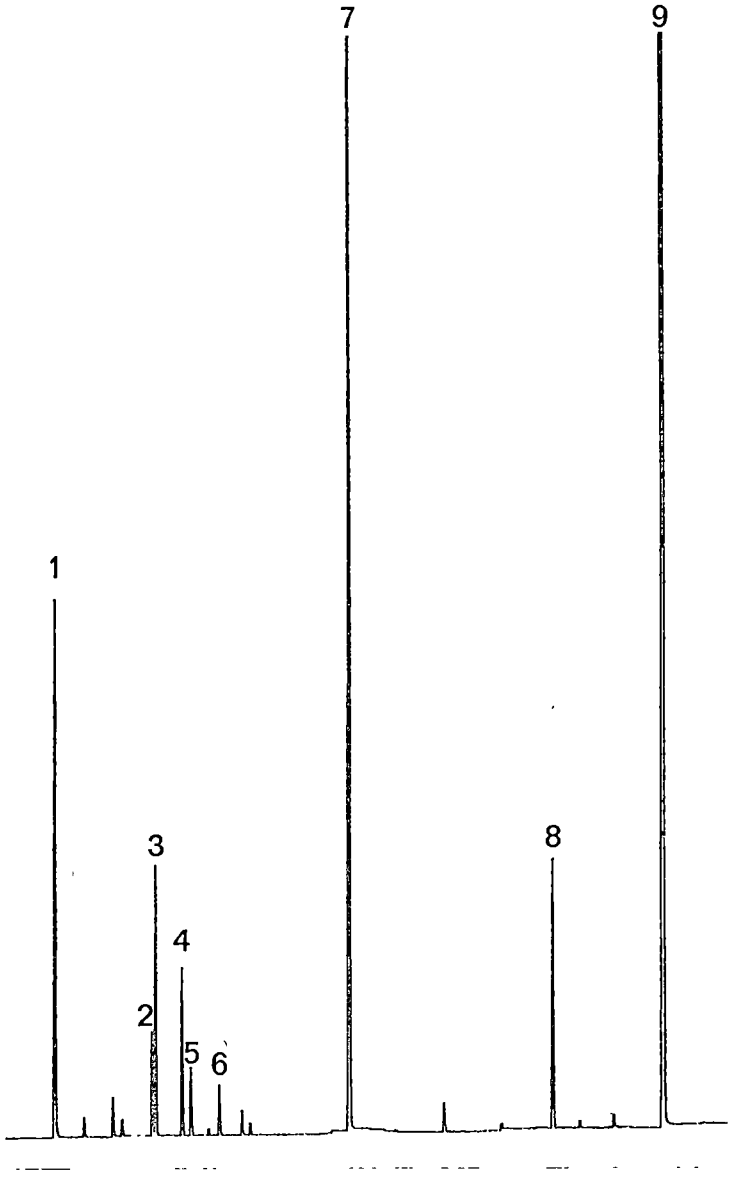


Figure III.2.3.

Gas chromatogram Fennel oil.

Hewlett-Packard 5880A, 50m BP20 column, Helium carrier, 50°C to 220°C at 6°C, flame ionization detector.

1 Alpha-pinene
2 Myrcene
3 Alpha-phellandrene
4 Limonene
5 Beta-phellandrene (+ some cineole)
6 Cis-beta-ocimene
7 Fenchone
8 Estragole
9 Trans-anethole

3 Glasshouse-growth room experiments

3.1 Glasshouse

Plants were grown in an air conditioned glasshouse at the Horticultural Research Centre, University of Tasmania, Hobart. Temperature control was maintained between 15 to 25°C, and relative humidity maintained above 50 percent by injection of water sprays into the air circulation system.

No artificial lighting was provided in the glasshouse, natural photon flux densities (PFD) varied from 600μmole m^{-2} s^{-1} to 1200μmole m^{-2} s^{-1} as determined using a Lambda Instruments LI-185 Quantum meter.

3.2 Growth Rooms

Growth rooms were each 1.5 * 4 m in size, containing trolleys which could be programmed to move between the growth rooms and the glasshouse at any particular time (Plate III.2). The rooms were light proof and lined with 50mm thick polystyrene slabs. Recirculation of the main glasshouse air through the rooms maintained the temperature and humidity constant relative to the glasshouse.

Artificial lighting was used to extend the natural daylength conditions when required. This lighting consisted of 2 Mazda 100W incandescent lamps and white fluorescent lamps distributed evenly and suspended 2.0m above the pots, this arrangement provided a PFD of 100μmole m^{-2} s^{-1} at 200mm above the pot surface.

Irrigation was applied to plants on the trolleys through an overhead mist system. The plants received 10 mins. at 10:00 a.m. and during summer months again at 3:00 p.m. This programme ensured that all pots were brought up to field capacity daily.

All glasshouse and growth room plants received nutrient solution (N.Hoaglands) once per week. Plant pests, mainly aphids, were controlled with Maldison solution.

The potting mixture for all experiments consisted of a mixture of equal volumes of coarse sand and peat moss. Equal amounts of dolomite and limil were added to this potting mixture to bring the pH to approximately 6.5. Osmocote (3 to 4 month slow release) was added at a rate of 1g per $40*10^{-3}m^3$ of mixture.

Plate III.2.

Growth room facilities at the Horticultural Research Centre, University of Tasmania. Moveable trolleys controlled by time clocks enabled movement of plants between the main glasshouse and growth rooms. In these rooms temperature and photon flux density and the integral could be controlled. Overhead irrigation provided even watering of pots.

4 Field experiments

4.1 Commercial crop areas

All field trials were located in commercial plantings of Foeniculum vulgare Mill., at three sites in Southern Tasmania. The three sites were:

i) "Rotherwood", Ouse located in the Upper Derwent Valley area of Tasmania.

ii) "Strathayr", Richmond in the Coal River Valley.

iii) "Glenleith", Plenty in the Lower Derwent Valley.

All three crops were subjected to the normal cultural practices adopted by commercial producers. A brief outline of these cultural practices will be provided.

4.2 Sowing

Crops were sown in late September at a rate of 2kg of seed per hectare with 100 kg/ha of 50:50 lime super. A plant density of 10 plants per m^2 was to be obtained by this sowing rate.

4.3 Fertiliser and Irrigation

The general recommendation for all crops was 350 to 400 kg/ha of N:P:K fertiliser (3:6:8), followed by two side-dressings of 50 to 75 kg/ha of ammonium nitrate. The amount of fertilizer applied depended on soil analysis results. Generally 4 to 6 irrigations of 30 to 40 mm each were applied. Irrigation was applied by overhead sprinklers using travelling irrigators.

4.4 Herbicides

Weed control during seedling establishment was important. Herbicides used were trifluralin applied presowing and incorporated. Linuron was applied pre crop emergence or after the two leaf stage as a general weed control measure. Propazine applied post sowing and prometryne or linuron applied post emergence. Diquat or Paraquat was used in early spring to control seedling fennel in established fennel crops.

4.5 Pests and diseases

The most damaging disease which occurs in fennel is caused by the fungal organism Cercosporidium punctum. This disease was controlled by the application of Bavistin (carbendazim) and Polyram (mancozeb), applied at a rate of 400gm/ha and 2 kg/ha respectively.

The major insect pests identified in fennel are cut worm, thrips, fennel and carrot aphid and potato mirids. Control measures were recommended as the need arose.

5 Scanning Electron Microscopy (SEM)

Material selected for SEM was fixed in gluteraldehyde (4 percent W/W) in sodium phosphate buffer (0.1M, pH 7.2) for 18 to 24 hours at 4°C. The fixed specimens were then rinsed with phosphate buffer and dehydrated in an alcohol series, then washed in acetone. Specimens were then critical point dried in a Polaron critical point drier (CPD). After mounting on electron microscope stubs using conductive paint the specimens were sputter coated with gold. Observations were carried out with a Phillips 505 SEM at approximately 15 kv. Electron micrographs were recorded using a Rolex 120 mm camera and Ilford FP4 film and developed in Ilford ID-11. Specimens were stored on stubs in a vacuum desiccator.

6 Observational Measurements

Throughout all field and glasshouse experiments, a number of standard measurements and observations were made to examine the growth and development of the plant.

(a) Vegetative: Two non destructive height measurements were recorded:

i) The overall height, a measure from the ground or pot surface to the highest portion of the plant.

ii) The height to the growing point, a measurement again from the ground or pot surface to the point of emergence of the last leaf or the primary umbel. This was a measurment of the elongation of the main stem.

(b) Floral: In experiments investigating the effects on umbels, all emerged umbels were removed, counted and weighed. Destructive measurement during elongation and initiation required careful removal of all leaf initials with a scalpel to excise the apex. These samples were examined under a stereo microscope or by SEM.

(c) General: Other measurements were: the number of emerged leaves and the total number of nodes present and fresh and dry weights per unit area of vegetative and floral material. All dry weight determinations were conducted at 70°C for 48 hours.

7 Statistics

An analysis of variance (ANOVA) table was calculated for each growth measurement and oil yield and oil component for all experiments. Where values were significant, the Least Significant Difference (LSD) was calculated at the five percent probability level.

For any experiments which utilized time as a treatment, and were significant, a test of homogeneity of the variances was calculated using the Duncans multiple range test, determining the least significant range (LSR).

IV

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

IV.A Field investigation of changes in oil yield and composition.

A.1 Comparison of first and second year crops.

1.1 Introduction

The object of this study was to investigate oil production and oil composition of fennel grown as a commercial crop and to compare first and second year crops.

1.2 Materials and Methods

A commercial crop of 8ha of fennel (variety C22) consisted of 2 ha in its first year of production and 6 ha in its second year of production. The two areas were subsampled, distilled and the oil analysed according to the methods outlined in the General Materials and Methods.

Sampling commenced on November 21 in the second year crop and on January 3 in the first year crop. The difference in commencement dates was necessary due to the difference in development of the first year spring sown crop compared to the established second year crop. The particular dates were chosen on the basis of umbel emergence. The oil yield and composition were examined from whole plant and umbel samples. Only six compounds were examined in the oil samples in this experiment. Sampling ceased on March 8 for both crops.

1.3 Results

1.3.1 Change in oil yield from whole plants.

The total amount of oil present (expressed as kg per ha) increased steadily for both the first and second year crops during the period of observation. Initially the amount of oil present in the

first year crop was significantly less than the oil yield from the second year crop. By January 31 no significant difference in oil yield was observed between the crops (Figure IV.A.1.1). This was due to more rapid rate of accumulation of oil by the first year crop over the month of January. For the next 3 weeks, no significant difference between the oil yields of both crops was recorded. Only on February 22 did the first year crop exceed the oil yield from the second year crop.

Maximum oil yield of 200.7 kg per ha and 167.5 kg per ha were obtained for the first and second year crops respectively. The apparent decrease in oil yield exhibited by both crops in the last three weeks was not significant when examined by the Duncans multiple range test. That is, no significant difference was noted between the means in each crop over the final 3 weeks of observation.

During the months of January and February there was no significant difference between the percent oil yields, either on a fresh or dry weight basis, between the first and second year crops (Figure IV.A.1.2 and IV.A.1.3).

The change in percent oil yield on both a fresh weight and dry weight basis reflected the same trend in both crops. But for both determinations, the first year crop yielded higher than the second year crop during the period from March 1 to March 8. These maximum percent yields coincided with the period of overall maximum oil yields. The maximum percent oil yields were, fresh weight basis, 0.52 percent and 0.35 percent for the first and second year crops respectively. The maximum percent yield on a dry weight basis was 1.72 percent and 1.09 percent for the first and second year crops respectively.

1.3.2 Change in oil yield from umbels.

Percent oil yields from umbel material were much higher than from whole plants (Figure IV.A.1.4). The percent oil yield from the umbels of the first year crop was significantly less than the second year umbels until February 22. The percent oil yield was not significantly different over the next 7 days. The maximum percent oil yield for umbels was 2.42 percent for the first year crop and 2.59 percent for the second year crop.

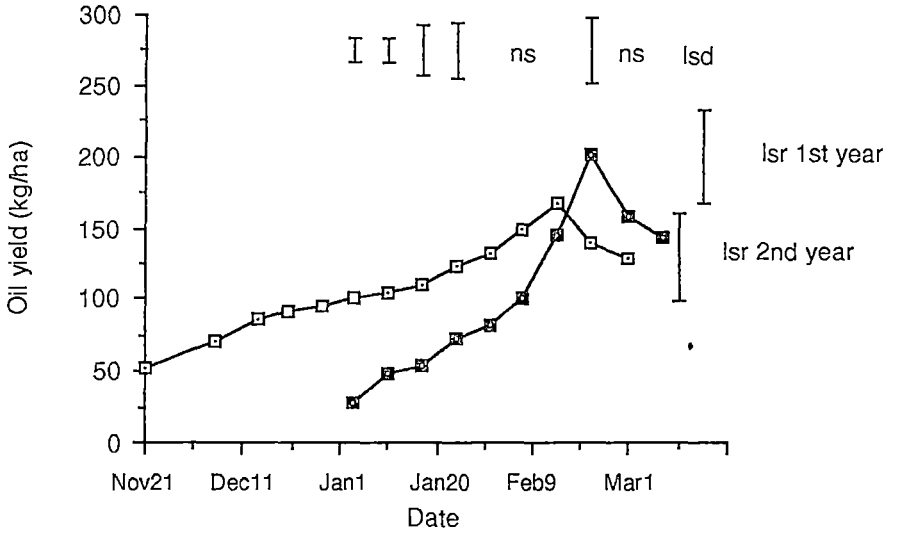
Figure IV.A.1.1
Comparison of oil yield (kg/ha) between first and second year crops.

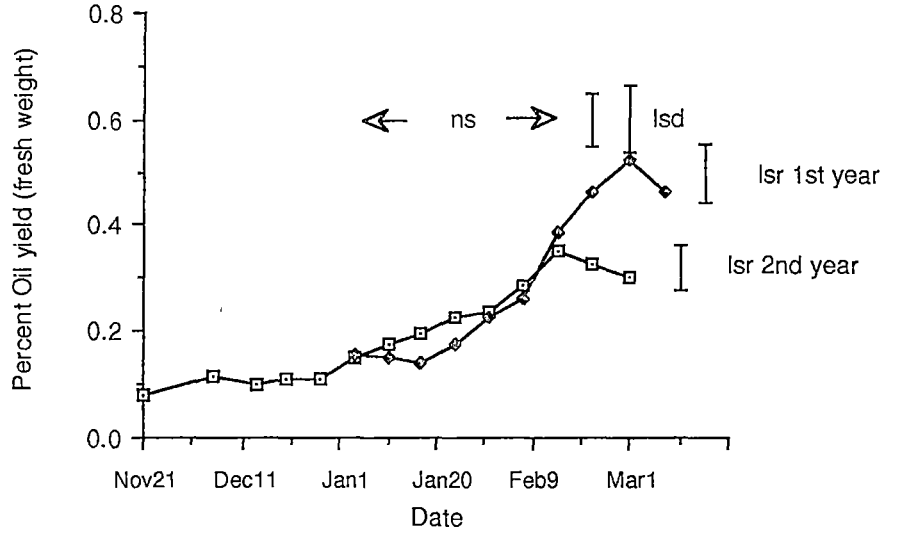
-■- First year crop

-□- Second year crop

Figure IV.A.1.2
Comparison of percent oil yield (on a fresh weight basis) between first and second year crops.

Figure IV.A.1.3
Comparison of percent oil yield (on a dry weight basis) between first and second year crops.


Oil yield (kg/ha)
300
250
200
150
100
50
0
ns
ns
lsd
lsr 1st year
lsr 2nd year
Nov21
Dec11
Jan1
Jan20
Feb9
Mar1
Date


Percent Oil yield (fresh weight)
0.8
0.6
0.4
0.2
0.0
ns
lsd
lsr 1st year
lsr 2nd year
Nov21
Dec11
Jan1
Jan20
Feb9
Mar1
Date

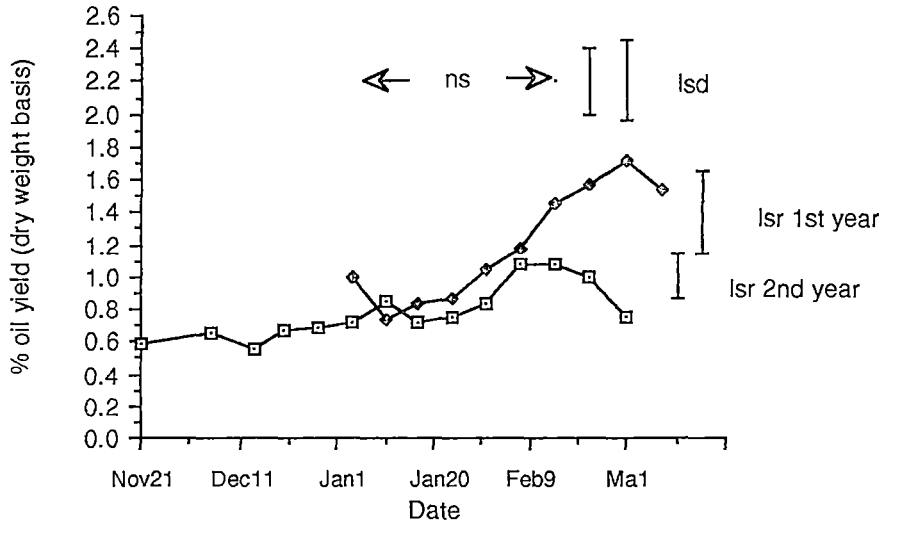
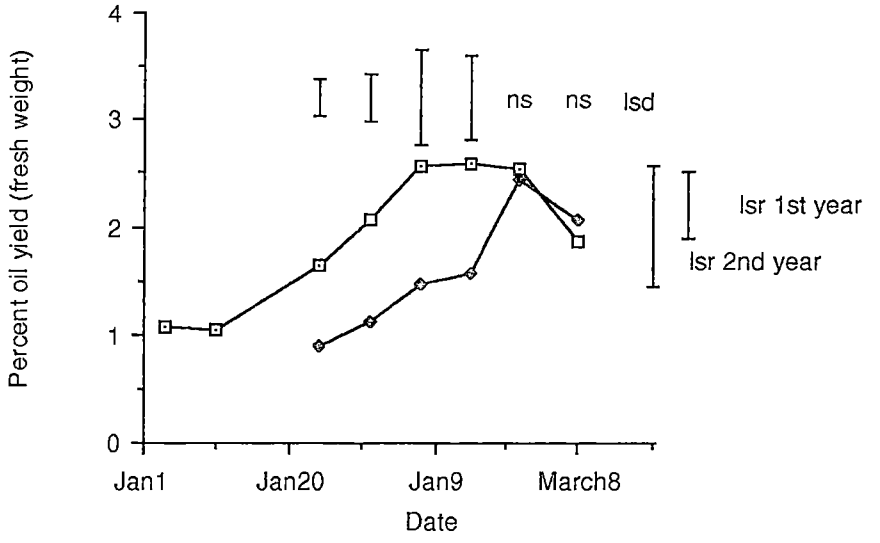

% oil yield (dry weight basis)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ns
lsd
lsr 1st year
lsr 2nd year
Nov21
Dec11
Jan1
Jan20
Feb9
Ma1
Date

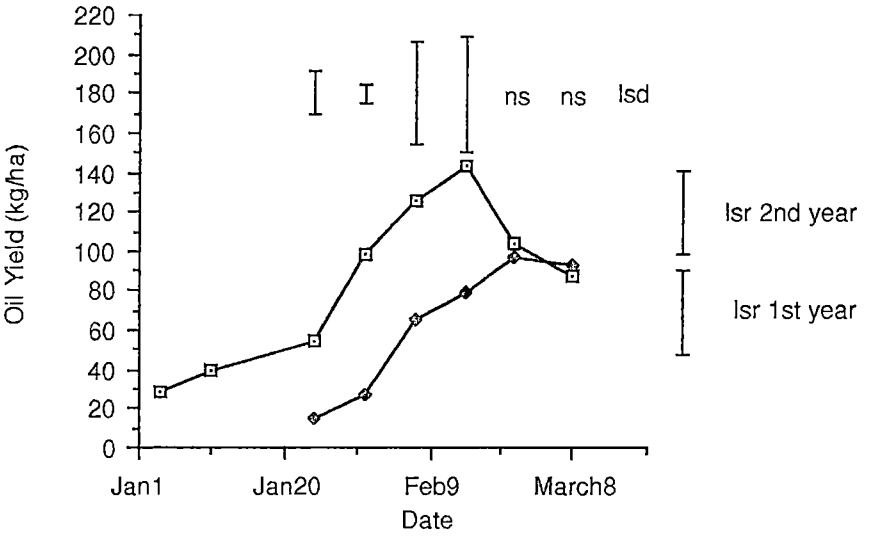
Figure IV.A.1.4
Change in percent oil yield (on a fresh weight basis) from umbel samples of a first and second year crop.


First year crop

Second year crop

Figure IV.A.1.5
Change in oil yield (kg/ha) from umbel samples of a first and second year crop.


Percent oil yield (fresh weight)
4
3
2
1
0
ns
ns
lsd
lsr 1st year
lsr 2nd year
Jan1
Jan20
Jan9
March8
Date


Oil Yield (kg/ha)
220
200
180
160
140
120
100
80
60
40
20
0
ns
ns
lsd
lsr 2nd year
lsr 1st year
Jan1
Jan20
Feb9
March8
Date

The oil yield from the umbels of the first year crop was significantly lower than the second year crop up to February 22. These results followed the same trends indicated by the results of the change in percent oil yield no significant difference detected from March 1 to March 8 (Figure IV.A.1.5).

A maximum oil yield per unit area from umbels produced by the second year crop was greater than the first year crop, 143.6 and 96.48 kg per ha respectively. The maximum for the second year crop was reached on February 22 whilst the first year crop maximum was recorded 1 week later. The rate of accumulation of oil was similar to the whole plant subsamples for both crops.

During the last 2 weeks of observation, from March 1 to March 8, the oil yield from the umbels of the second year crop decreased rapidly.

1.3.3 Change in oil composition from whole plants

The changes in composition of the main components of the oil from the whole plant from both the first and second year crop are given in Table A.1.1.

Alpha-pinene: In the second year crop levels were high, generally 20 to 23 percent during November to January. Subsequently the levels decreased markedly to approximately 8 to 11 percent. The levels detected for the first year crop changed erratically during the first 3 weeks of January. Levels fluctuated from 13.79 percent to 5.91 percent, this was followed by an increase to 13.15 percent and then decreased again to 6.69 percent on January 24. After this initial fluctuation the levels of alpha-pinene did not change significantly for the duration of the experiment.

Limonene: The second year crop exhibited a maximum level of 2.33 percent on December 20. A significant decrease to 1.05 percent was observed on February 7. The changes observed in limonene content in the first year crop were different to those of the second year crop. The lowest level, 1.17 percent, was obtained 17 days earlier, on January 21. The content was observed to increase to the highest level, 2.04 percent on March 15.

TABLE IV.A.1.1

Change in oil composition 1st year crop, whole plant.

Date	a-Pinene	Limonene	a-Phellandrene	Fenchone	Estragole	Anethole
3/1/85	13.79	1.57	10.62	8.73	2.51	59.14
10/1/85	5.91	1.20	8.91	13.92	2.33	61.06
17/1/85	13.15	1.57	12.73	8.59	2.18	54.89
24/1/85	6.69	1.17	9.22	13.55	2.35	60.34
31/1/85	7.99	1.29	8.70	15.36	2.22	58.24
7/2/85	12.06	1.48	11.13	10.32	2.12	55.15
14/2/85	11.66	1.55	12.85	12.54	2.19	53.69
22/2/85	13.95	1.38	13.90	13.72	2.08	50.93
1/3/85	13.90	1.77	12.43	11.97	1.90	48.93
8/3/85	12.91	1.87	10.16	12.61	1.95	54.05
15/3/85	13.39	2.04	8.56	11.99	2.38	52.05
LSR	6.54	0.55	5.05	4.03	0.36	8.1

Change in oil composition of 2nd year crop, whole plant.

Date	a-Pinene	Limonene	a-Phellandrene	Fenchone	Estragole	Anethole
21/11/84	16.99	1.93	14.64	3.81	2.05	49.28
5/12/84	19.78	1.74	14.28	3.08	1.97	48.38
14/12/84	20.73	1.86	18.57	4.23	1.77	48.98
20/12/84	23.11	2.33	11.51	6.59	1.89	49.22
27/12/84	22.52	1.68	9.08	5.21	2.58	50.68
3/1/85	23.03	1.44	7.00	6.50	2.37	51.02
10/1/85	11.49	1.52	9.23	9.26	2.01	58.10
17/1/85	11.37	1.48	13.95	10.17	2.10	53.79
24/1/85	14.59	1.67	9.50	10.56	2.05	51.94
31/1/85	10.43	1.18	7.91	12.71	2.23	59.72
7/2/85	8.37	1.05	9.06	20.63	2.31	53.63
14/2/85	11.40	1.36	8.33	17.73	2.44	54.14
22/2/85	15.67	1.31	10.41	18.48	2.08	45.94
1/3/85	13.06	1.28	9.35	18.96	2.33	49.06
8/3/85	12.67	1.24	6.38	16.60	2.46	56.95
15/3/85	11.63	1.23	7.24	13.97	2.39	58.36
LSR	8.53	0.54	7.18	5.86	0.51	7.66

Alpha-phellandrene: Levels of this compound were initially high in the second year crop. The highest level was 18.57 percent on December 14. After this date the level of alpha-phellandrene decreased significantly to a minimum value of 6.38 percent on March 8. In the first year crop no significant change in alpha-phellandrene content was observed during January and February. A maximum level of 13.9 percent occurred on February 22. The only significantly lower value was obtained on March 8.

Fenchone: The levels of this compound present in the second year crop increased steadily from a minimum value of 3.08 percent on December 5 to a maximum of 20.63 percent on February 7. A significant decrease was observed from this date to the completion of the experiment on March 15. In the first year crop during January the levels of fenchone fluctuated from 8.73 percent to 13.92 percent then down to 8.59 percent. From January 24 until the completion of the experiment no significant change was observed in the levels of fenchone in the oil from whole plants.

Estragole: In the second year crop the changes in the levels of this compound were small, ranging from the lowest value of 1.77 percent on December 14 to a maximum value of 2.58 percent on December 27. Similar small changes were observed in the first year crop. The highest value was 2.51 percent on January 3 and the lowest value was 1.9 percent on March 1.

Anethole: The levels of anethole in the second year crop increased during the month of December and into January from 49.28 percent to 58.1 percent on January 10. The maximum level attained was 59.72 percent on January 31. In February the levels fluctuated with an increased level observed only on March 15. In the first year crop the maximum level of anethole, 61.06 percent, occurred on January 10. During February and into March no significant difference was recorded in anethole content.

1.3.4 Change in oil composition from umbels

The changes in composition of the main components of oil from the umbels of both first and second year crops are given in Table IV.A.1.2.

Alpha-pinene: For the second year crop the highest level of alpha-pinene, 10.16 percent, was recorded on January 3. After this date the level of alpha-pinene declined rapidly. No significant difference was noted over the period from January 17 to March 8. The level of alpha-pinene present in the first year crop did not change significantly during the experiment.

Limonene: The changes in the content of this compound were very small in the second year crop and not significant in the first year crop. In the second year crop the highest value recorded was 2.01 percent on January 17. In the first year crop levels were approximately 1.5 percent.

Alpha-phellandrene: In the second year crop the maximum level was 8.06 percent on January 17. Two weeks later the level had dropped significantly to 4.19 percent and remained statistically the same up to the conclusion of the experiment. For the first year crop the highest level of alpha-phellandrene was recorded on January 10, 17.02 percent. No significant change resulted until February 7. After this date levels steadily decreased to a minimum value of 4.6 percent at the conclusion of the experiment.

Fenchone: Levels of fenchone present in the second year crop increased from a minimum value of 8.96 percent on January 17 to a maximum value of 24.93 percent on March 1. Similar changes were observed in the first year crop. An increase from 7.49 percent on January 10 to 13.18 percent on March 1.

Estragole: Changes in the levels of estragole were small in both crops. The range for the second year crop was 2.23 percent to 2.75 percent and for the first year crop was 1.53 percent to 2.3 percent.

TABLE IV.A.1.2

Change in oil composition 1st year crop, umbels.

Date	a-Pinene	Limonene	a-Phellandrene	Fenchone	Estragole	Anethole
3/1/85	9.23	1.42	9.40	7.49	1.85	56.48
10/1/85	8.56	1.52	17.02	7.63	1.53	55.81
17/1/85	8.99	1.37	12.07	12.20	2.04	54.88
24/1/85	11.96	1.58	15.03	9.79	2.07	52.42
31/1/85	10.24	1.40	10.26	11.12	2.30	57.76
7/2/85	9.92	1.48	12.34	11.45	2.25	55.80
14/2/85	7.57	1.73	9.55	12.48	1.79	57.00
22/2/85	8.17	1.79	6.36	11.27	2.03	60.25
1/3/85	6.93	1.31	8.07	13.18	2.00	62.56
8/3/85	7.15	1.68	4.67	12.16	2.15	59.90
LSR	6.07	0.54	5.92	5.10	0.43	8.69

Change in oil composition 2nd year crop, umbels.

Date	a-Pinene	Limonene	a-Phellandrene	Fenchone	Estragole	Anethole
3/1/85	10.16	1.69	7.86	13.24	2.45	60.84
10/1/85	8.39	1.71	7.77	14.12	2.23	59.76
17/1/85	7.52	2.01	8.06	8.96	2.65	61.40
24/1/85	3.39	1.38	5.34	18.29	2.36	65.64
31/1/85	5.43	1.66	4.19	16.66	2.51	66.66
7/2/85	4.14	1.52	3.10	22.11	2.45	63.99
14/2/85	4.22	1.65	2.93	21.37	2.61	63.67
22/2/85	3.71	1.20	2.28	23.99	2.75	63.53
1/3/85	2.45	1.18	2.57	24.93	2.60	63.22
8/3/85	3.66	1.13	2.55	24.16	2.64	63.25
LSR	5.60	0.52	3.20	7.91	0.45	11.74

Anethole: No significant change was recorded in the content of anethole in the umbels of the second year crop. Levels remained at approximately 60 percent to 63 percent. In the first year crop the minimum value of 52.42 percent was recorded on January 24. The levels increased to 62.56 percent on March 1.

1.4 Discussion

Generally the percent oil yields obtained were in agreement with those obtained by most workers. A yield of 2.5 percent from umbels is in agreement with Ashraf and Bhatty (1975). From the end of February and into the first week of March a similar percentage oil yield from umbels did not vary between the first and second year crops. Variation was only encountered in the early stages of development indicating the differences in the rate of development between the established crop and new crop. A newly sown crop must undergo an establishment phase whilst the second year crop has had a full year to become well established.

Percent oil yields from whole plant subsamples did vary between the first and second year crops. The first year plants achieved higher percent yields on both a dry and fresh weight basis. The contribution to percent oil yield from the umbels was lower in the first year crop which indicates that the vegetative portions of the plant are able to produce more secondary metabolites in their first season of growth than in subsequent years. Alternatively the first year crop may have a higher proportion of leaf to umbels than a second year crop.

The contribution by the umbels towards the overall oil yield per unit area was also lower for the first year crop. The second year crop was able to achieve the higher whole plant oil yield as a result of increased production of plant material per unit area. Such an increase in dry matter production would be possible in the second year once the plant had established a deep rooting system and a greater number of shoots. For a second year crop a period of active vegetative growth would ensue from the point of first harvest through the winter months increasing the root development of the plant.

Many changes in the composition of the oil from both whole plants and umbels occurred throughout the maturation of the crop. Generally

the changes in the composition of the umbels were also observed in the oil from whole plants. This was due to the umbels producing the majority of the oil, particularly in the second year crop. This effect was not as pronounced in the first year crop.

The levels of anethole from the umbel material were higher than those of the whole plant, in agreement with Embong et al. (1976), but only 10 percent greater, not 30 percent as indicated by these workers. In addition higher levels of limonene and fenchone observed by Embong et al. (1976) were not present in the variety C22.

Similarities to the changes recorded by El-Genghaihi and Hornok (1978) in dill were observed in the umbel with the terpinenes, alpha-pinene and alpha-phellandrene decreasing. A similar decrease in the level of limonene occurs in dill, but this did not occur in the fennel umbel.

Fenchone was the only compound observed to increase substantially during the maturation of the crop, particularly in the umbels. In the second year crop the levels of fenchone were higher than those from the whole plant, but the first year crop contained very similar levels. Anethole content did increase in the oil from umbels of the first year crop but remained constant in the umbels of the second year crop.

IV.A.2 The contribution of each umbel order to overall oil yield and oil composition.

2.1 Introduction

Previous examination of other members of the Apiaceae have shown that the various umbel orders mature at different rates, (Literature Review, Section III.2.1). As a result of this uneven maturation, the period of maximum oil yield is the cumulative effect of the maximum oil yield of each umbel order. The yield component of each individual umbel order must be identified to determine the overall yield profile of a crop.

2.2 Materials and Methods

The crop examined in this experiment was the variety C22 in its first year of production at Richmond in Southern Tasmania. A total of nine harvests were taken over the period of umbel maturation, January 20 to April 2. Four replications each of ten plants were harvested and separated into each individual umbel order. All samples were examined for oil yield and composition by the techniques outlined in the General Materials and Methods. In the first four harvests taken, only the number of umbels emerged was determined as insufficient material was available for oil yield determination.

2.3 Results

2.3.1 Number of umbels and oil yield

Figure IV.A.2.1 indicates the number of umbels of each umbel order present during the major oil yielding period of the crops growth. The numbers of primary umbels rapidly attained an already pre-determined maximum, dependent only on the number of plants examined. The number of secondary and tertiary umbels behave similarly. The quarternary umbels reached a maximum mean number per plant on February 27. After this date the number gradually declined.

This decline was due to abortion of the seed followed by senescence of the whole umbel. During the period of maximum oil yield, March 20, the largest number of umbels present were of the third order. The next largest number of umbels present at this stage were the quarternaries, then secondaries and finally the primary.

Table IV.A.2.1 Change in % oil yield (fresh weight)

Date	Primary	Secondary	Tertiary	Quarternary	LSD
17/2	1.99	1.52	1.42	1.51	ns
27/2	2.13	1.91	1.62	1.21	0.32
10/3	2.17	2.25	1.82	1.38	0.59
20/3	2.15	1.78	2.25	2.13	ns
2/4	3.19	2.11	2.01	2.15	ns
LSR	1.00	0.66	0.45	ns	

The percent oil yield, on a fresh weight basis, was significantly different between umbel orders over the period February 27 to March 10 (Table IV.A.2.1). During this period the percent oil yield of the quarternary umbels was significantly lower than the percent yield from the primary or secondary umbels. No difference in percent oil yield was detected between the primary, secondary and tertiary umbels. At all other harvest dates no significant differences were recorded.

For the primary umbel the percent oil yield on a fresh weight basis increased only on April 2 whilst at all other harvest dates no significant change occurred. A maximum percent oil yield for the secondary umbels was detected on March 10, whilst the maximum from the tertiary umbels was 10 days later. No significant change in percent oil yield was observed from the quarternary umbels during the period of observation.

Table IV.A.2.2 contains percent oil yield data expressed on a dry weight basis from the umbel orders. Only on February 27 was there a significant difference in percent oil yield between the umbel orders. At this date the primary and secondary umbels yielded significantly higher than the tertiary umbels which, in turn, yielded higher than the quaternary umbels.

Table IV.A.2.2 Change in % oil yield (dry weight)

Date	Primary	Secondary	Tertiary	Quarternary	LSD
17/2	8.13	6.22	5.79	4.74	ns
27/2	8.24	7.61	6.51	4.75	1.08
10/3	7.89	8.81	7.06	5.37	ns
20/3	7.05	6.17	8.53	8.22	ns
2/4	6.77	5.47	7.21	7.16	ns
LSR	ns	2.10	2.13	2.97	

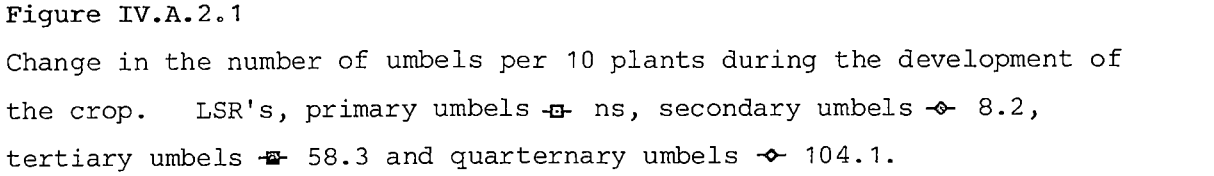
For the primary umbel the percent oil yield on a dry weight basis did not change significantly throughout the experiment. The secondary umbels reached a significantly higher yield on March 10 whilst the tertiary umbels produced a maximum 10 days later. The quarternary umbels also produced their significantly highest percent oil yield on a dry weight basis on March 20.

The oil yield per umbel for each umbel order is shown in Figure IV.A.2.2. No significant difference was observed between the tertiary and quarternary umbels in oil production. The secondary umbels produced slightly more oil than these two orders whilst the primary was the highest oil producing structure within the floral canopy.

The primary and secondary umbels both reached maximum oil yield on March 10. No significant change in the oil yield of the primary umbel occurred over the next 10 days whilst the secondary umbels maintained a constant yield until April 2. The tertiary and quarternary umbels reached significantly higher oil yields on March 20 and no significant decreases were observed until April 2.

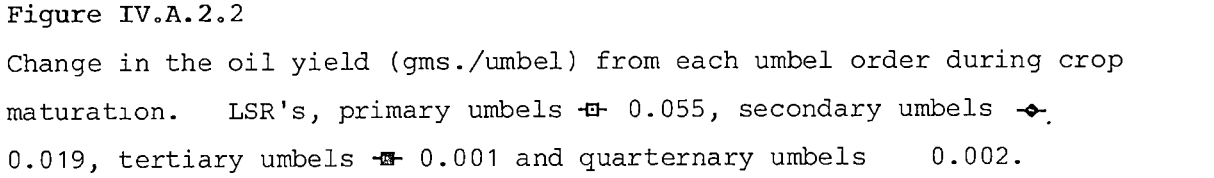
The oil yield from each umbel order was also expressed on a per plant basis (Figure IV.A.2.3). The major oil yielding group was the secondary umbels followed by the tertiary umbels. The secondary umbels contributed significantly more oil than the tertiary umbels on February 27 and March 10, at all other harvest dates no difference was detected. The total oil yield by the primary umbel was not significantly different to the total contribution by the quarternary

Figure IV.A.2.1

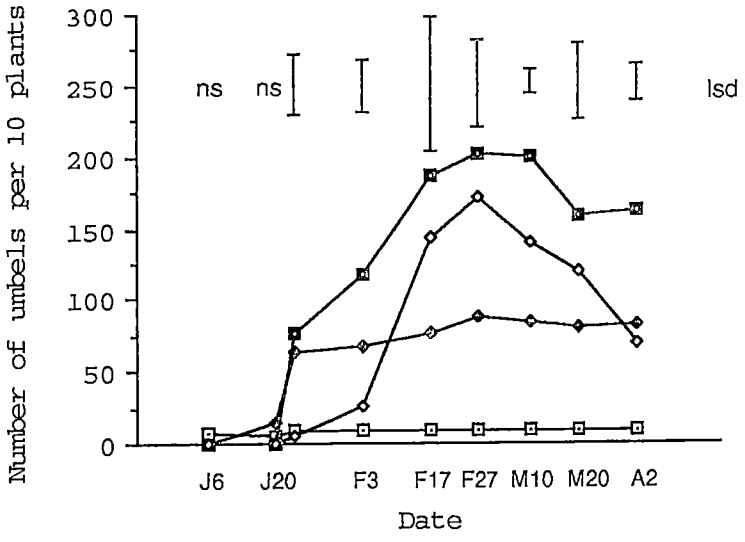


Change in the number of umbels per 10 plants during the development of the crop. LSR's, primary umbels ns, secondary umbels 8.2, tertiary umbels 58.3 and quarternary umbels 104.1.

Figure IV.A.2.2



Change in the oil yield (gms./umbel) from each umbel order during crop maturation. LSR's, primary umbels 0.055, secondary umbels 0.019, tertiary umbels 0.001 and quarternary umbels 0.002.


Number of umbels per 10 plants
300
250
200
150
100
50
0
ns
ns
lsd
J6
J20
F3
F17
F27
M10
M20
A2
Date

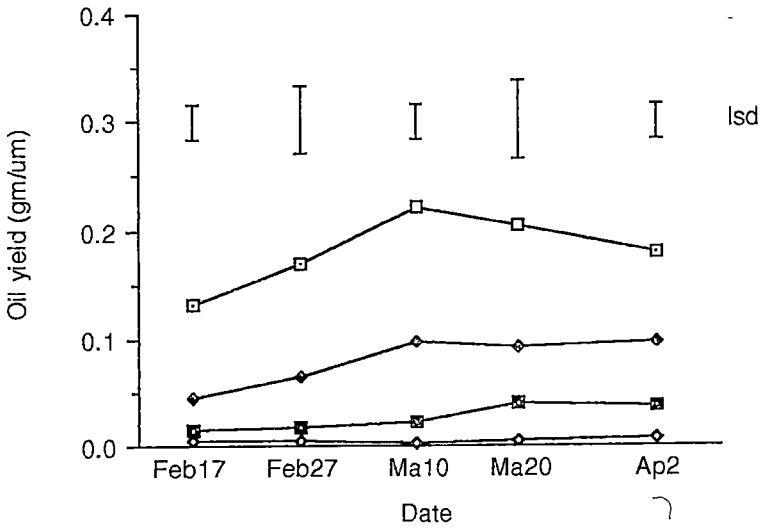
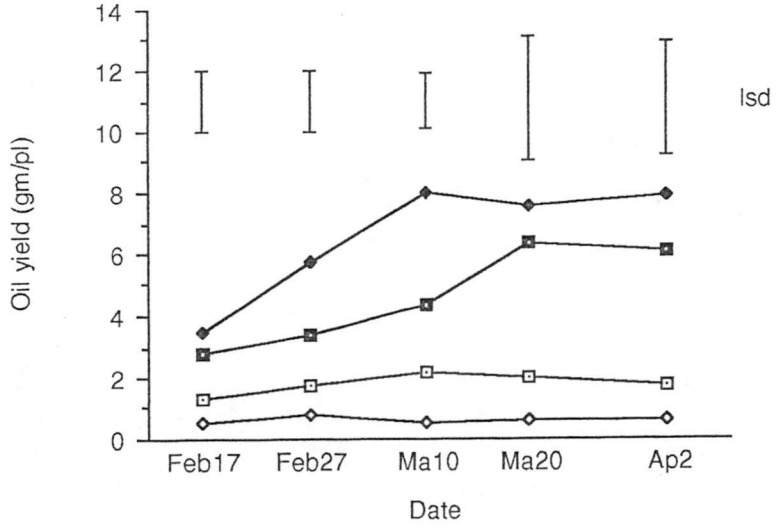

Oil yield (gm/um)
0.4
0.3
0.2
0.1
0.0
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date

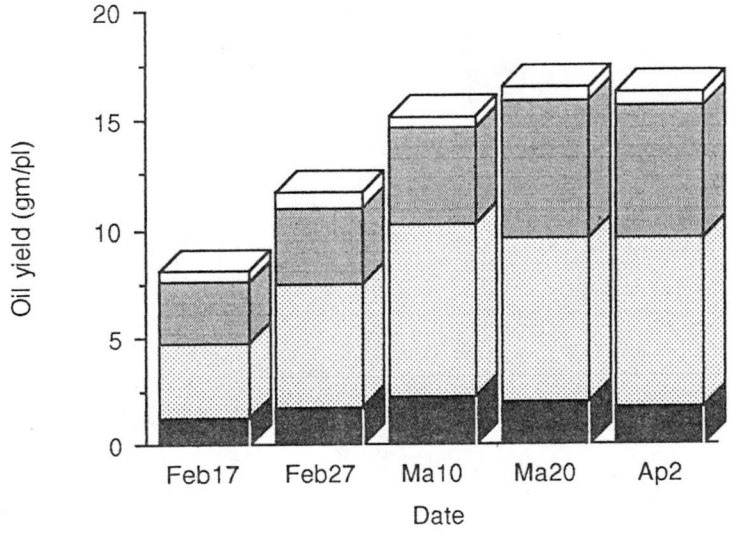
Figure IV.A.2.3

Change in oil yield on a per plant basis from each umbel order during crop maturation. LSR's primary umbels -⊡- 0.5, secondary umbels -◈- 1.88, tertiary umbels -■- 2.74 and quarternary umbels -◇- ns.

Figure IV.A.2.4

The accumulative effect of the oil yield from each umbel order during crop maturation. Primary umbel ▪, secondary umbels ⬚, tertiary umbels ▩ and quarternary umbels □.


14
12
10
8
6
4
2
0
Oil yield (gm/pl)
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date


20
15
10
5
0
Oil yield (gm/pl)
Feb17
Feb27
Ma10
Ma20
Ap2
Date

umbels at any harvest date. The oil yield from the quarternary umbels per plant did not change significantly during the experiment whilst the primary umbel increased in oil yield to a maximum over the period March 10 to 20. No significant change in oil yield from the tertiary umbels occurred over the period February 17 to March 10. After this period the oil yield increased significantly. The oil yield per plant from the secondary umbels increased significantly until March 10, then no significant change was observed up to April 2.

Figure IV.A.2.4 indicates the cumulative effect of the oil yield from each umbel order towards the overall oil yield. The maximum oil yield per plant occurred on March 20. At this time the contribution to total umbel oil yield was as follows:

Primary umbel	12.3 percent
Secondary umbels	45.4 percent
Tertiary umbels	38.4 percent
Quarternary umbels	3.8 percent

2.3.2 Oil composition

The results of the analysis of oil composition from the primary, secondary, tertiary and quarternary umbels during this experiment are shown in Figure IV A.2.5 (a to i).

Myrcene: (Figure IV.A.2.5.a)

Significant differences in the levels of myrcene between the umbel orders were recorded over the period of February 27 to March 20. On February 27 no difference between the content of myrcene in the oil from the secondary, tertiary and quarternary umbels was detected. The oil from the primary umbel was significantly lower in myrcene content than that of the tertiary umbels. In fact, at no stage of the experiment were the primary and secondary umbels significantly different in the content of myrcene in their oil.

On March 10 both the quarternary and tertiary umbels were significantly higher in myrcene content than the primary umbels. Ten days later the tertiary umbels were not significantly different in myrcene content to the primary umbels, only the quarternary umbels

were higher. This change was due to the myrcene content of the tertiary umbels decreasing steadily over the period from February 17 to March 20 to levels similar to the primary umbels. During the same period the mycrene content in the quarternary umbels increased to March 10 then decreased until April 2. At this time no difference in mycene content was detected between any umbel order.

Cis-beta-Ocimene: (Figure IV.A.2.5.b)

The quarternary umbels contained higher levels at all stages of maturation. All umbel orders decreased in cis-beta-ocimene content over the period February 17 to April 2, but at different rates. The quarternary umbels decreased the most followed by the tertiary umbels then the secondary umbels. The primary umbels decreased only a very small amount in content of cis-beta-ocimene content over this period.

Beta-phellandrene (plus cineole): (Figure IV.A.2.5.c)

Levels of these two compounds gradually decreased during the period of observation for all umbel orders except quarternary umbels. The levels present were highest in oil from the lower orders. At the commencement of observations and ten days later the only significant difference was between the quarternary and primary umbel levels. On March 10 the secondary umbel levels resembled the primary whilst the quarternary and tertiary umbels were also statistically the same. By March 20 the percent beta-phellandrene (plus cineole) content in the tertiary umbel oil had decreased further which resulted in no significant difference between the first three orders. All contained approximately 0.5 percent. Only the quarternary umbel oil still contained a significantly higher percentage of these compounds, 1.0 percent.

Limonene: (Figure IV.A.2.5.d)

Very small significant differences between the limonene content for all four orders was observed throughout the experiment. During the period from March 10 to 20, the limonene content of the tertiary and quarternary umbel oil was significantly higher than the primary and secondary umbel oil.

The content of limonene present in the oil from primary, secondary and tertiary umbels decreased steadily with time. For the quarternary umbel oil the limonene content did not change significantly during the experiment.

Alpha-phellandrene: (Figure IV.A.2.5.e)

On February 17 the quarternary umbel oil contained significantly higher amounts of alpha-phellandrene than any other umbel order. From this period onwards no significant difference in level of alpha-phellandrene was detected in the primary, secondary and tertiary umbel oil. Only the quarternary umbels contained significantly higher levels.

Alpha-phellandrene content decreased for all umbel orders during the period of observation, but as with the change in cis-beta-ocimene the rate of decrease was greater the higher the umbel order. The levels of alpha-phellandrene in the oil from the primary umbel decreased from 2.22 percent to 1.2 percent whilst the levels detected in the oil from the quarternary umbels decreased from 10.52 percent to 1.73 percent.

Alpha-pinene: (Figure IV.A.2.5.f)

The content of alpha-pinene was not significantly different between the primary, secondary and tertiary umbels at any harvest date. The quarternary umbel oil was also not significantly different to the other umbel orders on February 17, 27 and April 2, but on March 10 and 20 the content of alpha-pinene was higher for the quarternary umbels.

The alpha-pinene content for primary umbels decreased significantly from February 27 to April 2 whilst for the secondary umbels the decrease was over an earlier period, from February 17 to March 20. The tertiary umbel oil increased in alpha-pinene level from February 17 to 27 then decreased to a constant level at March 20. The quarternary umbels exhibited the same increase as the tertiary umbels but continued to increase to a maximum of 7.9 percent on March 10, then decreased rapidly by April 2.

Fenchone: (Figure IV.A.2.5.g)

Throughout the experiment the levels of fenchone for each umbel order remained constant. The only significant differences in the level of this compound were detected between the primary, secondary and tertiary umbel orders. The levels fluctuated only a small amount, maintaining a level of approximately 20 percent. The

Figure IV.A.2.5.a.

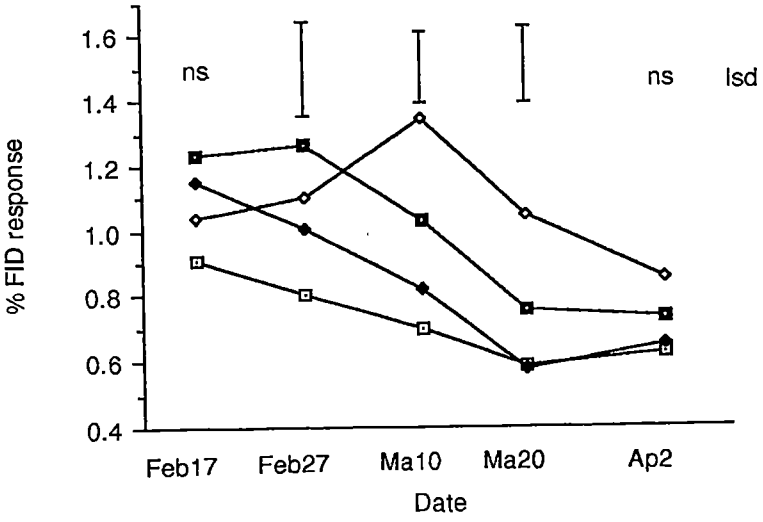
Change in the levels of Myrcene present in oil from the first four umbel orders. LSR's, primary umbel ⊡ 0.29, secondary umbels ◆ 0.22, tertiary umbels ◙ 0.17 and quarternary umbels ◇ 0.31.

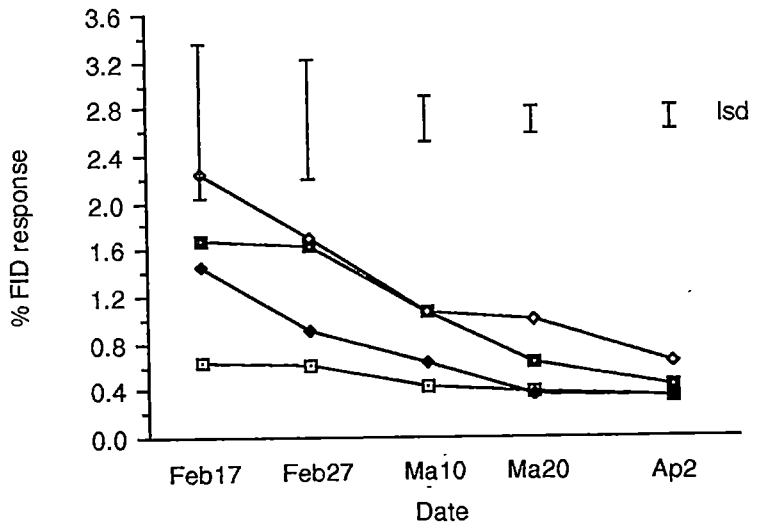
Figure IV.A.2.5.b.

Change in the levels of Cis-beta-Ocimene present in oil from the first four umbel orders. LSR's, primary umbel ⊡ 0.25, secondary umbels ◆ 0.89, tertiary umbels ◙ 0.83 and quarternary umbels ◇ 0.84.

Figure IV.A.2.5.c.

Change in the levels of Beta-Phellandrene (+Cineole) present in oil from the first four umbel orders. LSR's primary umbel ⊡ 0.18, secondary umbels ◆ 0.20, tertiary umbels ◙ 0.36 and quarternary umbels ◇ ns.


% FID response
1.6
1.4
1.2
1.0
0.8
0.6
0.4
ns
ns
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date


% FID response
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date

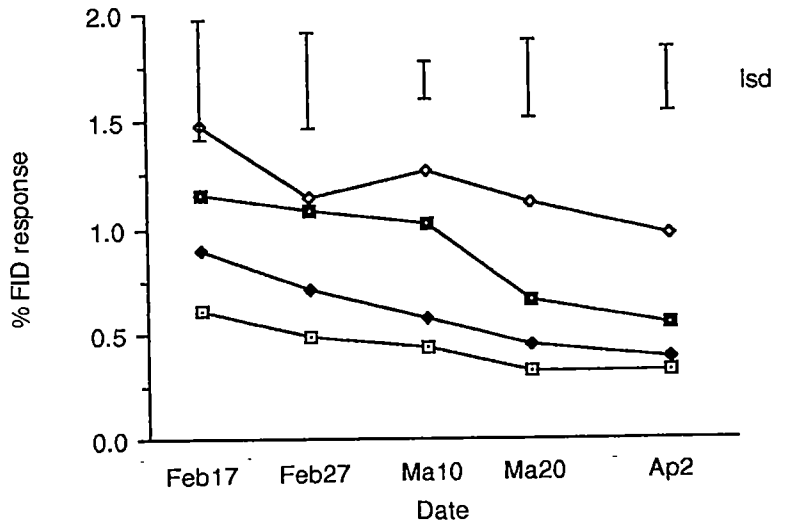

% FID response
2.0
1.5
1.0
0.5
0.0
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date

Figure IV.A.2.5.d.

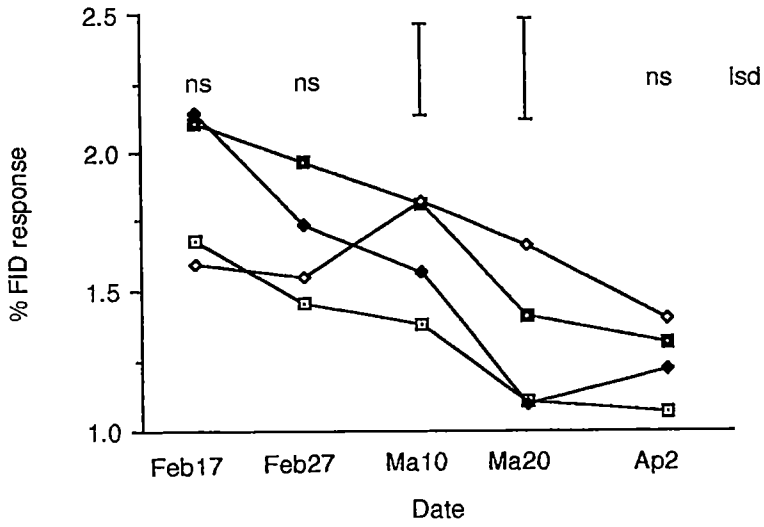
Change in the levels of Limonene present in oil from the first four umbel orders. LSR's primary umbel -⊡- 0.53, secondary umbels -◈- 0.39, tertiary umbels -▣- 0.24 and quarternary umbels -◇- ns.

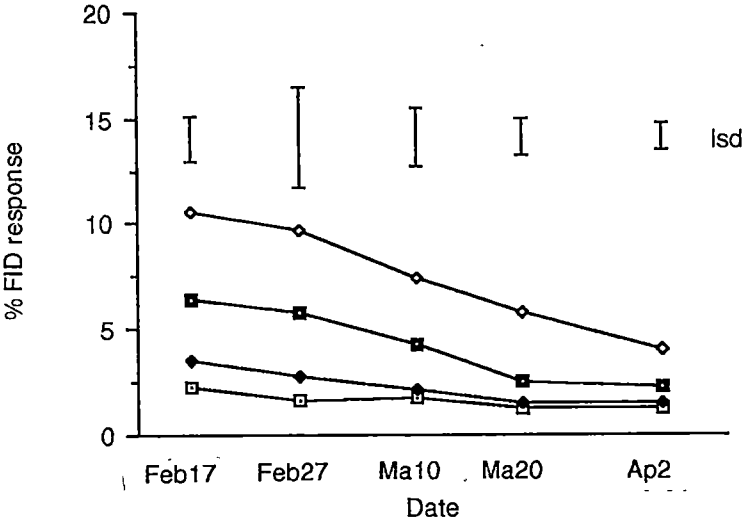
Figure IV.A.2.5.e.

Change in the levels of Alpha-Phellandrene present in oil from the first four umbel orders. LSR's primary umbel -⊡- 0.65, secondary umbels -⊡- 0.65, tertiary umbels -▣- 2.86 and quarternary umbels -◇- 4.77.

Figure IV.A.2.5.f.

Change in the levels of Alpha-Pinene present in oil from the first four umbel orders. LSR's primary umbel -⊡- 1.81, secondary umbels -◈- 1.01, tertiary umbels -▣- 2.45 and quarternary umbels -◇- 2.66.


2.5
2.0
1.5
1.0
% FID response
ns
ns
ns
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date


20
15
10
5
0
% FID response
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date

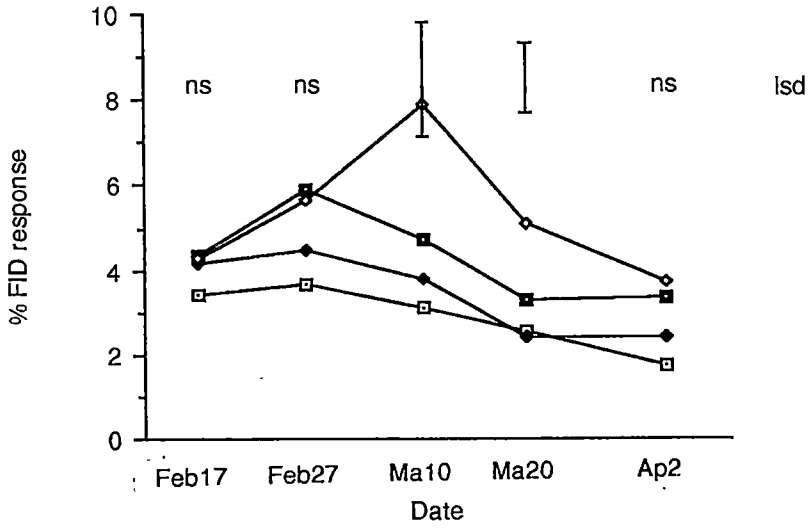
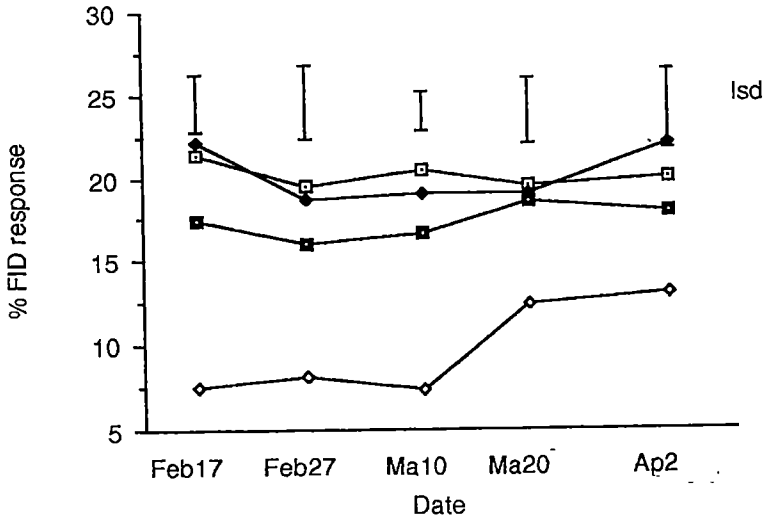
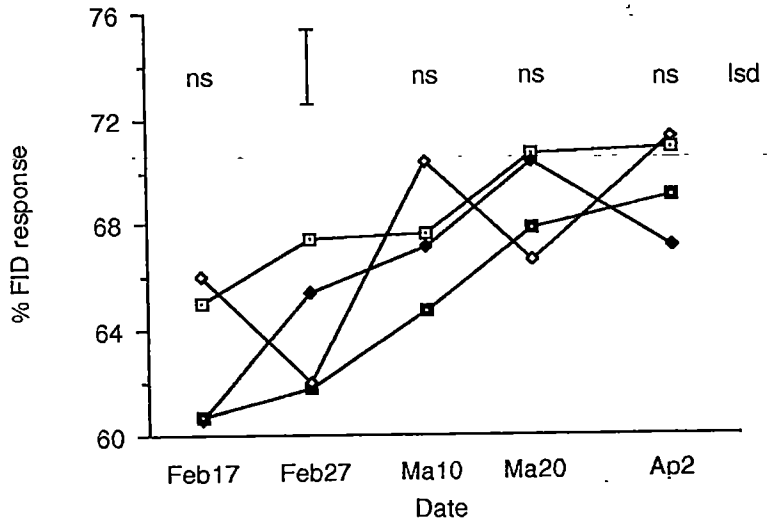

10
8
6
4
2
0
% FID response
ns
ns
ns
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date

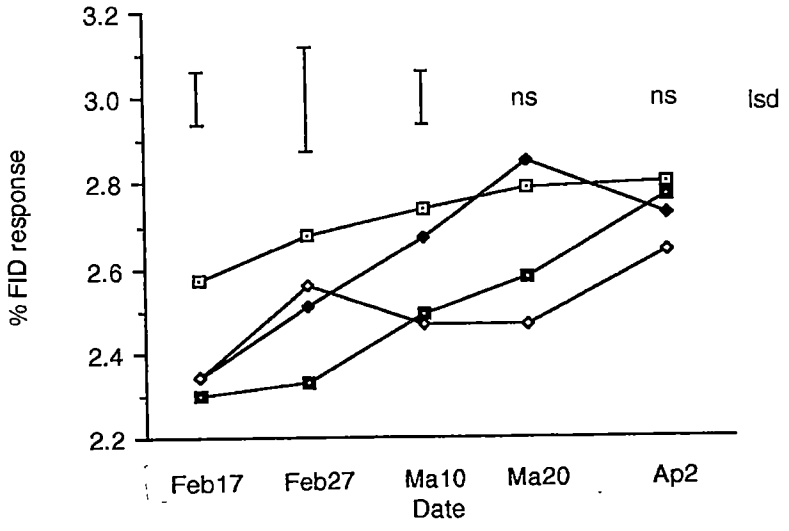
Figure IV.A.2.5.g.
Change in the levels of Fenchone present in oil from the first four umbel orders. LSR's all not significant, primary umbel ⊡ , secondary umbels ◇ , tertiary umbels ■ and quarternary umbels ◇ .

Figure IV.A.2.5.h.
Change in the levels of Anethole present in oil from the first four umbel orders. LSR's primary umbel ⊡ 2.90, secondary umbels ◇ 5.23, tertiary umbels ■ 3.57 and quarternary umbels ◇ 6.91.

Figure IV.A.2.5.i.
Change in the levels of Estragole present in oil from the first four umbel orders. LSR's primary umbel ⊡ 0.20, secondary umbels ◇ 0.18, tertiary umbels ■ 0.28 and quarternary umbels ◇ ns.


30
25
20
15
10
5
% FID response
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date


76
72
68
64
60
% FID response
ns
ns
ns
ns
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date


3.2
3.0
2.8
2.6
2.4
2.2
% FID response
ns
ns
lsd
Feb17
Feb27
Ma10
Ma20
Ap2
Date

quarternary umbel oil exhibited significantly lower level of fenchone than the other three umbel orders throughout the experiment.

Anethole: (Figure IV.A.2.5.h)

The levels of anethole increased throughout the course of the experiment for all umbel orders, except the quarternary umbels. In this order the final level of anethole was higher than the initial levels, but the changes during this period were erratic. No significant difference was recorded between any of the umbel orders for all harvests other than on February 27 when the primary and secondary levels were significantly higher than the quarternary and tertiary. Mean levels of anethole were in the range of 60 to 65 percent initially and increased to 65 to 70 percent at the conclusion of the experiment.

Estragole: (Figure IV.A.2.5.i)

On February 17 no significant difference in the content of estragole was recorded for secondary, tertiary and quarternary umbels. The oil contained approximately 2.3 percent. Only in the oil from the primary umbel were significantly higher levels of estragole detected. A maximum of 2.57 percent. After a further 10 days, the oil from the primary umbel contained significantly higher levels of estragole than the tertiary oil. On March 10 similar results were obtained except that the estragole content of the oil from the quarternary umbels was also lower. No significant differences in estragole levels were observed between the umbel orders after this time.

Increases in the levels of this compound in all orders, except quarternary, were recorded for the duration of the experiment resulting in a final content of approximately 2.65 percent to 2.8 percent for all orders. The rate of increase was higher for the secondary and tertiary umbels.

2.4 Discussion

The total production of oil from each umbel follows the sequence of the orders, decreasing from primary through to quarternary. Such

a difference in oil production may be due to the differences in time available in which to achieve maturation. The lower orders emerge progressively later in respect to the primary umbel. Other contributing factors may be fewer rays and less seed in the lower order umbels. Another explanation may be competition for assimilates between the orders. The primary umbel is possibly apically dominant in respect to assimilate balance. Investigation of assimilate partitioning may provide an insight into the variation in oil yield per umbel.

The percent oil yield on a fresh weight basis for the primary umbels increased markedly from March 20 to April 2 due to seed maturation. The natural maturation process of the seed includes a final stage of lignification of the seed coat in readiness for abscission from the rays. During this period the moisture content decreases. No actual increase in oil production occurs, as was indicated when oil yield was expressed on a dry weight basis.

The decrease in the number of quarternary umbels per plant was a result of the senescence of umbels that lacked fertilized seed. This was possibly due to insufficient pollinators present or lack of pollen. Because of the progressive nature of anther production and protandry in fennel inflorescences, no pollen may have been available when the flowers of the quarternary umbel were receptive. This situation is unlikely due the large spread of flowering time observed within the seed line under examination. Environmental conditions during the period in which the stigma was receptive may have been unfavourable for fertilization.

The maximum oil yield per unit area from a whole plant was on March 20. This yield was mainly due to the cumulative effect of the percent oil yield and the number of secondary and tertiary umbels present at this time.

Porter et al. (1983) indicated that the primary and secondary umbels were the main producers of oil in dill. In fennel the secondary and tertiary orders were determined to be the major contributors. The importance of these findings in respect to the cultural practices during umbel development will be discussed later.

The compounds alpha-pinene, alpha-phellandrene, cis-beta-ocimene, beta-phellandrene, myrcene and limonene were present in increasingly higher amounts with increasing umbel order. So that the quarternary umbel oil contained the highest level of these compounds proceeded

down to the lowest levels in the primary umbels. Such results indicates that these compounds are present in greater amounts during early development of an umbel and gradually decrease with maturation. The rate of decrease could be used as an indication of the stage of maturation of an umbel order.

During maturation, myrcene levels may initially increase to a maximum then decrease. This is indicated by the results from the oil of quarternary umbels. The period of observation for compositional changes did not commence at a sufficiently early stage of development in the other umbel orders to detect similar changes in the levels of myrcene. Only the decreasing phase was detected.

Similar changes may occur in the content of alpha-pinene during maturation of an umbel. But again the increasing phase was only detected in the quarternary umbels.

The only compounds which increased markedly in all orders during the experiment were anethole and estragole. Fenchone levels remained constant. The range of changes in estragole levels varied from 2.3 percent to 2.9 percent during the experiment. This did not account for any large change in the composition of the oil from a whole plant. Anethole content generally increased in all umbels during maturation of the seed. The range of levels detected was from 60.5 percent to 70 percent of the total FID response at the conclusion of the experiment.

IV.B. Investigation of the effect of photoperiod on flowering and development.

1.1 Introduction

Detailed studies on the photoperiodic requirements for floral initiation and the initiation process have not previously been reported for fennel. The following experiments examine the morphological changes that occur at the meristem and the conditions influencing floral induction.

1.2 Materials and Methods

2.1 Determination of the critical photoperiod for flower initiation.

2.1.1 Under field conditions.

Ten plants (variety C25) were harvested at random from a commercial field in Southern Tasmania, at weekly intervals. Samples were taken from September 3 to the October 24 and examined by dissection under a stereo microscope for signs of termination of the apical vegetative bud by differentiation into a floral apex. Apices were treated and examined by SEM as described in the General Materials and Methods.

2.1.2 Under glasshouse conditions

Fennel seedlings (var. C25) at the two true leaf stage, were transferred to the growth rooms described in the General Materials and Methods where they were subjected to the following conditions to examine the photoperiodic responses:

(1)	10 : 14	Day : Night
(2)	13 : 11	10 hrs daylight, 3hrs incandescent light
(3)	16 : 8	10 hrs daylight, 6hrs incandescent light
(4)	Night break	10 hrs daylight + four 15 min periods at 3 hour intervals during the dark period (again incandescent light)

These conditions were generated by programming the growth rooms as indicated in Appendix IV.B.1.1. The spacing of the night breaks was distributed evenly throughout the dark period. All growth rooms were maintained at constant glasshouse temperatures (indicated in the General Materials and Methods). Four plants from each treatment were labelled. The number of nodes, leaves, overall height, height to the apex, and umbel differentiation were determined for these plants at ten day intervals beginning 60 days after commencement of the experiment.

2.2 Determination of the length of the juvenile phase.

Seedling fennel plants (var. C25) at the two expanded leaf stage of development, were transferred to inductive photoperiods (as determined in experiment 2.1), in the growth rooms descibed in the General Materials and Methods. Plants were harvested at approximately 5 day intervals, from 8 to 74 days after the commencement of the experiment. At each harvest plants were examined for height, leaf number, node number and number of initiated umbels by dissection (described in the General Materials and Methods).

2.3 Determination of the number of inductive cycles required for umbel initiation.

Fennel plants (var. C25) were grown under non-inductive conditions in a growth room until the juvenile phase was completed (as determined in experiment 2.1 and 2.2). All plants were then transferred to another growth room where they were subjected to one of a number of inductive photoperiods ranging from 2 to 25 days. After treatment, the plants were returned to non-inductive photoperiods. The number of inductive cycles required for umbel initiation was determined by monitoring umbel initiation and development.

1.3 Results

3.1.1 Initiation in the field.

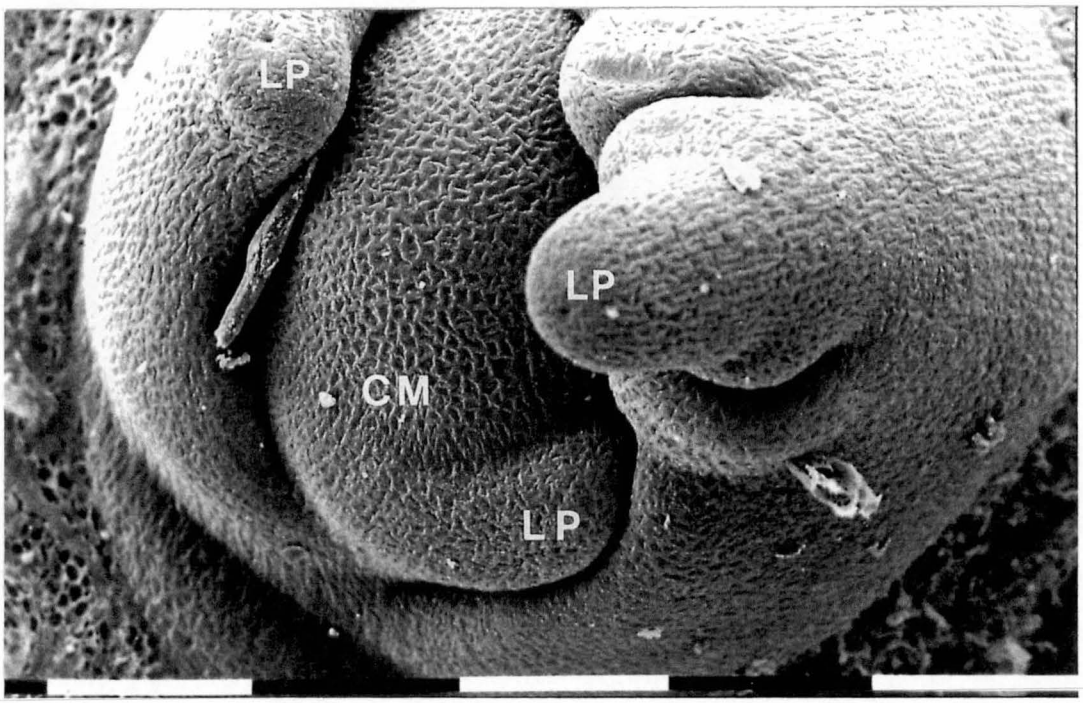
From the scanning electron microscope data (Plates IV.B.1 and IV.B.2) it was apparent that primary umbel initiation occurred in the field during the period October 17 to 27. The daylength at this time

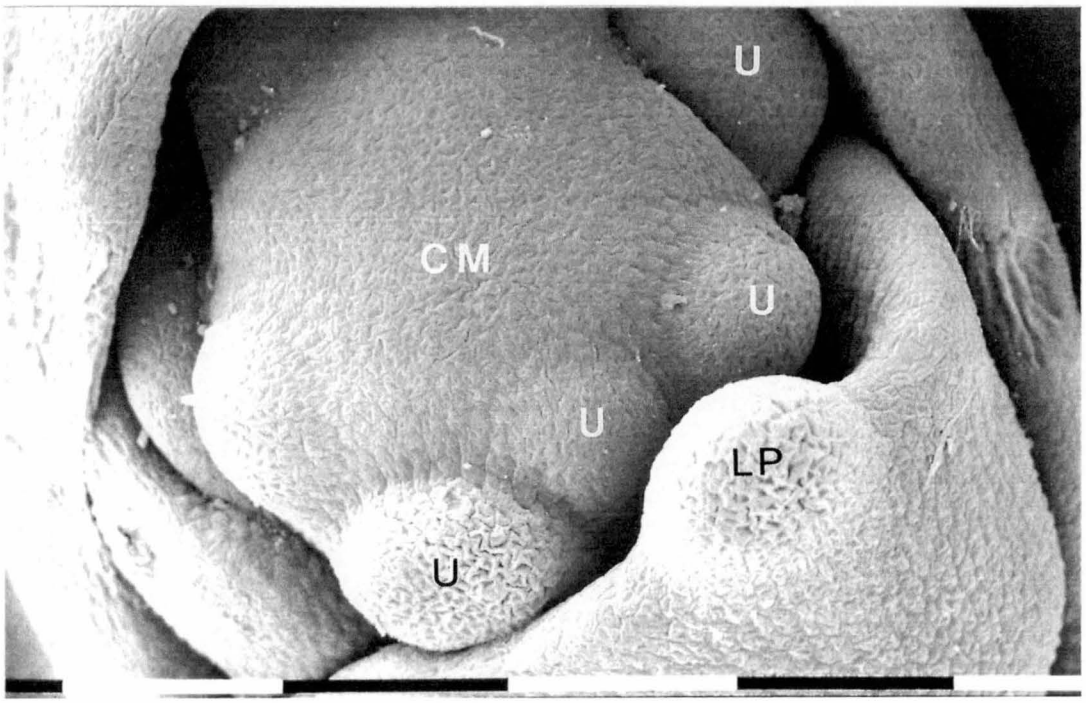
Plate IV.B.1.

A scanning electron micrograph of the meristematic region dissected from the developing main shoot of a fennel plant from the field during the first week of October. The differentiation of vegetative primordia from the central meristem (CM) can be observed as small peaked structures, these are the leaf primordia (LP). (Bar 0.01mm)

Plate IV.B.2.

The change from vegetative differentiation to floral differentiation was first detected in the third week of October. The floral primordia is distinguished the presence of a number of lobed structures. These are umbellet primordia (U). (Bar 0.01mm)


LP
LP
CM
LP


U
CM
U
U
LP
U

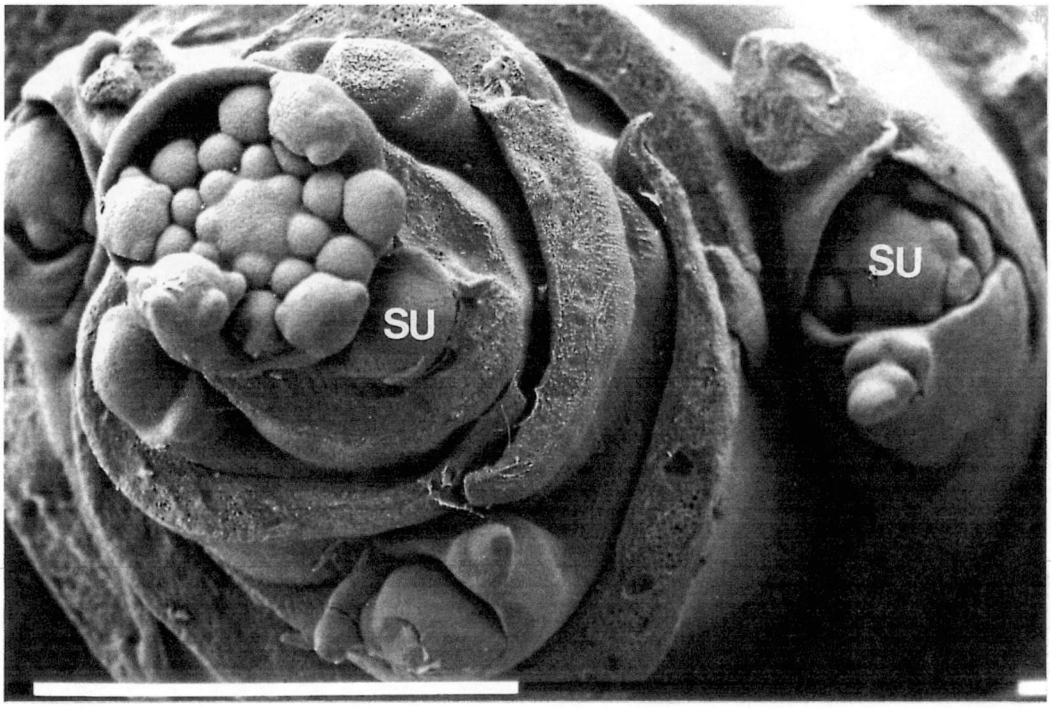


Plate IV.B.3.

A scanning electron micrograph of the dissected meristem 5 days after initial detection of floral initiation. The primary umbel has rapidly differentiated and individual umbellets are clearly visible. The umbellet primordia broadens and the primordia of the petals, stamens and carpels arise in acropetal succession. At this stage the secondary umbels have also initiated (SU). The basal secondary umbels are more developed than those nearer the primary umbel. (Bar 1mm)

was 13.5 hours and plants had 8 or 9 fully expanded leaves and 15 or 16 nodes below the meristem.

The change from a vegetative apex to a floral apex occurs rapidly. Prior to the appearance of the primordia of unbellet rays, the apical meristem was observed as a single peaked shaped form typical of a leaf primordia as in Plate IV.B.3. Umbellet primordia first appeared as round swollen structures of undifferentiated tissue which divide rapidly. After 5 days, rays of the terminating primary inflorescence were clearly discernable (Plate IV.B.2). Umbellet primordia develop centripetally and proceed to develop to form individual flowers.

Within 5 days of the observed change of the apical meristem into a floral structure, it was apparent that the meristems in the leaf axils were differentiating to form rays of the secondary umbels (Plate IV.B.3). Secondary umbels were initiated in a basipetal order.

3.1.2 Critical photoperiod

Generally, the photoperiod treatments imposed, did not affect leaf or node number during the course of the experiment (Figure IV.B.1.1 and IV.B.1.2). The only statistically significant difference obtained was 70 days after sowing. On this date the plants treated with 13 hour photoperiod had a slightly lower number of nodes and expanded leaves than all the other differently treated plants. At all other harvest dates no significant differences were observed.

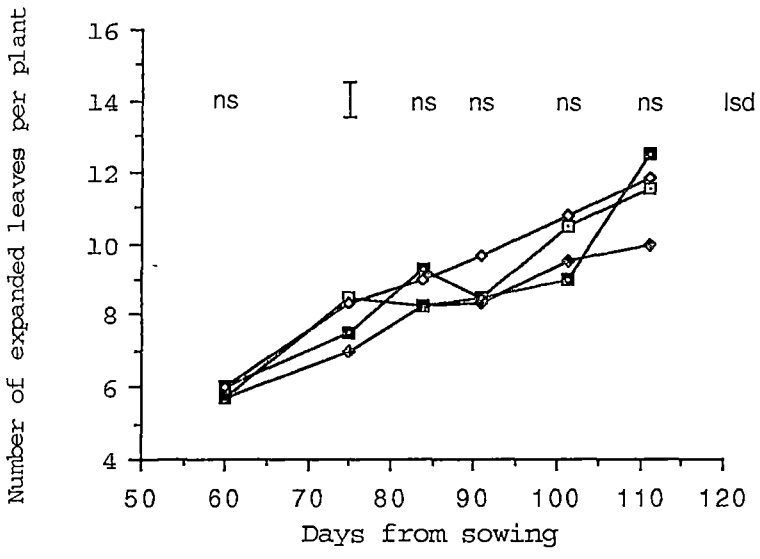
The most pronounced effect of photoperiod was on plant height. After 90 days, plants grown under long photoperiods rapidly increased in height relative to short day plants (Figure IV.B.1.3 and IV.B.1.4). The 16:8 and night break treatments were not significantly different to each other. But both elongated more than the 13:11 and 10:14 treatments.

The decrease in overall height of short day plants, after 90 days, was due to a change in leaf angle, as these plants assumed a pronounced rosette habit. Figure IV.B.1.4 indicates that no stem elongation resulted for the 13 and 10 hour photoperiod.

The rapid increase in plant height of long day plants coincided with the period of primary umbel initiation. Under the 16:8 and night break treatments, secondary umbel initiation was observed to commence when primary umbel initiation had occurred. The initiation

Figure IV.B.1.1.
Effect of different photoperiod regimes on the number of leaves present during the first 120 days of development of a fennel plant. 10 hours daylight: 14 hours dark ⊡ , 10 hours daylight plus 3 hours of incandescent light: 11 hours dark ◈ , 10 hours daylight plus 6 hours of incandescent light: 8 hours dark ▣ and 10 hours daylight plus four 15 minute periods of incandescent light during 14 hours dark ◈ .

Figure IV.B.1.2.
Effect of different photoperiod regimes on the number of nodes during the first 120 days of development of a fennel plant. 10 hours daylight: 14 hours dark ⊡ , 10 hours daylight plus 3 hours of incandescent light: 11 hours dark ◈ , 10 hours daylight plus 6 hours of incandescent light: 8 hours dark ▣ and 10 hours daylight plus four 15 minute periods of incandescent light during 14 hours dark ◈ .


Number of expanded leaves per plant
16
14
12
10
8
6
4
ns
ns
ns
ns
ns
lsd
50
60
70
80
90
100
110
120
Days from sowing

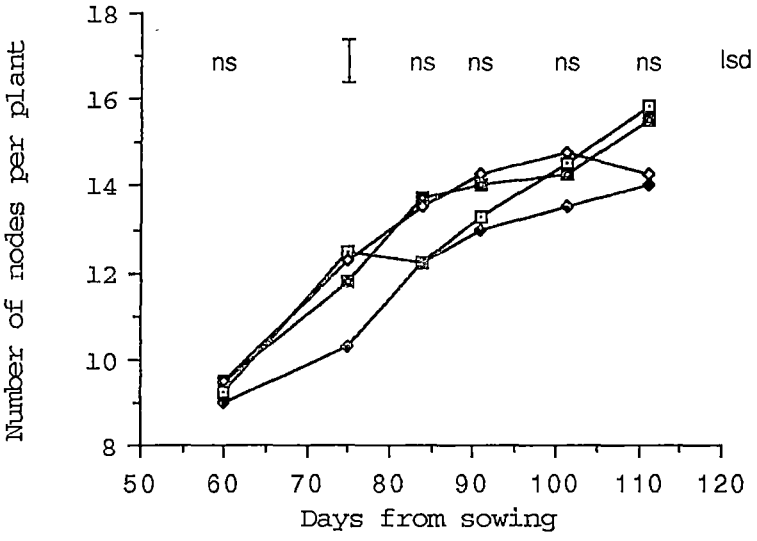
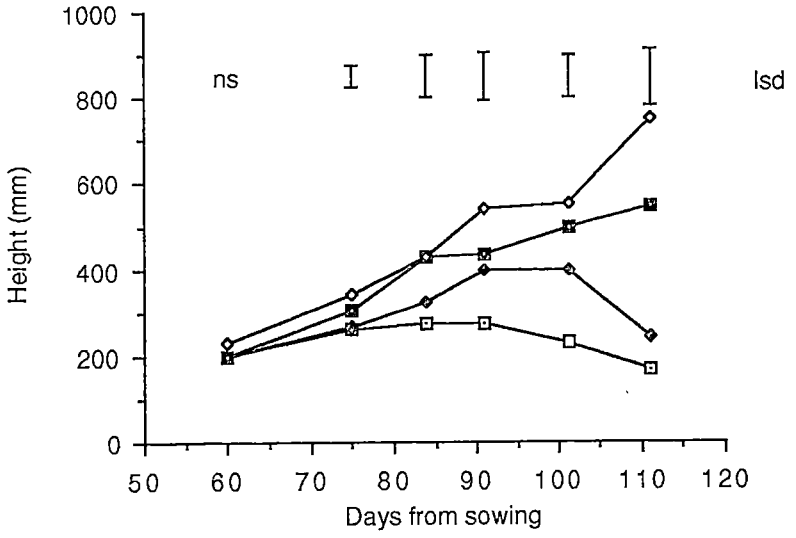
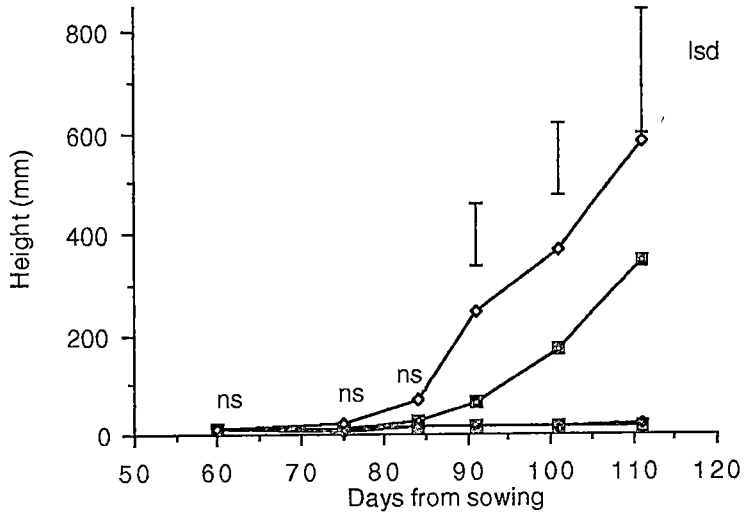

Number of nodes per plant
18
16
14
12
10
8
ns
ns
ns
ns
ns
lsd
50
60
70
80
90
100
110
120
Days from sowing

Figure IV.B.1.3.
Effect of different photoperiod regimes on the elongation of the main stem during the first 120 days of development of a fennel plant. 10 hours daylight: 14 hours dark ⊡ , 10 hours daylight plus 3 hours of incandescent light: 11 hours dark ⊙ , 10 hours daylight plus 6 hours of incandescent light: 8 hours dark ■ and 10 hours daylight plus four 15 minute periods of incandescent light during 14 hours dark ⊙ .

Figure IV.B.1.2.
Effect of different photoperiod regimes on the overall height of fennel plants during the first 120 days of development. 10 hours daylight: 14 hours dark ⊡ , 10 hours daylight plus 3 hours of incandescent light: 11 hours dark ▣ , 10 hours daylight plus 6 hours of incandescent light: 8 hours dark ⊙ and 10 hours daylight plus four 15 minute periods of incandescent light during 14 hours dark ⊙ .


1000
800
600
400
200
0
Height (mm)
ns
lsd
50
60
70
80
90
100
110
120
Days from sowing


800
600
400
200
0
Height (mm)
lsd
ns
ns
ns
50
60
70
80
90
100
110
120
Days from sowing

of secondary umbels was complete within 20 days. In contrast, plants grown under shorter photoperiods, 13:11 and 10:14, did not initiate umbels (Table IV.B.1 ').

Table IV.B.1. Photoperiod effect on flowering
Total number of inflorescences

	91 days from sowing		101 days from sowing		111 days from sowing	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
10:14	0	0	0	0	0	0
13:11	0	0	0	0	0	0
16:8	3	3	4	5	4	20
Night Break	4	8	4	16	4	17

3.2 Juvenile phase

Photoperiod conditions used for this experiment were long days consisting of 10 hours of natural daylight plus 6 hours of photosynthetically active light from a mixture of mercury vapour and fluorescent lighting (as detailed in the General Materials and Methods.

Floral initiation was observed to occur once 8 expanded leaves were present and 12.8 nodes were present (Table IV.B.2). The mean number of umbels present on the meristem increased rapidly from 0 to 1.8 after 5 days then to 4.0 after a further 5 days. A significant increase in the number of expanded leaves and the number of nodes was detected at this time. After initiation was observed, no significant increase was detected in the number of leaves and number of nodes until 29 days after initiation was detected.

Table IV.B.2. Examination of Juvenile phase.

Number of days start of treatment	Height to Apex(cm)	Number of Leaves	Number of Nodes	Number of Inflorescences
8	13.7	3.7	6.3	0
13	13.7	4.0	7.0	0
20	16.3	5.0	8.0	0
24	18.7	6.0	9.0	0
29	27.3	6.3	11.3	0
34	70.0	6.3	10.7	0
40	81.0	7.0	12.0	0
45	161.7	8.0	12.8	1.8
49	235.0	9.3	14.7	4.0
53	260.0	8.7	14.0	4.7
57	228.3	8.7	13.7	3.7
60	355.0	10.0	14.7	4.0
64	386.7	9.7	15.0	4.3
74	676.7	12.3	15.0	7.3
LSR	79.0	.7	.7	1.2

A period of rapid stem elongation was observed when initiation occurred.

3.3 Inductive cycles

Individual plants varied in their response, but general indications were that a minimum of 10 cycles of long photoperiod were required for initiation of the primary and secondary umbels (Table IV.B.1.3). The minimum number of cycles for initiation of tertiary inflorescences was 15. No significant effect was observed on the rate of elongation for 2 to 10 cycles, only the treatment of 15 cycles or more resulted in a significant elongation compared to the non induced control. The effect of these treatments can be seen in Plate IV.B.4, but again plants varied in their response.

Plate IV.B.4.

Effect of variation in the number of inductive cycles of photoperiod on the rate of elongation and development of fennel.

Control (SD) 5 cycles 10 cycles 15 cycles 20 cycles 25 cycles

Table IV.B.3. Examination of number of inductive cycles

Number of Cycles	Height of Apex after 37 days(cm)	Height of Apex after 63 days(cm)	Number of inflorescences Primary	Secondary	Tertiary
0	4.7	5.6	0	0	0
2	5.0	6.2	0	0	0
5	5.8	7.1	0	0	0
10	7.1	33.7	.4	1.7	0
15	13.5	63.7	.8	3.6	3.0
20	17.5	74.5	.9	4.1	4.0
25	27.1	93.0	1.0	4.7	4.6
LSD	5.5	29.3	.3	1.6	3.8

1.4 Discussion

Examination of the differentiating meristem by scanning electron microscopy enables precise examination of the change in morphology of the fennel plant from vegetative to floral. Once this event is triggered a period of rapid differentiation of other apices was observed. This demonstrated the strength of the initiation response once perceived by the plant. Partial floral expression was only possible over a period of 5 to 10 days. No reversion of a floral bud to a vegetative bud was observed.

The critical photoperiod for umbel initiation in fennel is greater than 13 hours. Since the night break treatment resulted in umbel initiation, it is apparent that a phytochrome mediated photoperiodic effect is involved, and not simply a photosynthetic effect due to increasing daylength. Stem elongation appeared to coincide with umbel initiation. Therefore, in the field, initiation could be detected by the extent of elongation of the main stem.

It is not clear from these results whether elongation is under direct photoperiodic control, or is a consequence of initiation, or vice versa. Application of phytohormones may allow a more detailed examination of these two responses.

It is apparent from the data presented, that fennel exhibits a

juvenile phase. This juvenile phase was terminated when the plants had developed a minimum of 8 fully expanded leaves or a minimum of 12.8 nodes. This result is in agreement with the concept of minimum leaf number required before the plant is receptive to inductive photoperiod (Holdsworth 1955). In comparison, Naylor (1941) reported that one fully expanded leaf was sufficient to induce flowering in dill.

With respect to the number of inductive cycles, 40 percent of plants initiated primary umbels after only 10 cycles whereas 25 cycles were required for 100 percent initiation. Similarly, more than 10 cycles were required for maximum initiation of secondary and tertiary umbels. That is the variety C25 would appear to require more than the 5 or 7 cycles required for full initiation in Florence fennel (Mol 1981). The diversity observed in response to variation of inductive cycles may indicate that considerable genetic diversity may exist in the commercial seed line C25. For further experiments in the glasshouse with small numbers of plants, diversity may be reduced by production of clonal plants by tissue culture methods.

IV.C. Investigation of the use of growth regulatory substances to control elongation, flowering and development.

C.1 Pot trial evaluation of a number of growth regulatory chemicals.

1.1 Introduction

No comprehensive study of chemical manipulation of fennel as a crop has been reported in the literature. In order to initiate a major investigation it was first necessary to screen a number of different growth regulatory substances to provide an understanding of their affect on the growth and development of the fennel plant. From such a preliminary study a suitable compound or group of compounds may be selected for more detailed investigation. The choice of compound(s) would relate to their potential in the manipulation of flowering and elongation, thereby allowing control of the umbel canopy and possibly secondary metabolite production.

1.2 Materials and Methods

Seedlings (var. C22) were transplanted 16 days after sowing into 150mm pots containing potting media described in the General Materials and Methods. Equal packing density of pots was ensured by weighing at field capacity to a standard weight. Plants were grown in an outdoor wire netting enclosure and irrigated by automatically controlled overhead sprinklers. The treatments applied were as follows:

1. Applied as a soil drench
   PP333 (Paclobutrazol)
   EL500 (Flurprymidol)

2. Applied as foliar sprays.
   Alar (Daminozide)
   Terpal C (Mepiquat chloride)
   Ethrel (Ethephon)
   Atrinal (Dikegulac-sodium)
   CCC (Chlormequat chloride)
   GA (Gibberellic acid, number 3 nomenclature)
   NAA (Naphthylacetic acid)
   Kinetin (a cytokinin)
   PP333 (Paclobutrazol)
   EL500 (Flurprymidol)

The treatments were applied at three logarithmic rates, 1, 10 and 100 mg of active ingredient (a.i.) per plant. Only duplicates of each treatment were used, results are expressed as the mean but no statistical analysis was performed. All chemicals were applied in 20 ml of water, three times, seven days apart. This resulted in a total of 3, 30 and 300 mg a.i. per plant applied for each of the above treatments. Treatments commenced when the plants were at the 6 expanded leaf stage of growth (60 days after sowing). No statistical data was presented as only duplicates of each treatment were made. Only observational data was collected to aid in the selection of a suitable compound(s). Effects on elongation were measured by recording the overall height of the plants over a 70 day period, commencing at initiation. The effect on umbel number and development was also observed during this period.
The first harvest was performed 14 days after treatment.

1.3 Results

Most treatments produced a marked effect almost immediately after application, either as a change in the elongation or leaf angle of the plant.

Overall height records are shown in Figure IV.C.1 (a to l). The change in height of the control is presented on each graph. Data on the number of umbels per plant and the time of flowering relative to the control is given in Table IV.C.1.

GA_3 (Figure IV.C.1.a):

All three application rates had no effect on the time to anthesis of the primary umbel. Low levels of GA_3 decreased the number of secondary umbels, as well as delayed flowering of all umbel orders. Similar effects were observed on the tertiary umbels. Although Figure IV.C.1.a indicates that the 3 and 30 mg treatments decreased plant height, this was an artifact due to wind damage. The plants actually grew taller but were very etiolated and had weak tips which subsequently broke off. Plants treated with the high rate of GA_3 also encountered the same fate but the overall increase of the remaining stem still exceeded the height of the control. Because of the loss of elongated tips which enclosed developing umbels, the umbel data is not an accurate indication of the effect of GA_3 on flowering.

Ethrel (Figure IV.C.1.b):

Application of the highest rate of Ethrel decreased the overall height substantially when compared with the control. This can be attributed to the plants remaining in a purely vegetative phase for 2.5 months longer than the control plants. Initiation occurred at such a late stage that only the primary umbel was able to develop.

Kinetin (Figure IV.C.1.c):

The highest rate of this cytokinin produced an increase in the number of main stems from the basal area of the plants. Increased rate of application from 3 to 30 mg a.i. per plant increased the time to flowering of secondary and tertiary umbels. All rates initially suppressed the overall height of the plants. Initial observations indicated that a decrease in overall height of the plant resulted from increased rate of application of kinetin. But the effectiveness of applied kinetin decreased with time. By the completion of the experiment all plants were a similar height.

NAA (Figure IV.C.1.d):

A moderate decrease in overall height was obtained with the 3 and 30 mg treatments, with little effect on the number of secondary and tertiary umbels. The 30 mg rate increased the number of main stem shoots, whilst the highest rate caused severe distortion and finally death.

Alar (Figure IV.C.1..e):

The 30 and 300 mg treatments were successful in reducing the overall height of the plants by a considerable amount. 3 mg a.i. per plant did not affect the overall height. No major affects were recorded in respect to umbel number or development.

CCC (Figure IV.C.1.f):

Application of CCC at 30 and 300 mg a.i. per plant resulted in a reduction in height. The magnitude of these reductions were similar to Alar treated plants. The initial reduction in height was similar for all rates of CCC applied. The measurement 70 days after commencement of the experiment indicated that the plants treated with 3 mg a.i. were only slightly shorter than the control plants. Again no major effects were recorded in respect to umbel number or development.

Atrinal (Figure IV.C.1.g):

Increased rate of active ingredient decreased the number of secondary umbels but had a minimal effect on the tertiary umbels, other than delaying flowering. The highest rate of Atrinal prolonged the vegetative phase for a period of 2 months. This resulted in plants which appeared very similar to those treated with the high rate of Ethrel.

Terpal C (Figure IV.C.1.h):

The 300 mg treatment resulted in a similar effect to Kinetin, producing 6 main stems from the basal meristem. However, the number of secondary umbels did not increase compared to the control plants due to the low yield of umbels per stem. The number of tertiary umbels was not increased by the larger number of main stems. The effect of this compound on height was noticeable only at the 30 and 300 mg levels, the latter decreased the overall height by 50 percent in relation to the control.

PP333 (Figure IV.C.1.i and j):

Application of this chemical to the foliage induced earlier umbel development for all orders. The time to emergence of the umbels decreased as the rate of active ingredient applied increased. The 30 and 300 mg treatments decreased overall height very markedly. The 3

Figure IV.C.1.a.

Effect of GA_3 application on the overall height of fennel plants.

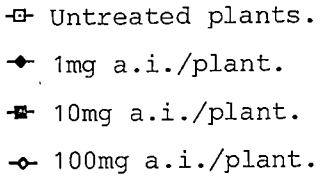
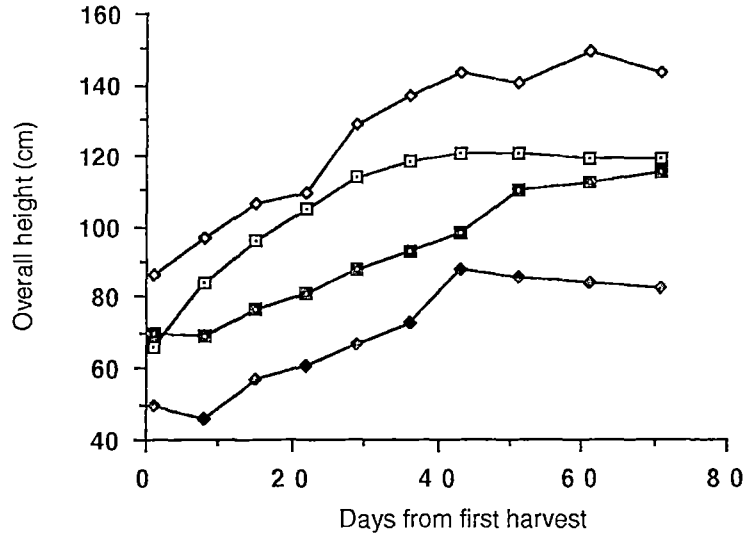


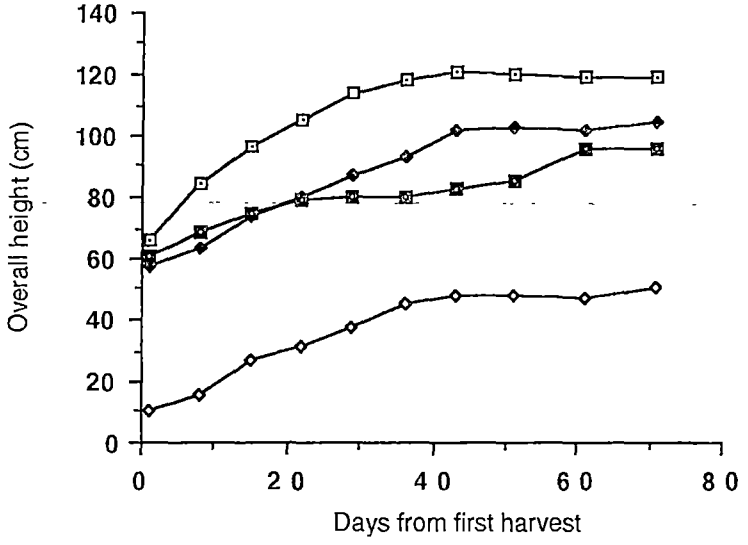
Figure IV.C.1.b.

Effect of Ethrel application on the overall height of fennel plants.

Figure IV.C.1.c.

Effect of Kinetin application on the overall height of fennel plants.


160
140
120
100
80
60
40
Overall height (cm)
0
20
40
60
80
Days from first harvest


140
120
100
80
60
40
20
0
Overall height (cm)
0
20
40
60
80
Days from first harvest

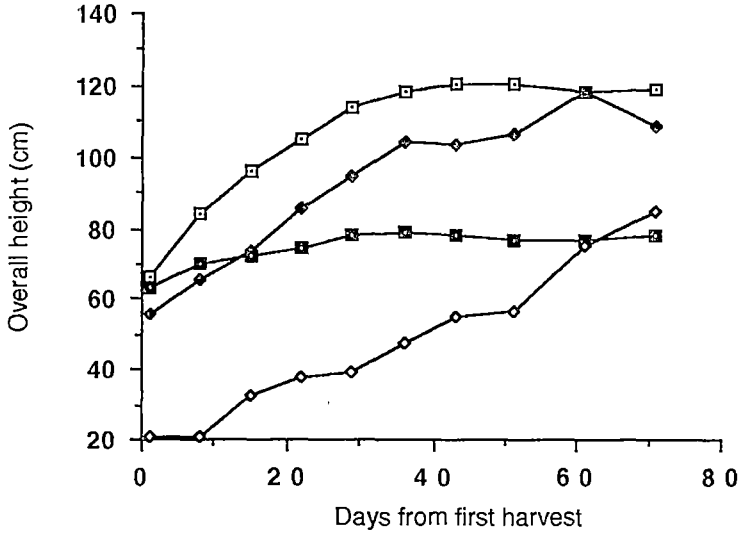

140
120
100
80
60
40
20
Overall height (cm)
0
20
40
60
80
Days from first harvest

Figure IV.C.1.d.

Effect of NAA application on the overall height of fennel plants.

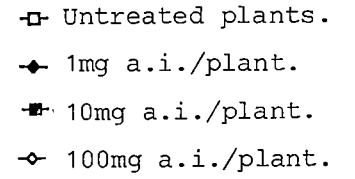
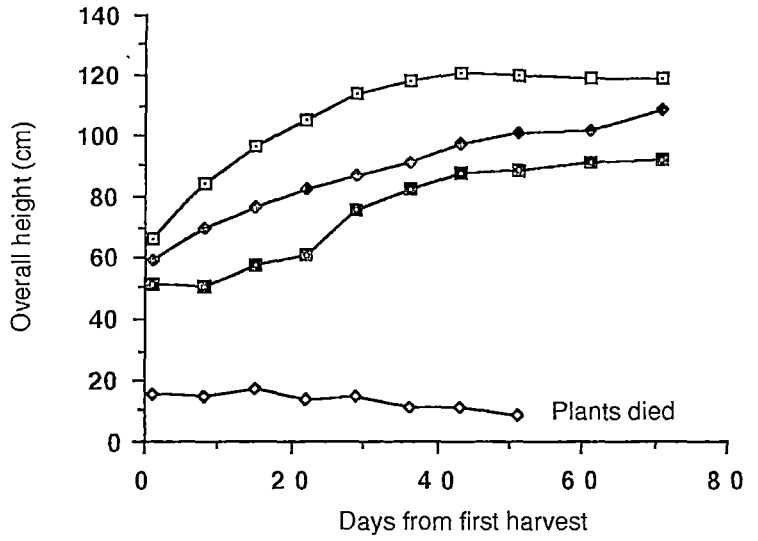


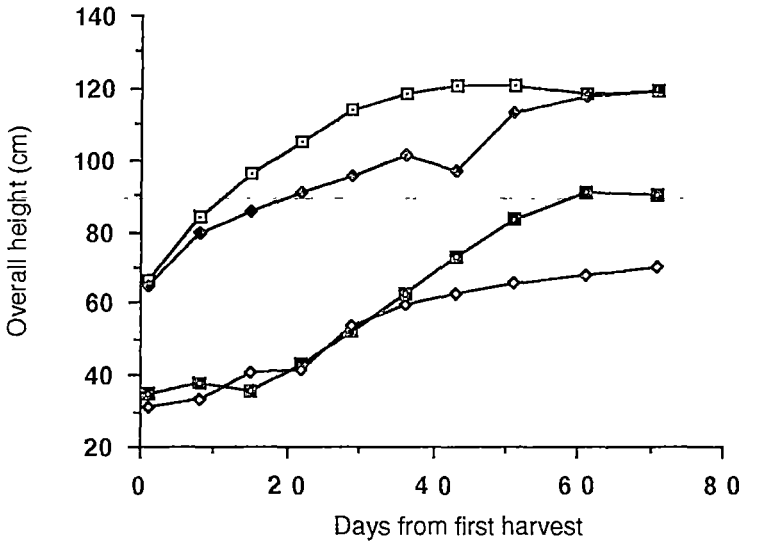
Figure IV.C.1.e.

Effect of Alar application on the overall height of fennel plants.

Figure IV.C.1.f.

Effect of CCC application on the overall height of fennel plants.


Overall height (cm)
140
120
100
80
60
40
20
0
0
20
40
60
80
Days from first harvest
Plants died


Overall height (cm)
140
120
100
80
60
40
20
0
20
40
60
80
Days from first harvest

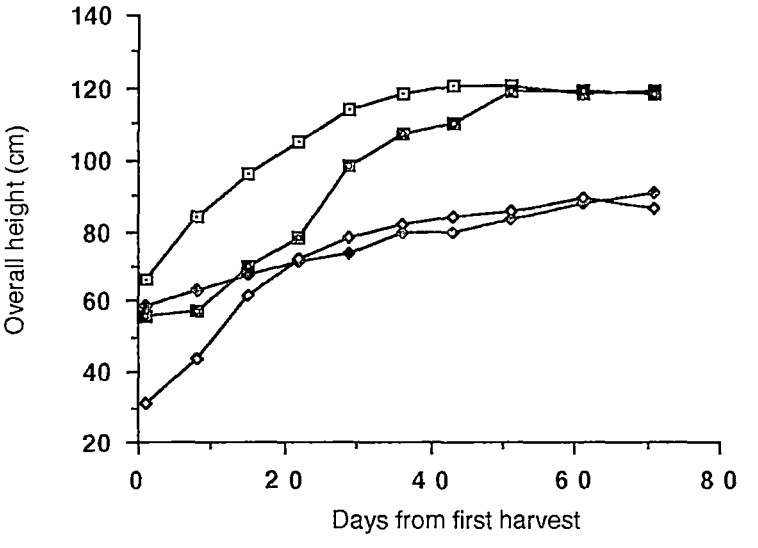

Overall height (cm)
140
120
100
80
60
40
20
0
20
40
60
80
Days from first harvest

Figure IV.C.1.g.

Effect of Atrinal application on the overall height of fennel plants.

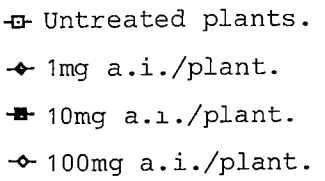
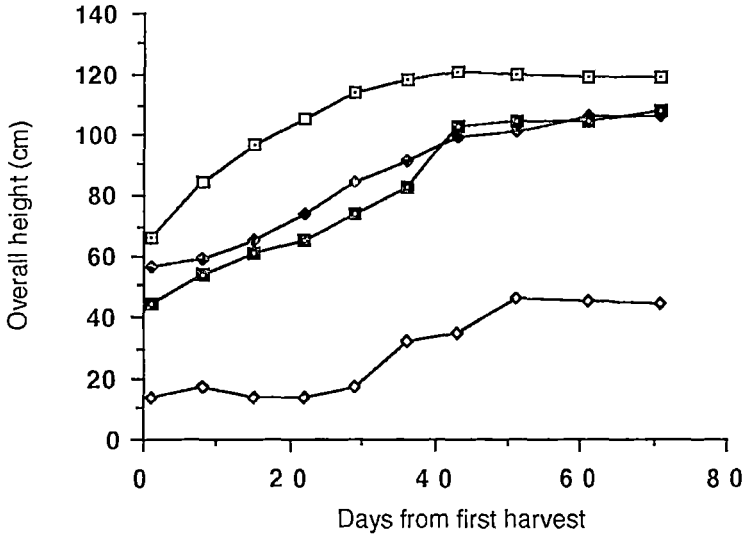


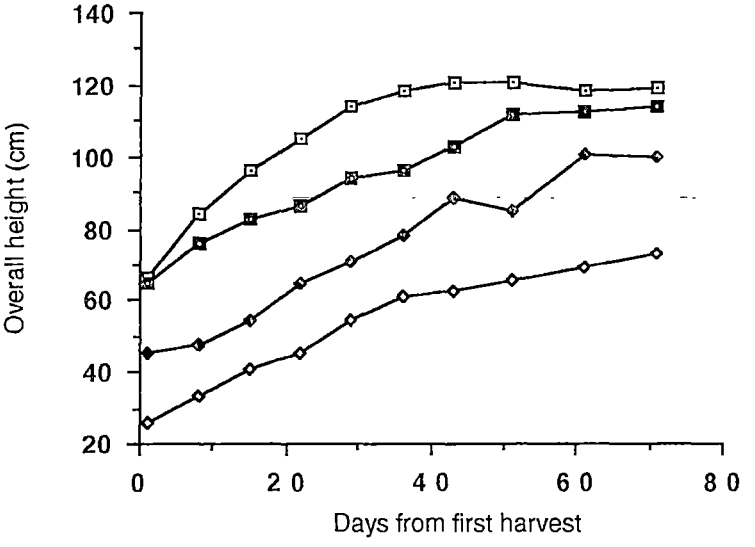
Figure IV.C.1.h.

Effect of Terpal-C application on the overall height of fennel plants.

Figure IV.C.1.i.

Effect of PP333 as a foliar application on the overall height of fennel plants.


Overall height (cm)
140
120
100
80
60
40
20
0
0
20
40
60
80
Days from first harvest


Overall height (cm)
140
120
100
80
60
40
20
0
20
40
60
80
Days from first harvest

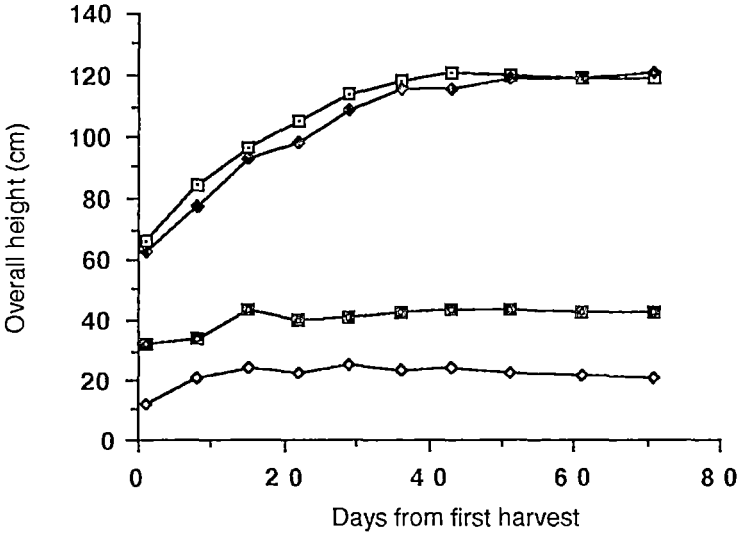
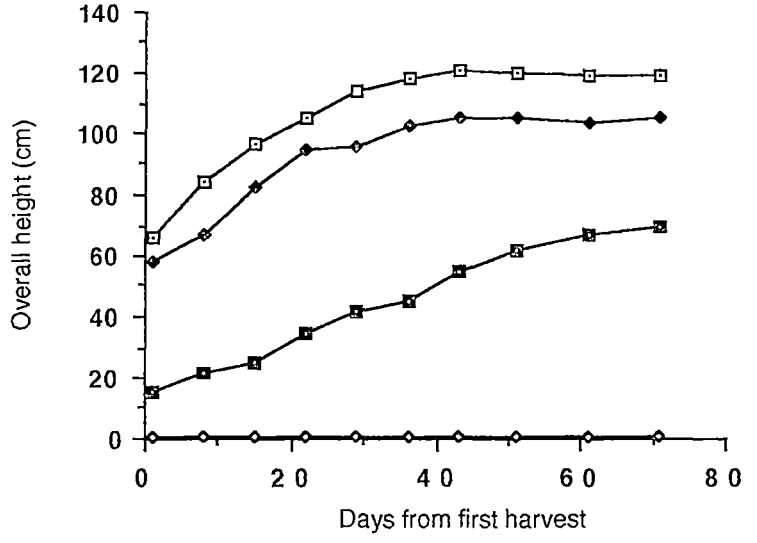

Overall height (cm)
140
120
100
80
60
40
20
0
20
40
60
80
Days from first harvest

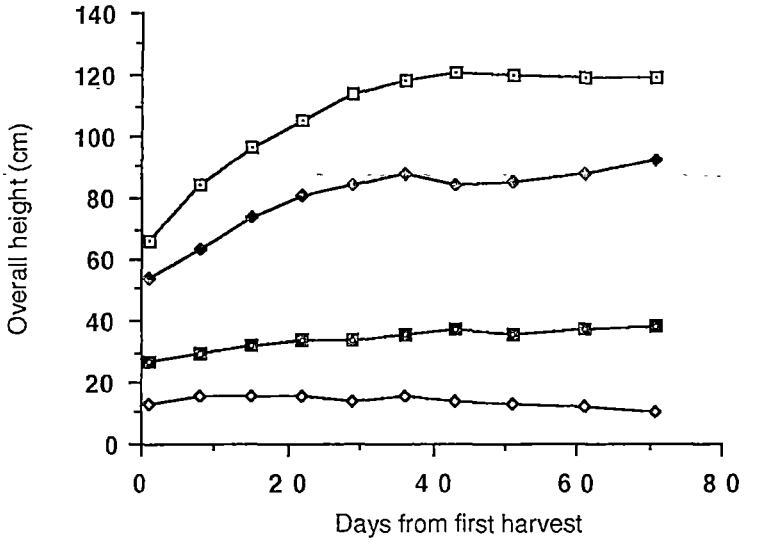
Figure IV.C.1.j.
Effect of PP333 as a soil application on the overall height of fennel plants.

- Untreated plants.
- 1mg a.i./plant.
- 10mg a.i./plant.
- 100mg a.i./plant.

Figure IV.C.1.k.
Effect of EL500 as a foliar application on the overall height of fennel plants.

Figure IV.C.1.l.
Effect of EL500 as a soil application on the overall height of fennel plants.


Overall height (cm)
140
120
100
80
60
40
20
0
0
20
40
60
80
Days from first harvest


Overall height (cm)
140
120
100
80
60
40
20
0
0
20
40
60
80
Days from first harvest

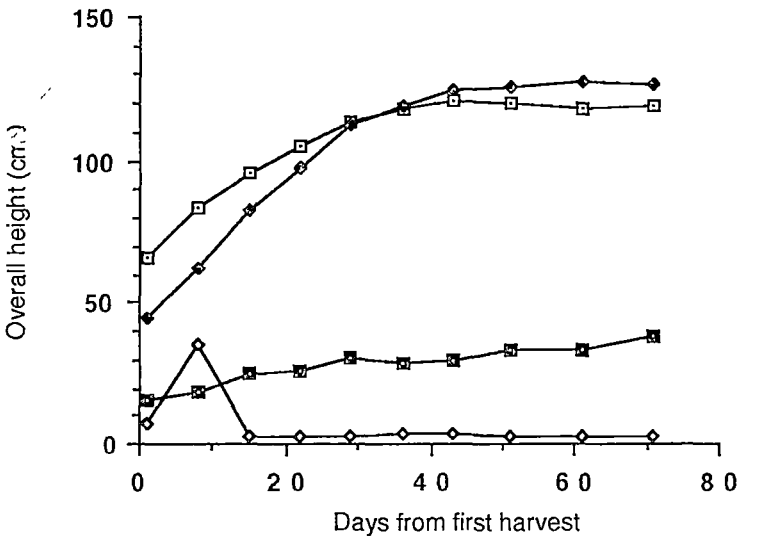

Overall height (cm)
150
100
50
0
0
20
40
60
80
Days from first harvest

Table IV.C.1.

Effect of 12 Growth Regulators on the number of umbels per plant

Treatment	Rate applied (mg a.i./pl)	Primary	Secondary	Tertiary
Control		1	8.5	16
Atrinal	1	1E	4.5	7.5L
	10	1L	3E	15E
	100	Vegetative throughout experiment		
PP333 foliar	1	1	5.5E	20L
	10	1E	6	8.5
	100	1E	6.5E	6E
PP333 soil	1	1	6.5E	11
	10	1L	6L	10L
	100	Too densely compressed to flower		
Ethrel	1	1E	6.5	15.5
	10	1E	6E	12.5
	100	1L	0	0
Terpal-C	1	1	6.5E	13.5E
	10	1E	7L	8
	100	6L	7.5	14.5L
GA3	1	1	3	4.5
	10	1L	4.5L	2L
	100	1L	6.5	2
Kinetin	1	1	4.5	9.5
	10	1	4	0
	100	4.5L	4.5L	0
Alar	1	1	4.5	14.5L
	10	1L	5	7.5
	100	1L	7.5	19.5
CCC	1	1E	7L	15L
	10	1	4.5E	13.5L
	100	1	10.5	18E
EL500 foliar	1	1	8.5	17E
	10	1	6.5	10E
	100	1	5.5E	12E
EL500 soil	1	1L	10.5L	17
	10	1L	6.5	4L
	100	Too densely compressed to flower		
NAA	1	1	4.5	12
	10	3.5	6.5	16
	100	Plants died		

Note: E indicates flowering earlier relative to the corresponding order of the control and L indicates later flowering.

mg treatment remained the same as the control. Soil applied PP333 delayed the time to flowering of all umbels when applied at the 30 mg rate. Soil application of PP333 produced a greater reduction in height than the same rates as applied to the foliage. Inhibition of stem elongation and umbel development by the highest rate was extreme. A very small compressed plant only 25 mm high resulted.

EL500 (Figure IV.C.1.k and l):

Increased rate of applied active ingredient to the foliage did not affect the number of secondary or tertiary umbels greatly. The higher rates only resulted in earlier flowering of these umbel orders. Soil applied EL500 decreased the height more effectively than the foliage application at equivalent rates. At the highest rate the height reduction was extreme and similar to the highest rate of PP333. Growth was compresssed to such an extent that the number of umbels present were difficult to determine. Foliar application resulted in an almost linear decrease in height with increased rate of active ingredient. The soil application at a rate of 30 mg a.i. per plant, delayed flowering as well as decreased the number of tertiary umbels.

1.4 Discussion

Moderate reduction in height was achieved with Terpal C, Alar, CCC, Ethrel and Kinetin when applied at a total amount per plant of 30 mg a.i. Application of these chemicals at the 300 mg a.i. per plant rate produced detrimental affects on flowering and development. Terpal C and Kinetin at such rates induced differentiation of the basal meristematic region increasing the number of main stems. These plants resembled a fennel plant in its second year of growth. In the first year of growth a single stem elongates. After senescence, the plant regrows from the base with 5 or 6 new shoots in the second year. NAA produced a similar effect at a lower rate than Terpal C or kinetin, but death resulted at the highest rate. This high dosage could be compared to the mode of action of a hormonal herbicide.

No conclusions could be made on the affect of applied GA_3 on flowering due to mechanical damage by wind to the top portion of the excessively elongated plants. Further investigation is necessary to determine the effects of applied GA_3 on flowering. The rate, as well

as the timing of the application may provide an insight into the elongation and flowering responses in fennel.

Major reductions in overall height were achieved with EL500 and PP333. The application of these growth retardants to the soil improved their effectiveness. This increase in activity indicated that the major mode of uptake in fennel for EL500 and PP333 was through the root system. This is in agreement with the literature supplied by the manufacturing companies of these compounds. The difference in action between the foliar and soil drench applications was possibly due to differences in the rate at which the chemical reaches the site of action. A foliar spray will be more rapidly absorbed through the cuticle. In comparison, a soil drench would be slower acting due to the chemical leaching throught the soil before being actively absorbed by the roots. Similar effects were absorbed for both PP333 and EL500 between the activity when applied in both these modes.

Technical information from manufacturing companies has indicated that PP333 exhibits some antifungal activity. Unfortunately no observations could be made in respect to activity against Cercosporidium in fennel as no disease problems were encountered within this experiment.

The application of these growth retardants on fennel as a commercial crop may enable a reduction in the height of the floral canopy and a reduction in the amount of vegetative material present. Such improvements would allow more efficient harvesting. A study of the effectiveness under field conditions as well as a more detailed examination of the effects on oil yield is necessary.

IV.C.2 Preliminary investigation of the effectiveness of EL500, PP333 and Atrinal in the field.

2.1 Introduction

The two growth retardants Flurprymidol (EL500) and Paclobutrazol (PP333) were examined in the previous pot trial and found to produce favourable changes in the growth habit of fennel. Atrinal, at certain moderate concentrations also produced some desirable changes in the development of the plant. To further evaluate the effects of these growth regulators, treatments were applied to a commercial field crop.

2.2 Materials and Methods

All treatments were applied to a commercial crop of variety C22 at Ouse, in the Derwent Valley.

	PP333	EL500	Atrinal
Rate 1:	2.6 kg a.i./ha	5.0 kg a.i./ha	6.25 kg a.i./ha
Rate 2:	0.86 kg a.i./ha	2.5 kg a.i./ha	3.1 kg a.i./ha
Rate 3:	0.29 kg a.i./ha	1.25 kg a.i./ha	1.56 kg a.i./ha
Rate 4:	0.09 kg a.i./ha	0.63 kg a.i./ha	0.78 kg a.i./ha
Rate 5:	0 kg a.i./ha	0 kg a.i./ha	0 kg a.i./ha

The above rates were determined from the preliminary screening trial and also from appropriate literature. All treatments were applied using a self propelled small plot sprayer, fitted with a 3.2 metre spray boom and calibrated to an output of 182 l/ha. Plot sizes were 3.2 metre by 8 metre in length with a 2 metre border between plots. Plots were randomized complete block design with 3 replications. All treatments were applied on the December 5, after floral initiation had occurred.

Ten plants were harvested per plot. Measurements taken were: height to the primary umbel, whole plant and umbel oil yields. The first harvest of each set of growth regulator treatments was taken on the following dates:

EL500	7th of February
PP333	15th of February
Atrinal	22nd of February

Different harvest dates were used to allow sufficient time for processing of the harvested material.

Promising results were obtained from EL500 at the first harvest. Consequently PP333 and Atrinal were not subsequently harvested again.

A further 3 harvests of the EL500 treated plants were performed on March 1, March 15 and April 6.

2.3 Results

The results of the harvest of the Atrinal treated plants are presented in Table IV.C.2.1.a. Increased concentration of this compound resulted in a significant reduction in elongation. Application at a rate of 0.75 kg a.i. per ha did not significantly affect the height compared to the control plants. But, as the rate was increased further to 3.1 kg a.i. per ha, a decrease in height was recorded. Unfortunately the affect of applying this growth retardant was severely detrimental to the oil yield. A 54 percent decrease in oil yield from the whole plant and 63 percent decrease from the umbels was recorded.

PP333 did not significantly reduce the height of the crop, (Table IV.C.2.1.b), at any of the applied rates. No significant effect on oil yield from either whole plant or from the umbels was observed. Further harvests of this series of treatments, and of the Atrinal treatments, was considered unnecessary.

Figure IV.C.2.1 indicates the effect of EL500 on the stem elongation of field plants. No significant difference was detected between the heights of the primary umbels of the control and the lowest rate of application, 0.6 kg a.i. per ha. The plants treated with the 1.25 and 2.5 kg a.i. per ha of EL500 were significantly shorter than the control, but no significant difference was observed between these two rates of application. Considerable reduction in the stem elongation resulted from the application of 5 kg a.i. per ha. A decrease from a mean of 105cm for the control to 65cm for this treatment was observed. The change in stem elongation with time for

Table IV.C.2.1.

a) Effect of Atrinal application in the field
Harvested on the 22nd of Feb.

Oil Yields

Rate kg a.i./ha	Height to primary cm	Whole plant yield kg/ha	% yield fresh wt.	Umbel yield kg/ha
0	102.9	117.63	1.35	68.2
0.75	113.1	70.47	0.95	35.31
1.5	86.7	57.41	0.89	34.53
3.1	72.8	75.66	1.14	39.89
6.25	68.3	55.05	1.16	25.02
LSD	29.7	30.5	ns	17.4

b) Effect of PP333 application in the field,
Harvested on the 15th of Feb.

Oil Yields

Rate kg a.i./ha	Height to primary cm	Whole plant yield kg/ha	% yield dry wt.	Umbel yield kg/ha
0	113.9	144.34	1.29	104.24
0.09	96.3	136.2	1.69	99.5
0.3	106.5	92.18	0.94	76.47
0.86	108.7	100.03	1.21	81.09
2.6	105.1	123.05	1.35	100.08
LSD	ns	ns	ns	ns

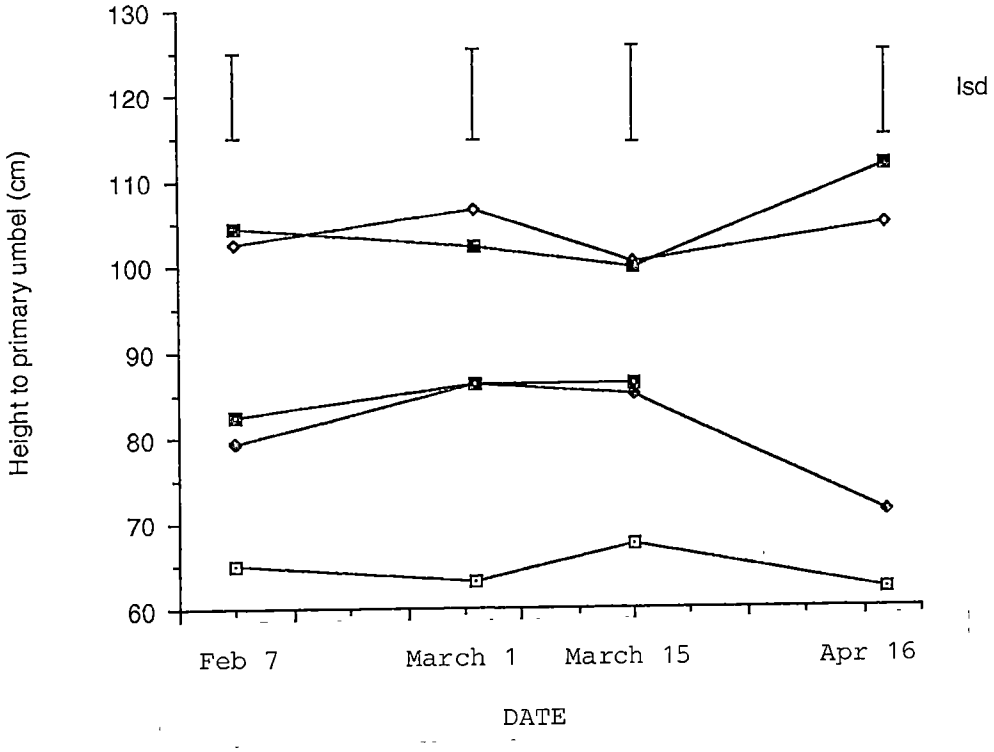


Figure IV.C.2.1.

Effect of EL500 application on the elongation of the main stem. Expressed as the height to the primary umbel. The following LSR's were obtained: Untreated plants ns, 0.6 kg a.i./Ha 11.3, 1.25 kg a.i./Ha ns, 2.5 kg a.i./Ha ns, 5 kg a.i./Ha ns.

Table IV.C.2.2 Effect of EL500 on the oil yield from fennel in the field.

Rate kg a.i./ha	Whole plant yield kg/ha			
	14/2	1/3	15/3	6/4
0	59.7	127.8	123.7	69.86
0.6	75.0	118.5	137.7	100.62
1.25	108.2	147.9	163.2	-
2.5	92.3	134.2	167.9	120.7
5	93.9	195.1	127.1	118.8
LSD	ns	ns	ns	ns

Rate kg a.i./ha	% yield dry wt.			
	14/2	1/3	15/3	6/4
0	1.14	1.63	2.17	1.04
0.6	1.07	1.57	1.75	1.23
1.25	1.37	1.8	2.23	-
2.5	1.22	1.87	2.12	1.85
5	1.27	2.74	2.23	1.71
LSD	ns	0.43	ns	ns

Rate kg a.i./ha	Umbel yield kg/ha			
	14/2	1/3	15/3	6/4
0	54.2	89.1	88.3	63.5
0.6	59.5	89.8	79.9	107.2
1.25	59	117.3	114.3	-
2.5	56.1	111.3	152.3	95.6
5	71.9	123	103.9	130.8
LSD	ns	ns	ns	ns

all treatments was not significant.

At all harvest dates the application of EL500 had no significant effect on the oil yield from whole plants or from umbels. On March 1, the 5 kg a.i. per ha treatment resulted in a significant increase in percent oil yield, on a dry weight basis, from whole plant samples. At all other harvest dates no significant differences were observed in percent oil yield (Table IV.C.2.2). No data for the 1.25 kg a.i. per ha was collected on April 6 due to these treatments being inadvertently harvested by a commercial harvester on March 15.

2.4 Discussion

The effect of Atrinal was detrimental to the oil yield of the plant although a useful reduction in height was observed. Even at the lowest rate, 0.75 kg a.i. per ha, a significant decrease in yield from whole plants and umbels resulted. The effect of increasing the rate of active ingredient did not result in any further decrease in oil yield. Much of this effect could be attributed to the observed increased abortion of the primary umbels. Shortly after application severe yellowing of the tips was observed. Damage to the primary umbel possibly occurred at this stage (Plate IV.C.2).

The performance of PP333 in the field was disappointing as no significant effects were recorded for all factors examined. The initial pot trial results indicated a reduction in height should have resulted. A possible explanation for the difference in effect between the pot trial and field trials would be the different time of application of this growth retardant in each of these experiments. In the pot trial application was at the 6th expanded leaf stage. As indicated in IV.B.1.2, the juvenile phase would not have been completed at this stage. Hence application was prior to initiation. In this field trial application was after initiation was observed.

Stem elongation was inhibited by the application of EL500. The ability to reduce the elongation response in fennel indicates the possibilities for further manipulation of the canopy design to enable more efficient harvesting. The levels of EL500 necessary to achieve a significant reduction in height were very high. But this may be reduced by application prior to initiation. Application at an earlier stage of development may have significant effects on the umbel

Plate IV.C.2.

Effect of Atrinal, 1 week after spray application to a field crop. Severe yellowing of the younger, more rapidly differentiating tissue can be observed.

initiation. However, data from the preliminary trial suggests that lower rates may not achieve the required inhibition in stem elongation.

The pot trial screening indicated soil application to be more effective, hence the major uptake was by the roots. In a field crop situation application direct to the soil may not be practical. To ensure rapid utilization of the growth retardant higher rates of water application may be necessary to wash the active ingredient into the soil.

To determine the effect of this growth retardant on umbel orders, oil composition etc. more detailed experimentation is required.

IV.C.3 Examination of the rates of application and the effect of different application times of EL500.

3.1 Introduction

A preliminary pot trial investigation was required to evaluate the effects of EL500 on fennel in more detail before undertaking a large scale field trial. The effect of varying the timing and rate of application of EL500 to individual plants was examined. The results of such experimentation was utilized in the design of a field trial.

3.2 Materials and Methods

3.2.1 Effect of different concentrations of EL500.

Fennel seedlings (var. C25) were transplanted into 150mm pots and placed in the exterior wire enclosure detailed in the General Materials and Methods.

7 application rates of EL500 per plant were utilized:

Control	0 mg a.i.
	5 mg a.i.
	10 mg a.i.
	15 mg a.i.
	20 mg a.i.
	25 mg a.i.
	30 mg a.i.

Treatments were applied to the plants at the 4 expanded leaves stage of development. All treatments were applied in 50 ml of aqueous solution, as soil applications. Treatments were only duplicated and results are observational. No statistical data was presented. The effect on stem elongation was recorded over a period of 170 days.

3.2.2 Effect of application of EL500 at different growth stages.

Fennel seedlings (var C25) were grown in the glasshouse described in the General Materials and Methods during the natural long day photoperiods of summer. All plants were grown in 150mm pots in the medium described in the General Materials and Methods.

The rate of applied EL500 for this experiment was 20 mg a.i. per plant in 50 ml of aqueous solution. All solutions were applied directly to the soil, ensuring even distribution over the pot surface. All treatments were replicated four times. The treatments were as follows:

Applied at:	2 expanded leaves	
	4 expanded leaves	7 days later
	6 expanded leaves	17 days from 2 leaf stage
	8 expanded leaves	28 days from 2 leaf stage
	10 expanded leaves	52 days from 2 leaf stage

The height to the last emerged leaf was recorded over a period of 130 days and the number of initiated umbels of each order were recorded.

3.3 Results

3.3.1 Effect of different concentrations of EL500

Application of EL500 to fennel plants decreased stem elongation. The highest rates had the most pronounced effect on elongation. The application of a 5 mg a.i./plant solution decreased elongation by 50 percent. Further reductions were achieved with the 10 to 25 mg a.i./plant treatments. (Figure IV.C.3.1). The highest rate applied decreased elongation by 90 percent with respect to the control plants.

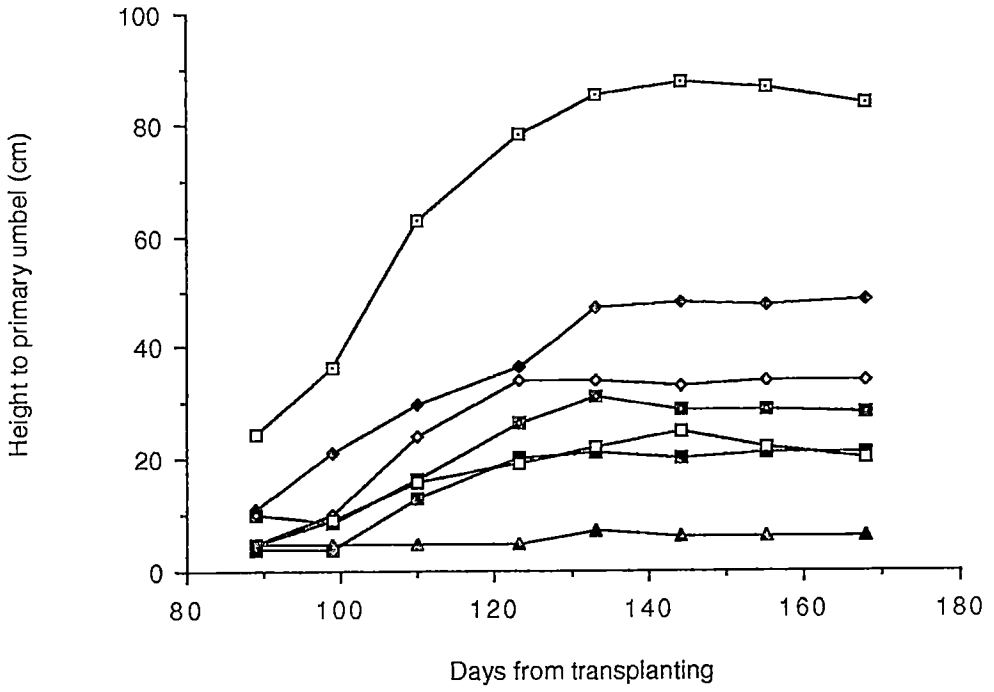


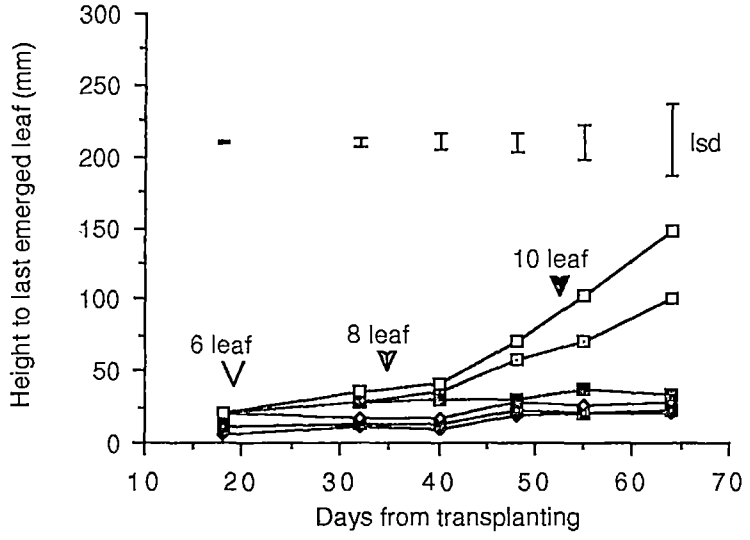
Figure IV.C.3.1.
Effect of different rates of applied EL500 on the elongation of the main stem, as measured by the height to the primary umbel. Untreated plants -□- , 5 mg a.i./plant -◇- , 10 mg a.i./plant -■- , 15 mg a.i./plant -◇- , 20 mg a.i./plant -■- , 25 mg a.i./plant -□- , 30 mg a.i./plant -▲- .

Figure IV.C.3.2.a.

Effect of application of EL500 at different stages of development of a fennel plant, during the first 65 days of growth. The elongation of the main stem was determined by measurement of the height to the last emerged leaf. Untreated plants ⊡ , application at 2 expanded leaves ◇ , 4 expanded leaves ■ , 6 expanded leaves ◇ , 8 expanded leaves ■ and 10 expanded leaves ⊡ .

Figure IV.C.3.2.b.

Effect of different application times of EL500 on elongation up to 127 days after transplanting. LSR's, untreated plants 140.9, 2 expanded leaves 15.7, 4 expanded leaves 21.7, 6 expanded leaves 69.8, 8 expanded leaves 80 and 10 expanded leaves 157.7.


Height to last emerged leaf (mm)
300
250
200
150
100
50
0
lsd
10 leaf
8 leaf
6 leaf
10
20
30
40
50
60
70
Days from transplanting

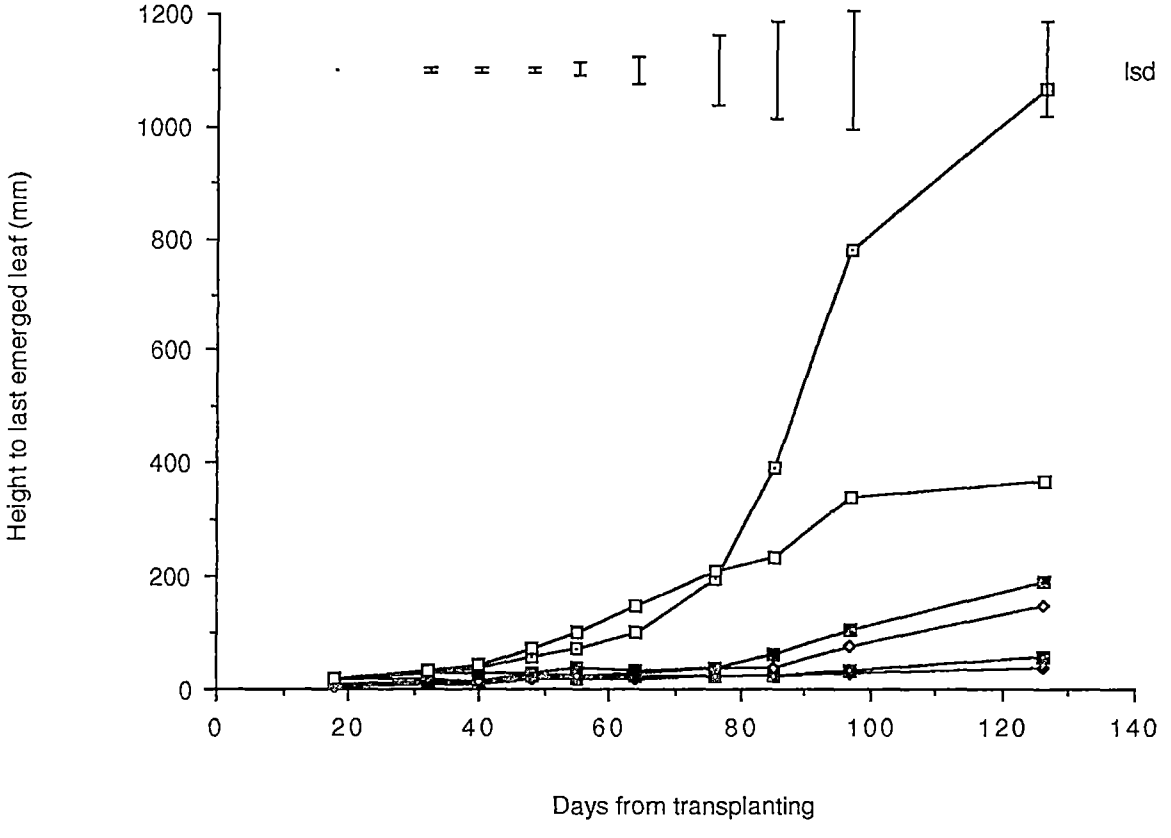
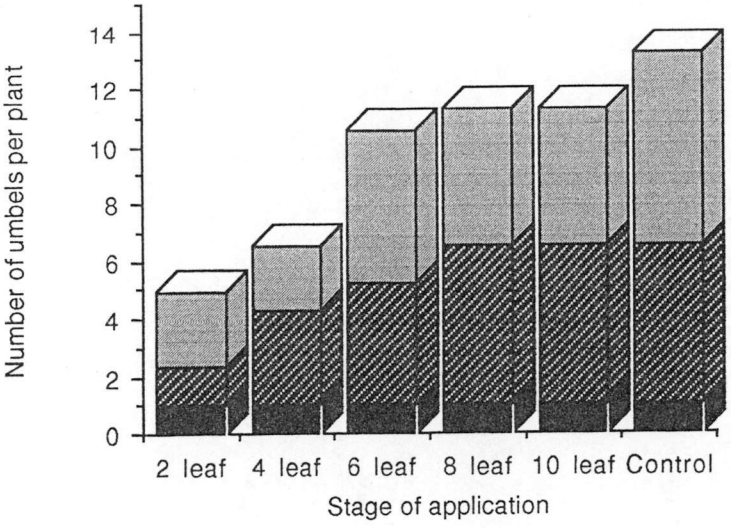

Height to last emerged leaf (mm)
1200
1000
800
600
400
200
0
lsd
0
20
40
60
80
100
120
140
Days from transplanting

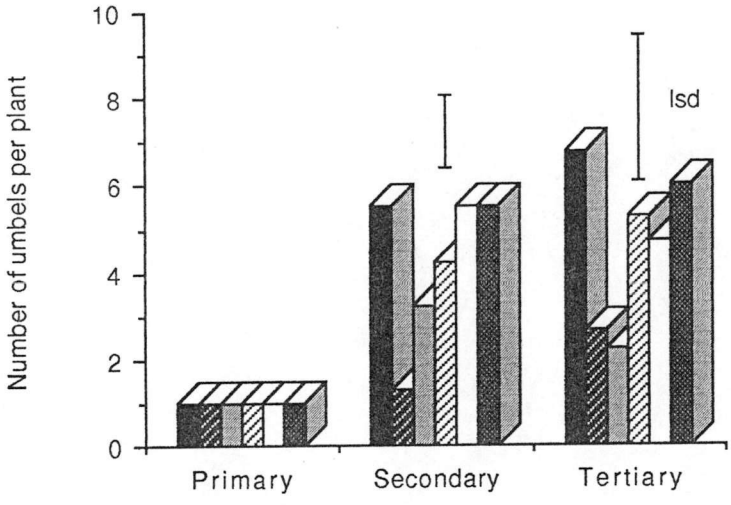
Figure IV.C.3.3.

Effect of different application time of EL500 on the number of umbels of each order per plant. Numbers of primary umbels ▩ , secondary umbels ▨ and tertiary umbels ▧ . LSD 4.88.

Figure IV.C.3.4.

The effect of application of EL500 at different stages of development on the total number of umbels per plant. No application ▩ , application at 2 expanded leaves ▨ , 4 expanded leaves ▧ , 6 expanded leaves ▨ , 8 expanded leaves □ and 10 expanded leaves ▩ .


Number of umbels per plant
0
2
4
6
8
10
12
14
2 leaf
4 leaf
6 leaf
8 leaf
10 leaf
Control
Stage of application


Number of umbels per plant
0
2
4
6
8
10
lsd
Primary
Secondary
Tertiary

3.3.2 Effect of EL500 applied at different growth stages

The effect of application of EL500 at the 2 expanded leaf stage through to the 8 expanded leaves was significant in reducing plant height as compared to the control (Figure IV.C.3.2.a). The reduction in height was approximately 70 percent after 65 days from transplanting. The reduced height was maintained for a further 60 days growth. There was no significant difference in the reduction of elongation of the main stem of plants treated with EL500 at either the 2, 4, 6 or 8 leaf stages at any time during the experiment. Application at a later stage in growth, at 10 expanded leaves, decreased the rate of elongation but not to the extent of the earlier applied treatments (Figure IV.C.3.2.b). Significant differences in height were only observed after 98 days from the commencement of the experiment. At this stage, the height of the main stem was significantly lower than the control plants and significantly higher than the 2, 4, 6 and 8 expanded leaf stages. All treatments elongated significantly over the period of observation but at decreasing rates as EL500 concentrations increased.

Application of EL500 at the earlier stages of development, 2 and 4 expanded leaves, reduced the number of tertiary and secondary umbels (Figure IV.C.3.3). When applied at the 6 or later leaf stages no significant effect on umbel number was observed.

The effect on total number of umbels per plant is shown in Figure IV.C.3.4. Application at the 2 and 4 expanded leaf stages significantly decreased the total number of umbels.

3.4 Discussion

This investigation of the effect of applying various rates of EL500 to fennel plants provided a useful working range of 10 to 25 mg a.i./plant. A rate of 30 mg a.i./plant decreased stem elongation markedly but decreased umbel number. Due to the nature of uptake of this growth retardant, through both roots and leaves, this working range may not necessarily relate to a per plant basis, but may be related to the concentration necessary in a set volume of soil for each plant. The application of EL500 to plants in the field would require careful calibration.

EL500 can be applied from the 6 expanded leaf stage onwards

without significantly affecting the umbel number but the later the application after this period the less is the effect on elongation. Application prior to initiation, that is, up to and including the 6 expanded leaf stage, resulted in decreased number of umbels. Application at the 8 leaf stage did not effect the number of umbels indicating that initiation had already commenced. Photoperiod experiments examining the stage of development at time of initiation indicated that at the 8 expanded leaf stage initiation of umbels was still proceeding. In this experiment initiation was completed by the time of application at the 8 expanded leaves.

The mode of action of this compound is reported to be based on an inhibitory role in the biosynthesis of Gibberellins. Application prior to initiation then decreases the levels of endogenous GA's during the period of change from vegetative to floral growth by the apical meristem. The results above thereby suggest an important role for the GA's in the floral evocation process in fennel.

Fennel plants treated early in development with high rates of EL500 demonstrated a compressed growth habit. These plants ceased to produce any large new leaves. The new leaves formed after EL500 application did not expand significantly, yet initiation of umbels proceeded, but at a reduced number. These umbels were observed to develop normally producing numbers of seed and seed size equivalent to untreated plants. Such results indicate that either

(I) the very reduced leaf area of the plant is sufficient for the supply of assimilates to developing seeds

(II) the umbels are able to assimilate as self supporting units

(III) both (I) and (II)

This aspect of fennel umbel development is examined in more detail in later sections.

Only data of umbel numbers per plant were recorded in this experiment, no quantitative measurements of seed size etc. were performed. The application of EL500 may have effects within the umbel such as altering the number of rays per umbel and seeds per ray. Further investigation of these parameters is necessary.

IV.C.4 Application of EL500 to fennel as a field crop and the residual effects in the following year.

4.1 Introduction

The preliminary field trial indicated that considerable reduction in the height of a fennel crop may be obtained with the application of the growth retardant EL500. This trial was not sufficient in size and replication to accurately determine effects on oil yield and composition.

To enable a meaningful field trial to be conducted a suitable site with minimal variation in soil type and slope was required. Another confounding factor when conducting field experimentation was plant density. Changes in plant density may produce a change in morphological characters. For example flowering in carrots is reported to be effected by the density. Gray and Steckel (1985) demonstrated that increased plant density increased the spread of flowering time of the primary umbels. This investigation attempted to minimize the influence of such variables to enable a more concise study of the effects of this growth retardant.

4.2 Materials and Methods

Fennel seed, variety C26, was sown with a Stanhay drill at Plenty, in the Derwent Valley. The seed was sown at a rate to produce approximately twice the required plant density. The rows assigned as treatments and also border rows were thinned by hand to 10 plants per m^2

The experiment was designed as a randomized complete block consisting of 4 blocks and 5 treatments, as follows:

Treatment A	0 g	a.i.EL500 per m^2
Treatment B	0.2 g	a.i.EL500 per m^2
Treatment C	0.4 g	a.i.EL500 per m^2
Treatment D	0.8 g	a.i.EL500 per m^2
Treatment E	1.6 g	a.i.EL500 per m^2

Rates higher than those used in pot trials were used to compensate for any problems which may be encountered by the increase in soil volume per plant in a field situation. Plot size was 8m long and 3 rows wide with 0.8m between rows. A 1m border between plots was maintained.

All treatments were applied utilizing a motorized sprayer unit, a 12 volt Flojet pump delivering 250 kpa and regulated by a piston type pressure relief valve. A 1.5m wide boom with three nozzle Teejet flat spray tips, 110^{o} delivered 178 l per ha. Each treatment was applied at 3 l per plot, when the plants had 8 expanded leaves and 12 nodes present.

4.2.1 Effect in the first year of growth

Four harvests of ten plants per plot were taken during the period of maturation of the crop in the first year. Height measurements to the highest primary and lowest umbels were recorded. Fresh weights and oil yields were determined for whole plant and each umbel order subsample, and the composition determined as per the General Materials and Methods.

4.2.2 Residual effect in the second year of growth

In the following season, the second year of this crop, a single harvest at the time of commercial harvest was performed. Again height measurements and oil yield determinations on whole plant and umbel samples were recorded. The composition of the oil samples was determined for all samples as per the General Materials and Methods.

4.3 Results

4.3.1 First year

4.3.1.1 Effect of EL500 on elongation.

At all harvest dates, EL500 applied at high rates decreased the height of the lowest umbel in the canopy (Figure IV.C.4.1.a). On February 9 no significant decrease in the height of the lowest umbel was recorded between the control plants and those treated with 0.2 gms a.i.per m^2. EL500 applied at a rate of 1.6 gms a.i.per m^2 decreased height by 28 percent.

No significant difference was recorded on February 9 in the heights to the primary umbel (Figure IV.C.4.1.b) Elongation of the main stem was not affected at this time. Only the 1.6 gms a.i.per m^2 rate decreased the height of the highest umbel (Figure IV.C.4.1.c).

On March 4, the 1.6 and 0.8 gms a.i.per m^2 treatments did not significantly affect the position of the umbels but all were significantly lower than the control. The 0.2 gms a.i.per m^2 treatment was only significantly different in the height of the lowest umbel.

The height of the umbels of the control plants reached a maximum on March 18. At this time no significant difference was recorded between the heights of all umbels of the 0.2 gms a.i.per m^2 treatment and the control plants. Further increase in the rate of EL500 applied significantly reduced the height of all umbels. For the height of the primary umbel this reduction was observed as 11 percent, 15 percent and 27 percent for the 0.4, 0.8 and 1.6 gms a.i.per m^2 treated plants respectively.

The heights to all umbels did not increase during the final harvests for the control, 0.2, 0.4 and 0.8 gms a.i.per m^2 treated plants. The 1.6 gms a.i.per m^2 treated plants continued to elongate further as shown by the increase in the height of the primary from March 18 to April 18. Again no effect on any of the umbels was observed at the 0.2 application rate but decreases in height of all umbels was recorded with further increased rate of applied EL500.

From the height records, a measure of the height of the total floral bearing portion of the crop was made, the difference between the highest and lowest umbels. No significant changes to these values were observed during the experiment (Table IV.C.4.1).

4.3.1.2 Effect of EL500 on fresh weight of whole plants

The total fresh weight of plant material per m^2 was not affected by the application of EL500 (Table IV.C.4.1). During the four harvest dates the total fresh weight of whole plants was approximately 2250 gm per m^2 .

Figure IV.C.4.1.a.

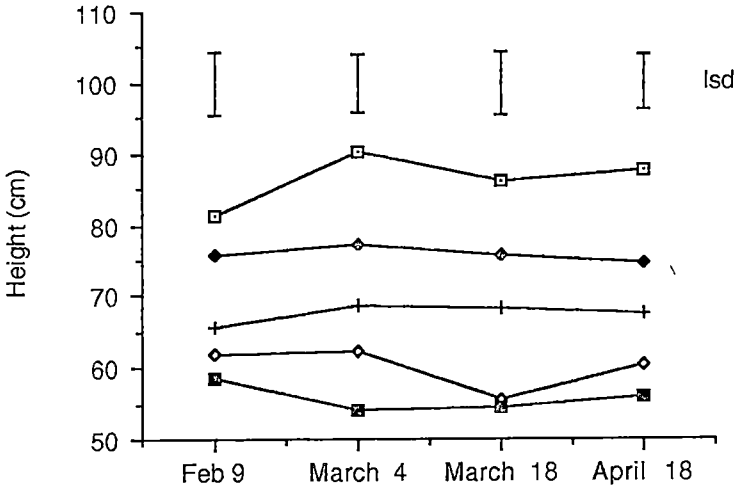
The effect of application of EL500 on the position of the umbels within the canopy was examined over a period of 75 days. Height measurement to the lowest umbel was recorded and presented. LSR's, untreated plants ⊡ 6.68, 0.2 g a.i. m^{-2} ◆ ns, 0.4 g a.i. m^{-2} + ns, 0.8 g a.i. m^{-2} ◇ 6.37 and 1.6 g a.i. m^{-2} ■ ns.

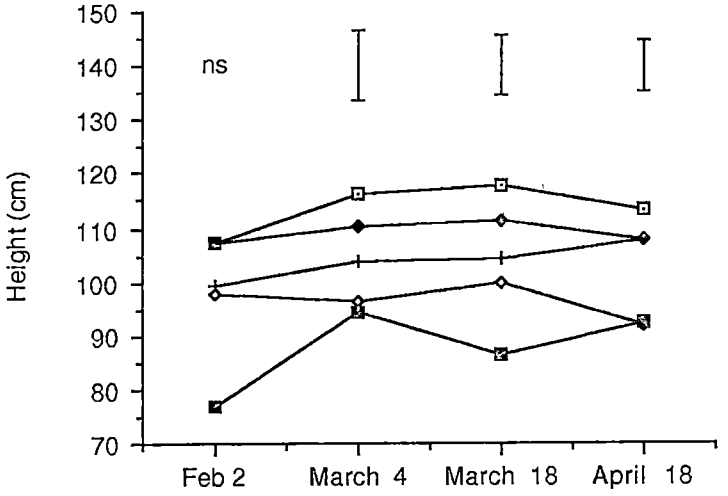
Figure IV.C.4.1.b.

Effect of EL500 on the height to the primary umbel. LSR's, untreated plants 9.68, 0.2 g a.i. m^{-2} ns, 0.4 g a.i. m^{-2} 9.68, 0.8 g a.i. m^{-2} ns, 1.6 g a.i. m^{-2} 21.64.

Figure IV.C.4.1.c.

Effect of EL500 on the height to the highest umbel. LSR's, untreated plants 7.21, 0.2 g a.i. m^{-2} ns, 0.4 g a.i. m^{-2} ns, 0.8 g a.i. m^{-2} ns, 1.6 g a.i. m^{-2} 10.64.


110
100
90
80
70
60
50
Height (cm)
lsd
Feb 9
March 4
March 18
April 18


150
140
130
120
110
100
90
80
70
Height (cm)
ns
Feb 2
March 4
March 18
April 18

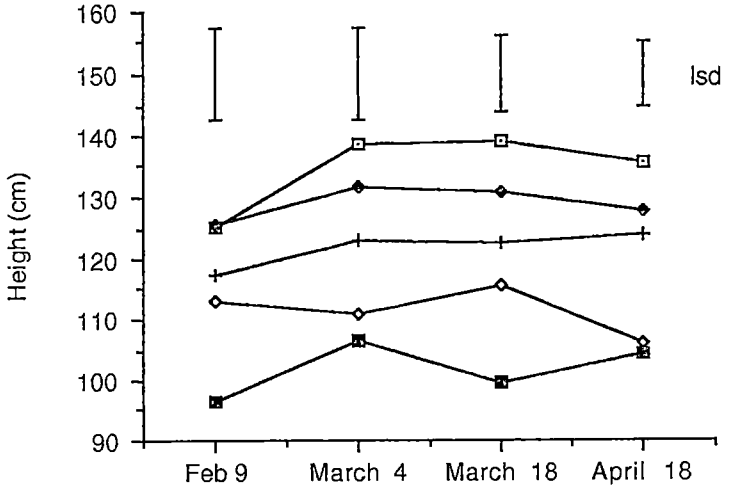
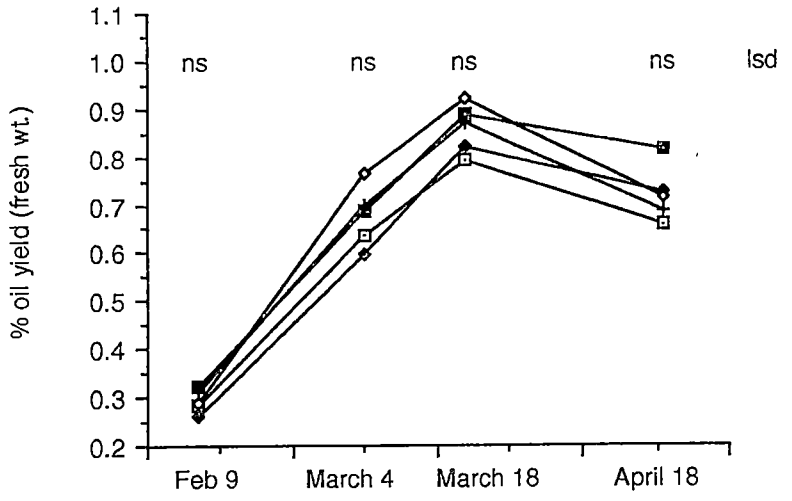

160
150
140
130
120
110
100
90
Height (cm)
lsd
Feb 9
March 4
March 18
April 18

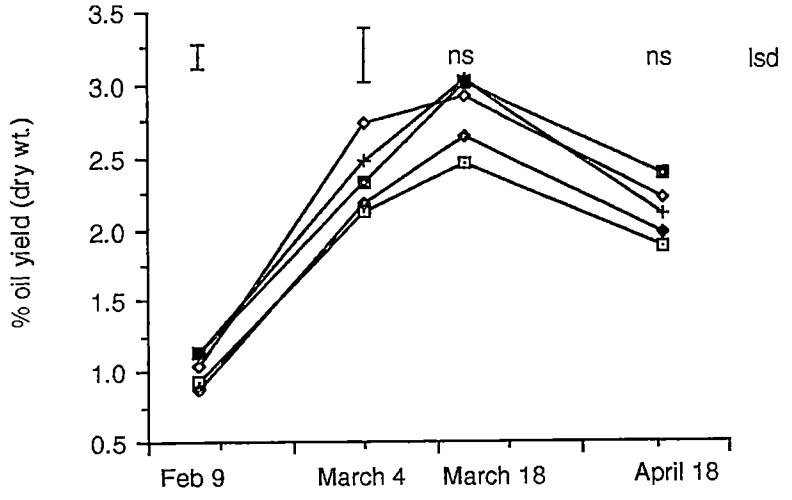
Figure IV.C.4.1.d.

The effect of EL500 on the % oil yield from whole plants, expressed on a fresh weight basis. LSR's, untreated plants ⊡ 0.107, 0.2 g a.i. m^{-2} ◇ 0.111, 0.4 g a.i. m^{-2} + 0.141, 0.8 g a.i. m^{-2} ◇ 0.190, 1.6 g a.i. m^{-2} ■ 0.119.

Figure IV.C.4.1.e.

The effect of EL500 on the % oil yield of whole plants, expressed on a dry weight basis. LSR's, untreated plants 0.326, 0.2 g a.i. m^{-2} 0.455, 0.4 g a.i. m^{-2} 0.612, 0.8 g a.i. m^{-2} 0.515, 1.6 g a.i. m^{-2} 0.548.


% oil yield (fresh wt.)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
ns
ns
ns
ns
lsd
Feb 9
March 4
March 18
April 18


% oil yield (dry wt.)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ns
ns
lsd
Feb 9
March 4
March 18
April 18

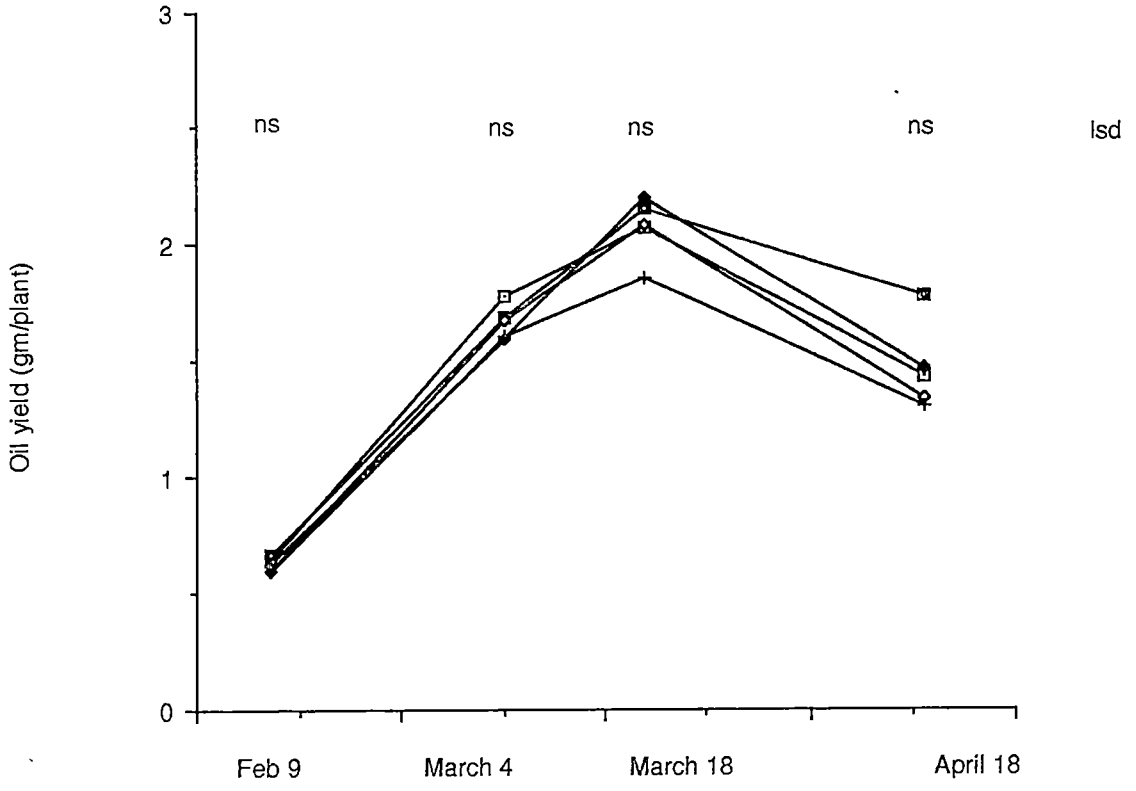


Figure IV.C.4.1.f.

The effect of EL500 on the oil yield from whole plants, expressed as grams per plant. LSR's, untreated plants ⊡ 0.44, 0.2 g a.i. m^{-2} ◇ 0.82, 0.4 g a.i. m^{-2} + 0.52, 0.8 g a.i. m^{-2} ◇ 0.54, 1.6 g a.i. m^{-2} ▣ 0.38.

Table IV.C.4.1.a.

Effect of EL500 on the fresh weight of whole plants (gms/10 plants).

	Treatment (gms a.i. per m^2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	2304	2456	2078	2036	1928	ns
4/3	2788	2648	2603	2180	2292	ns
18/3	2609	2667	2407	2256	2122	ns
18/4	2169	2020	2171	1868	1885	ns
LSR	ns	ns	ns	ns	ns	

4.3.1.3 Effect of EL500 on oil yield from whole plants

Applied EL500 did not effect the whole plant oil yield when expressed as a percentage of the total fresh weight of whole plants (Figure IV.C.4.1.d). All plants responded similarly with an increase in percent oil yield which reached a maximum on March 18. Percentage oil yield when expressed as a percent of the total amount of dry plant material present was significantly higher when plants were treated with 0.8 and 1.6 gms a.i.per m^2 of EL500. This result was recorded at on February 9 and March 4. Therafter no differences were recorded in yield between any treatments (Figure IV.C.4.1.e).

The oil yield expressed as grams per plant was not affected by EL500 (Figure IV.C.4.1.f). All treated plants reached maximum oil production at on March the 18th.

4.3.1.4 Effect of EL500 on oil composition from whole plants

The effects of the various application rates of EL500 on the composition of oil from whole plants are presented in Table IV.C.4.1.b to j. Levels of alpha-pinene, myrcene, cis-beta-ocimene and beta-phellandrene (+cineole) decreased with time in all treatments. No significant differences were noted between the various treatments. Alpha-phellandrene content decreased dramatically in all treatments as plants matured, the 1.6 and 0.4 gms a.i.per m^2 treated plants decreased more than the control plants to significantly lower levels on April 18. At no other stage, was any significant difference in alpha-phellandrene content detected.

No significant effect from application of EL500 was recorded on the levels of limonene, all plants responded similarly. The levels increased to maximum 70 days after initiation for the 0 and 0.2 g a.i. per m^2 treated plants whilst plants which received the higher application rates recorded an earlier maxima.

All three components, fenchone, estragole and anethole increased steadily in all plants during the period of observation. No effects of EL500 application were recorded.

4.3.1.5 Effect of EL500 on oil yield from each umbel order

The results of examination of the effect of EL500 on the yield of each umbel order are presented in Table IV.C.4.1.k. No effect of EL500 application was observed on numbers of umbels present, fresh weight of umbels, percent oil yield (fresh weight basis) or the amount of oil produced from each umbel for either primary, secondary or tertiary orders.

4.3.1.6 Effect of EL500 on oil composition for each umbel order

Analysis of the oil composition from each umbel order is presented in Table IV.C.4.1.l to n. The only significant change in the composition of the oil from the primary umbels was a decrease in fenchone levels for plants treated with 0.2, 0.4 and 0.6 gms a.i.per m^2 EL500. The umbels of plants treated with 1.6 gms a.i.per m^2 produced oil which was not different to that of the control. Estragole and anethole levels were higher in the primary umbels of plants treated with 0.2, 0.4, 0.6 gms a.i.per m^2. But, no effect was detected in the levels of these components at the highest rate of EL500 applied.

The application of EL500 decreased the levels of alpha-phellandrene in the oil from the secondaries, no other components were affected. No effects were observed for any components in the oil produced by the tertiary umbels.

Table IV.C.4.1

b) Effect of EL500 on the composition of oil from whole plants, mean values for Alpha-Pinene (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	16.94	14.22	14.33	14.66	15.77	ns
4/3	12.18	11.58	11.89	10.72	10.62	ns
18/3	11.55	10.09	8.89	10.36	9.67	ns
18/4	10.12	9.05	8.82	7.30	9.36	ns
LSR	2.61	3.98	2.18	2.34	3.24	

c) Effect of EL500 on the composition of oil from whole plants, mean values for Myrcene (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	1.67	1.52	1.66	1.62	1.73	ns
4/3	1.62	1.53	1.49	1.46	1.57	ns
18/3	1.62	1.47	1.37	1.57	1.32	ns
18/4	1.21	1.09	1.01	1.04	1.18	ns
LSR	0.23	0.32	0.24	0.38	0.21	

d) Effect of EL500 on the composition of oil from whole plants, mean values for Alpha-Phellendrene (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	13.84	12.64	14.15	13.36	14.30	ns
4/3	7.95	8.01	7.40	5.53	6.63	ns
18/3	4.49	4.68	4.30	3.46	3.58	ns
18/4	4.68	4.55	3.44	4.56	3.49	1.09
LSR	2.18	1.65	2.05	1.23	1.33	

Table IV.C.4.1 con't

e) Effect of EL500 on the composition of oil from whole plants, mean values for Limonene (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	1.63	1.50	1.65	1.47	1.68	ns
4/3	2.13	2.04	2.18	2.22	2.29	ns
18/3	2.26	2.19	2.08	2.42	2.02	ns
18/4	-1.62	1.53	1.50	1.66	1.69	ns
LSR	0.37	0.27	0.35	0.45	0.24	

f) Effect of EL500 on the composition of oil from whole plants, mean values for Beta-Phellandrene(+Cineole) (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	1.42	1.39	1.52	1.48	1.57	ns
4/3	1.29	1.31	1.31	1.15	1.37	ns
18/3	0.88	0.91	0.84	0.86	0.77	ns
18/4	0.80	0.68	0.58	0.83	0.56	ns
LSR	0.31	0.18	0.23	0.27	0.37	

g) Effect of EL500 on the composition of oil from whole plants, mean values for Cis-Beta-Ocimene (% total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	0.97	1.07	1.04	1.14	1.48	ns
4/3	0.95	0.99	1.29	0.85	1.35	ns
18/3	0.68	0.83	0.72	0.82	0.65	ns
18/4	0.49	0.50	0.46	0.52	0.46	ns
LSR	0.24	0.28	0.27	0.31	0.70	

Table IV.C.4.1 con't

h) Effect of EL500 on the composition of oil from whole plants, mean values for Fenchone (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	7.17	9.36	8.49	6.97	7.72	ns
4/3	16.77	15.86	17.23	18.64	18.38	ns
18/3	19.79	20.22	20.12	18.25	18.92	ns
18/4	17.85	18.39	19.46	19.11	19.39	ns
LSR	4.07	3.67	5.18	4.44	1.63	

i) Effect of EL500 on the composition of oil from whole plants, mean values for Estragole (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	1.95	2.11	2.06	2.08	1.93	ns
4/3	2.15	2.16	2.08	2.21	2.17	ns
18/3	2.31	2.21	2.32	2.32	2.37	ns
18/4	2.43	2.53	2.55	2.57	2.44	ns
LSR	0.21	0.23	0.02	0.02	0.23	

j) Effect of EL500 on the composition of oil from whole plants, mean values for Anethole (% of total FID response).

	Treatment (gms a.i. per m2)					
Harvest date	0	0.2	0.4	0.8	1.6	LSD
9/2	51.28	53.01	51.86	53.66	50.42	ns
4/3	51.91	53.45	51.71	54.11	52.54	ns
18/3	53.44	53.98	56.44	56.78	57.77	ns
18/4	58.22	59.34	59.91	60.44	59.28	ns
LSR	6.13	4.47	4.27	2.95	4.19	

Table IV.C.4.1.k.

Effect of EL500 on the primary, secondary and tertiary umbels in a field crop at time of commercial harvest.

PRIMARY UMBEL

Treatment	Number/plant	Weight/umbel	% Oil yield(fw)	gm Oil/umbel
0 g a.i./m2	1	4.48	3.87	0.175
0.2 g a.i./m2	1	5.07	5.25	0.264
0.4 g a.i./m2	1	5.29	5.09	0.233
0.8 g a.i./m2	1	5.26	3.32	0.189
1.6 g a.i./m2	1	4.85	5.14	0.238
LSD	ns	ns	ns	ns

SECONDARY UMBEL

Treatment	Number/plant	Weight/umbel	% Oil yield(fw)	gm Oil/umbel
0 g a.i./m2	7.1	3.24	2.69	0.085
0.2 g a.i./m2	7.3	2.73	3.64	0.097
0.4 g a.i./m2	7.4	3.06	3.92	0.107
0.8 g a.i./m2	6.8	3.12	3.09	0.096
1.6 g a.i./m2	6.9	3.79	2.94	0.109
LSD	ns	ns	ns	ns

TERTIARY UMBEL

Treatment	Number/plant	Weight/umbel	% Oil yield(fw)	gm Oil/umbel
0 g a.i./m2	14.8	0.95	2.26	0.021
0.2 g a.i./m2	13	0.89	2.55	0.022
0.4 g a.i./m2	11.5	0.95	2.24	0.021
0.8 g a.i./m2	12.1	0.76	1.93	0.015
1.6 g a.i./m2	12.7	0.84	1.98	0.016
LSD	ns	ns	ns	ns

Table IV.C.4.1 con't.

1) Effect of EL500 on the composition of oil from Primary umbels, mean values for analysis (% total FID response).

Compound	Treatment (gms a.i. per m2) 0	0.2	0.4	0.8	1.6	LSD
Alpha-Pinene	3.04	3.58	2.96	2.50	3.32	ns
Myrcene	0.91	0.82	0.76	0.91	0.85	ns
Alpha-Phellendrene	1.58	0.97	0.93	0.88	0.98	ns
Limonene	1.91	1.39	1.45	1.24	1.59	ns
Beta-Phellandrene + Cineole	0.65	0.47	0.54	0.52	0.56	ns
Cis-Beta-Ocimene	0.36	0.38	0.38	0.36	0.42	ns
Fenchone	25.39	20.86	21.30	20.81	24.31	3.43
Estragole	2.56	2.89	2.80	2.80	2.68	0.18
Anethole	62.04	67.35	67.73	68.86	64.11	4.31

m) Effect of EL500 on the composition of oil from Secondary umbels, mean values for analysis (% total FID response).

Compound	Treatment (gms a.i. per m2) 0	0.2	0.4	0.8	1.6	LSD
Alpha-Pinene	4.30	5.13	3.55	4.64	5.02	ns
Myrcene	1.02	1.03	0.92	0.81	1.07	ns
Alpha-Phellendrene	2.09	1.59	1.41	1.31	1.42	0.54
Limonene	2.08	1.82	1.82	1.89	1.92	ns
Beta-Phellandrene + Cineole	1.02	1.03	0.92	0.81	1.07	ns
Cis-Beta-Ocimene	0.52	0.46	0.46	0.48	0.56	ns
Fenchone	23.64	21.33	23.24	20.08	22.42	ns
Estragole	2.51	2.72	2.71	2.68	2.62	ns
Anethole	61.52	63.45	63.77	65.58	62.53	ns

Table IV.C.4.1 con't.

n) Effect of EL500 on the composition of oil from Tertiary umbels, mean values for analysis (% total FID response).

Compound	Treatment (gms a.i. per m2) 0	0.2	0.4	0.8	1.6	LSD
Alpha-Pinene	4.89	6.11	4.38	6.66	6.30	ns
Myrcene	1.14	1.12	0.98	1.01	1.13	ns
Alpha-Phellendrene	3.26	2.44	2.54	2.26	2.56	ns
Limonene	1.94	1.76	1.79	1.89	2.03	ns
Beta-Phellandrene + Cineole	0.86	0.71	0.78	0.88	0.79	ns
Cis-Beta-Ocimene	0.99	1.09	0.91	0.95	1.06	ns
Fenchone	21.67	18.62	18.84	17.73	21.01	ns
Estragole	2.41	2.59	2.62	2.51	2.38	ns
Anethole	61.05	64.39	66.03	64.48	61.40	ns

4.3.2 Residual effect of EL500

A year later the effect of EL500 on elongation was still noticeable but only from the areas treated with greater than 0.4 g a.i. per m^2 (Figure IV.C.4.2). The 1.6 gms a.i.per m^2 rate of EL500 decreased the height of the highest umbel by 19 percent. The height of the primary umbel was reduced by 23 percent and to the lowest umbel by 28 percent when compared with the untreated plants.

Table IV.C.4.2.a) Residual effect of EL500 on the whole plant yields in a field crop at time of commercial harvest.

Treatment	Total FW g/m^2	% Oil (FW)	% Oil (DW)	Oil g/plant
0 g a.i./ m^2	3024	0.67	1.93	2.03
0.2 g a.i./ m^2	3521	0.52	1.46	1.83
0.4 g a.i./ m^2	3118	0.64	1.77	1.99
0.8 g a.i./ m^2	3352	0.66	1.89	2.19
1.6 g a.i./ m^2	2092	0.67	1.68	1.54
LSD	ns	ns	ns	ns

The total fresh weight of plant material present per m^2 in the area treated the previous year was not affected by application of EL500. Percent oil yield on both a fresh and dry weight basis as well as the oil yield in gm per plant were not affected (Table IV.C.4.2.a).

Alpha-phellandrene levels were lower in whole plant oil from the 0.8 and 1.6 gms a.i.per m^2 treated areas. All other components remained the same for all treatments (Table IV.C.4.2.b).

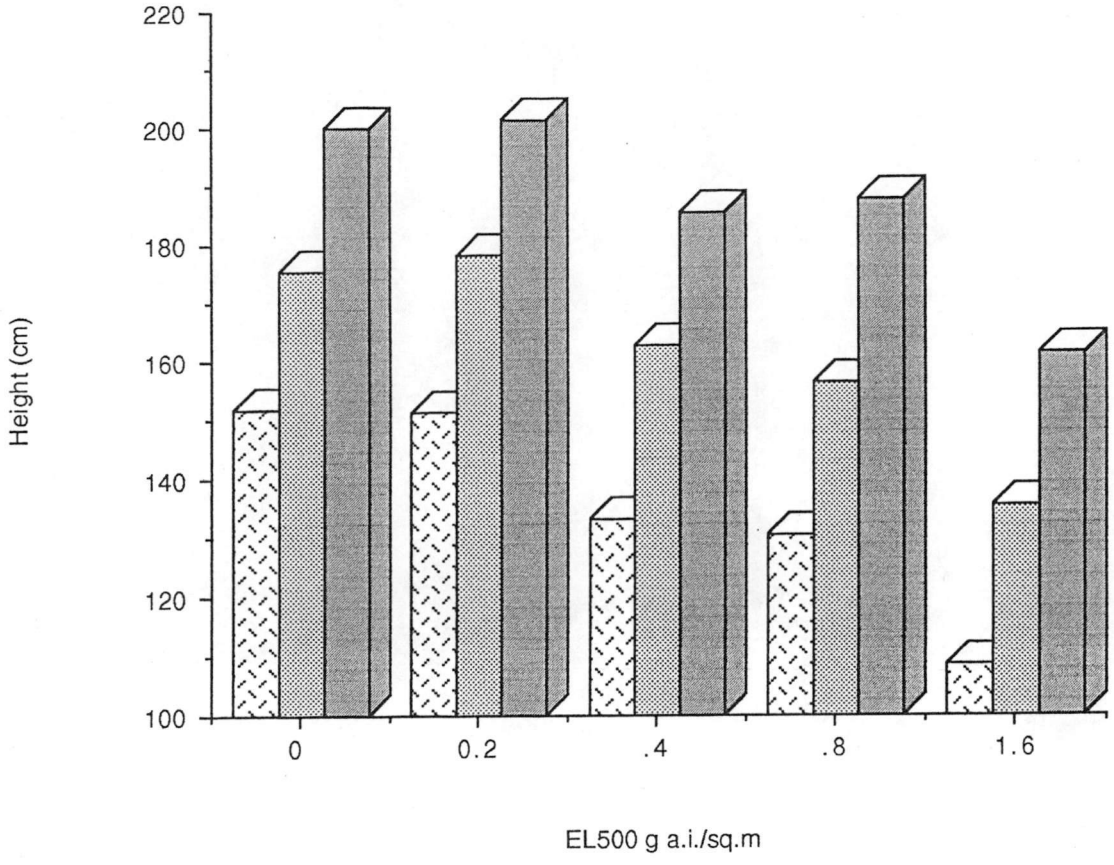


Figure IV.C.4.2.

The residual effect of EL500 in the following season on the heights to the lowest, primary and highest umbels within the canopy. LSD's, lowest umbel ▨ 16.9, primary umbel ▩ 15.9 and highest umbel ▩ 19.5.

Table IV.C.4.2.a) Residual effect of EL500 on the whole plant yields in a field crop at time of commercial harvest.

Treatment	Total FW g/m2	% Oil (FW)	% Oil (DW)	Oil g/plant
0 g a.i./m2	3024	0.67	1.93	2.03
0.2 g a.i./m2	3521	0.52	1.46	1.83
0.4 g a.i./m2	3118	0.64	1.77	1.99
0.8 g a.i./m2	3352	0.66	1.89	2.19
1.6 g a.i./m2	2092	0.67	1.68	1.54
LSD	ns	ns	ns	ns

b) Residual effect of EL500 on the composition of oil from whole plants, mean values for analysis (% total FID response).

	Treatment (gms a.i. per m2)					
Compound	0	0.2	0.4	0.8	1.6	LSD
Alpha-Pinene	8.72	7.83	6.96	7.39	7.34	ns
Myrcene	1.14	1.04	0.98	1.03	1.07	ns
Alpha-Phellendrene	5.11	5.51	5.10	3.34	3.37	1.76
Limonene	1.41	1.44	1.42	1.39	1.60	ns
Beta-Phellandrene + Cineole	1.00	1.03	1.03	0.84	0.95	ns
Cis-Beta-Ocimene	0.49	0.56	0.42	0.36	0.39	ns
Fenchone	16.37	15.69	17.14	15.92	16.08	ns
Estragole	2.62	2.62	2.71	2.81	2.69	ns
Anethole	60.10	61.64	60.56	62.78	62.29	ns

Table IV.C.4.2.b) Residual effect of EL500 on the composition of oil from whole plants, mean values for analysis (% total FID response).

	Treatment (gms a.i. per m^2)					
Compound	0	0.2	0.4	0.8	1.6	LSD
Alpha-Pinene	8.72	7.83	6.96	7.39	7.34	ns
Myrcene	1.14	1.04	0.98	1.03	1.07	ns
Alpha-Phellendrene	5.11	5.51	5.10	3.34	3.37	1.76
Limonene	1.41	1.44	1.42	1.39	1.60	ns
Beta-Phellandrene + Cineole	1.00	1.03	1.03	0.84	0.95	ns
Cis-Beta-Ocimene	0.49	0.56	0.42	0.36	0.39	ns
Fenchone	16.37	15.69	17.14	15.92	16.08	ns
Estragole	2.62	2.62	2.71	2.81	2.69	ns
Anethole	60.10	61.64	60.56	62.78	62.29	ns

4.4 Discussion

The growth retardant EL500, flurprymidol, was shown to be effective in the inhibition of extension growth of the fennel plant when grown in a field situation, provided the rate of application was 0.4 g a.i.per m^2 or greater. Inhibition of stem elongation increased as the application rate was increased. No detrimental effects on the oil yield or composition were noted for the range of rates utilized. Some changes in minor components were detected. The anethole yield was not changed by the use of this growth retardant. The possibility of increasing the anethole content using EL500 does exist. The primary umbels increased by 6 percent over the values for the untreated plants but no increase was observed for any other umbel order. The later orders may increase in anethole level as they mature and follow the similar pattern of the primary umbels.

The application of EL500 with a precise spray unit as well as attention to plant density in the trial area improved the variability between replications and also between blocks, no statistically significant block effect was apparent in the results.

EL500 was shown to have a residual effect in the second year of growth of the crop. Initially during the early regrowth of the plants in spring this effect appeared to be pronounced as indicated by

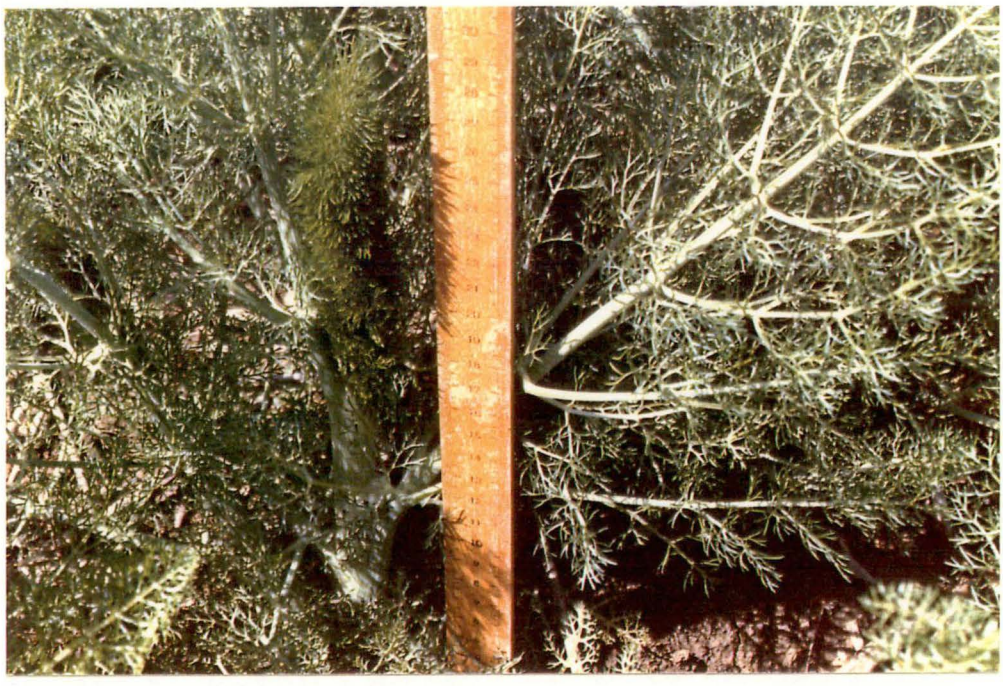
Plate IV.C.4.1 and IV.C.4.2. As the season progressed the initial inhibition effect lessened. Plants continued to elongate rapidly once the natural daylength exceeded inductive levels. The final effect on elongation was still measurable for the 0.8 and 1.6 g a.i. per m^2 treated areas. No effect on umbel number was detected as indicated by no significant effect on the oil yield per plant. Such a result was not expected after the effects observed in preliminary timing trials (IV.C.4.2). In these earlier series of experiments on plants in pots the presence of EL500 prior to initiation decreased the number of umbels initiated. A possible explanation for no effect on umbel number in this experiment is the larger volume of soil present as well as the effect of winter rain and irrigation diluted the EL500 available to the plant roots. Technical information from Elanco indicated that EL500 is only weakly adsorbed by soil and is readily desorbed back into solution (Appendix IV.C.4.1). In areas which receive 250 to 300 mm per annum and supplementary irrigation, a soil half-life of six months is expected. But despite this data from Elanco, the 1.6 g a.i. per m^2 decreased main stem elongation by 23 percent over the control plants in the second year compared to 27 percent in the first year of application.

Plate IV.C.4.1.

The residual effect of EL500 (1.6 gm a.i. m^{-2}) in the next season. The inhibition of stem elongation and leaf expansion is evident when compared to Plate IV.C.4.2 below.

Plate IV.C.4.2.

An example of the normal stage of development in Spring of an untreated plant adjacent to the EL500 (1.6 gm a.i. m^{-2}) treated plants.



IV.C.5 Effect of exogenous applications of Gibberellins under inductive and non-inductive conditions.

5.1 Introduction

Exposure of fennel to long photoperiods results in a change in growth habit from a compressed rosette to a rapid elongation phase of growth. The process of rapid stem elongation suggests that the major group of phytohormones involved in this response are the gibberellins. The application of EL500, thought to be an inhibitor of gibberellin biosynthesis, has been shown to reduce stem elongation. Some workers have indicated that the gibberellins are also important for umbel initiation (Section II.E.8).

This study endeavours to investigate the role of this phtyohormone group in elongation and umbel initiation by the application of GA_3 to the plant in varying concentrations and at different stages of development.

5.2 Materials and Methods

5.2.1 Effect of different concentrations of applied GA_3

Fennel seedlings (var. C25) were transplanted into 150mm pots containing potting media as described in the General Materials and Methods.

0.5 ml of GA_3 solutions, 0, 50, 75, 100, 200 mg/l, were applied twice. The first application was at the 6th true leaf emerged stage of development. The second application was a fortnight later. The method of application used was wetting a cotton bud with solution placed in the axil of the last emerged leaf. A technique in accordance with Hanisova and Krekule (1975). Four replications of each treatment were used.

The series of treatments were applied to two groups of plants, each in one of the growth rooms described in the General Materials and Methods. One room produced non-inductive conditions, 10 hours photoperiod and 14 hours night. A second growth room was used to subject fennel plants to inductive conditions, 16 hours photoperiod (10 hours daylight + 6 hours non-photosynthetic incandescent light) and 8 hours night.

5.2.2 Effect of application of GA_3 at different stages of development

Fennel plants in the same growth room units received two applications of 0.5 mls of GA_3 solution (200 mg/l), a fortnight apart. Application commenced at various growth stages, 2, 4, 6 and 8 expanded leaves.

In both experiments the cotton wool buds remained in the leaf axils for 5 days. The height to the last emerged leaf and umbel number were recorded over a period of 74 days.

5.2.3 Application of GA_3 as a continual exogenous source

A third GA_3 application experiment was conducted to examine the effect of continual fortnightly applications of 0.5 mls of a 200 mg/l GA_3 solution. The cotton bud technique was again used for application of GA_3 to fennel plants in a growth room generating the non-inductive conditions. The commencement of GA_3 application was at the 2, 4, 6, 8 and 10 expanded leaf stages.

5.3 Results

5.3.1 Effect of different concentrations of exogenous GA_3

Plants treated with 50, 100 or 200 mg/l solutions of GA_3 subjected to long photoperiod elongated more than both plants treated with 25 mg/l solution GA_3 and control plants (Figure IV.C.5.1.a). These three higher rates were not significantly different in height from each other over the period of observation.

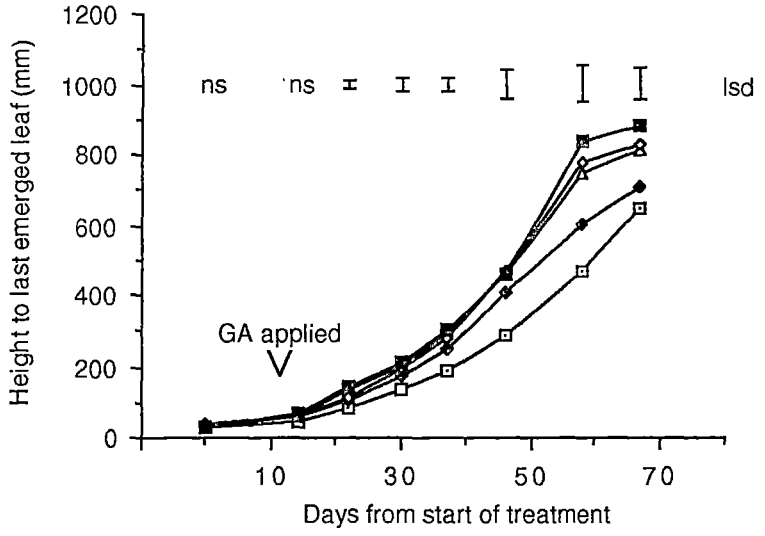
During the experiment, long photoperiods induced elongation in all treatments. The application of GA_3 solutions at concentrations greater than 50 mg/l increased the rate of elongation, this response was measurable after 20 days.

Figure IV.C.5.1.a.

The effect of GA_3 applied at various concentrations on the elongation of the main stem as measured by the height to the last emerged leaf. Plants subjected to long-days. LSR's, Untreated plants ⊡ 80.2, 25mg GA_3 per litre solution ◆ 150.5, 50mg GA_3 per litre solution △ 165.7, 100mg GA_3 per litre solution ○ 80.3 and 200mg GA_3 per litre solution ▣ 91.7.

Figure IV.C.5.1.b.

The effect of GA_3 applied at various concentrations on the elongation of the main stem as measured by the height to the last emerged leaf. Plants subjected to short-days. LSR's, Untreated plants 13.1, 25mg GA_3 per litre solution 15.6, 50mg GA_3 per litre solution 16.7, 100mg GA_3 per litre solution 20.2 and 200mg GA_3 per litre solution 19.5.


1200
1000
800
600
400
200
0
Height to last emerged leaf (mm)
ns
ns
lsd
GA applied
10
30
50
70
Days from start of treatment

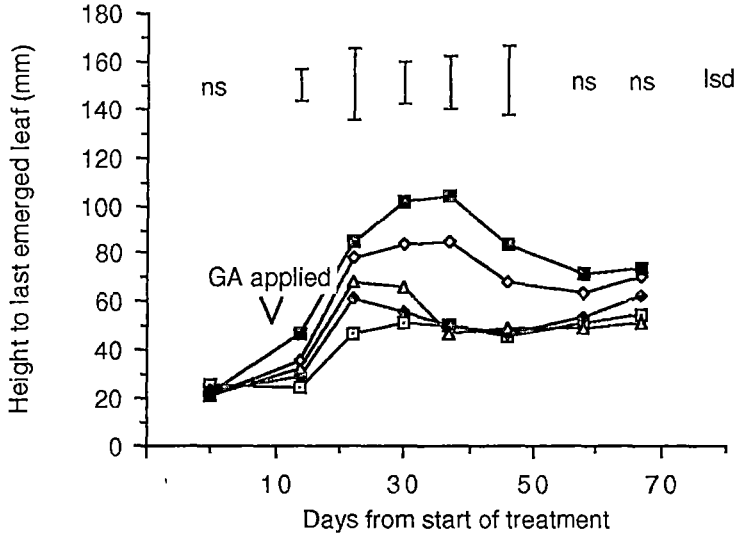

180
160
140
120
100
80
60
40
20
0
Height to last emerged leaf (mm)
ns
ns
ns
lsd
GA applied
10
30
50
70
Days from start of treatment

Table IV.C.5.1.a) Effect of different rates of application of GA_3 on umbel number under long photoperiod (85 days after commencement)

Treatment	Mean number of umbels per plant		
	Primary	Secondary	Tertiary
Control	1	1.75	0
25mg/l GA3	1	3.25	2
50mg/l GA3	1	4	1
100mg/l GA3	1	5	5.5
200mg/l GA3	1	5.25	7.75
LSD		2.27	3.38

Application of 100 and 200 mg/l GA_3 solutions increased the number of secondary and tertiary umbels present (Table IV.C.5.1.a). No other treatments affected umbel number.

Plants under non-inductive photoperiods treated with the same concentration series of GA_3 elongated rapidly after application. Elongation increased with the increased concentration of applied GA_3 solution. But no difference was noted between the response to the 100 and 200 mg/l GA_3 solutions (Figure IV.C.5.1.b). After a period of 50 days after application of GA_3 no differences in overall height were recorded as all plants reverted to a rosette form of growth. This decrease in the height shown by the high rates of GA_3 treated plants was due to an affect on leaf angle, producing a more erect leaf which in time reverted to almost a horizontal habit. The time for this reversion to a rosette habit to occur increased with increasing concentration of applied GA_3. No umbel initiation resulted from GA_3 application to plants under short days.

5.3.2 Effect of applying GA_3 at different stages of development

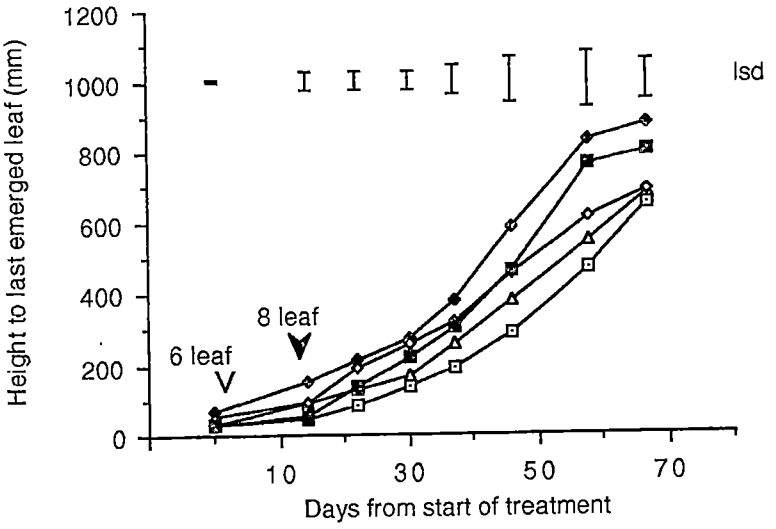
Plants under long photoperiod elongated more rapidly when GA_3 was applied at the 2 or 8 expanded leaf stage of development (Figure IV.C.5.2.a). Application of GA_3 at the 4 or 6 leaf stage did not

Figure IV.C.5.2.a.

The effect of GA_3 applied at various stages of development on the elongation of the main stem as measured by the height to the last emerged leaf. Plants subjected to long-days. LSR's, Untreated plants -⊡- 80.2, application at 2 expanded leaves 106.6, at 4 expanded leaves -△- 98.3, at 6 expanded leaves -◇- 134.8 and at 8 expanded leaves -■- 161.

Figure IV.C.5.2.b.

The effect of GA_3 applied at various stages of development on the elongation of the main stem as measured by the height to the last emerged leaf. Plants subjected to short-days. LSR's, Untreated plants 13.1, application at 2 expanded leaves 15.2, at 4 expanded leaves 33.6, at 6 expanded leaves 43.5 and at 8 expanded leaves 13.5.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1000
800
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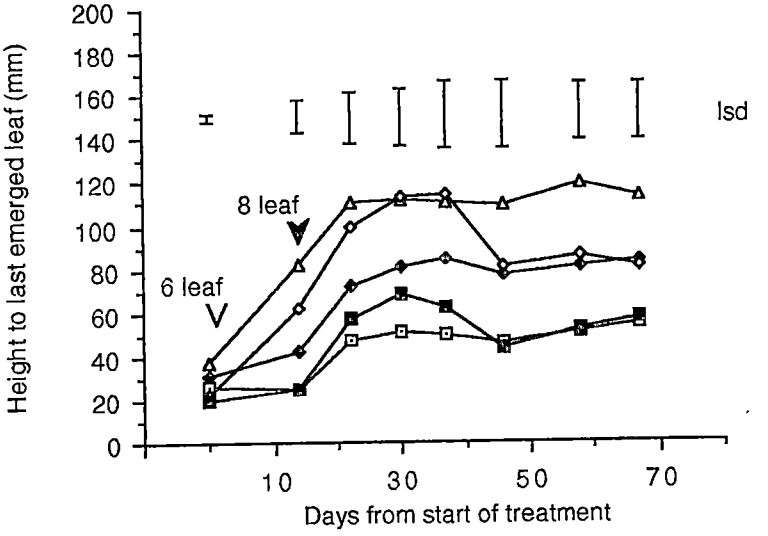
400
200
0
Height to last emerged leaf (mm)
lsd
8 leaf
6 leaf
10
30
50
70
Days from start of treatment


200
180
160
140
120
100
80
60
40
20
0
Height to last emerged leaf (mm)
lsd
8 leaf
6 leaf
10
30
50
70
Days from start of treatment

significantly affect elongation compared to the control. The affect on umbel number was similar with the 2, 6 and 8 leaf applied GA_3 treatments producing significantly more secondary and tertiary umbels than the control plants after 85 days (Table IV.C.5.1.b). Application at the 8 leaf stage under short photoperiods did not significantly affect elongation. But when applied at the 4 and 6 leaf stage, GA_3 increased elongation rate for a period of approximately 25 days followed by a cessation of elongation (Figure IV.C.5.2.b). Umbel initiation again did not occur under short photoperiod.

Table IV.C.5.1.b) Effect of application of GA_3 at different stages of development under long photoperiod (85 days after commencement)

Treatment	Primary	Secondary	Tertiary
Control	1	1.75	0
2 leaf	1	3.75	5.75
4 leaf	1	3.25	0
6 leaf	1	4.5	4.5
8 leaf	1	5	6
LSD		1.79	2.05

5.3.3 Effect of a continual exogenous supply of GA_3

Continual application of GA_3 to plants under short photoperiods resulted in increased rate of elongation. Plants elongated more when GA_3 was applied early in development, at the 2 or 4 leaf stage of development. Application at later stages resulted in increased elongation of the plant but at a slower rate (Figure IV.C.5.3). The fortnightly application of GA_3 to fennel plants under non-inductive photoperiod did not cause umbel initiation.

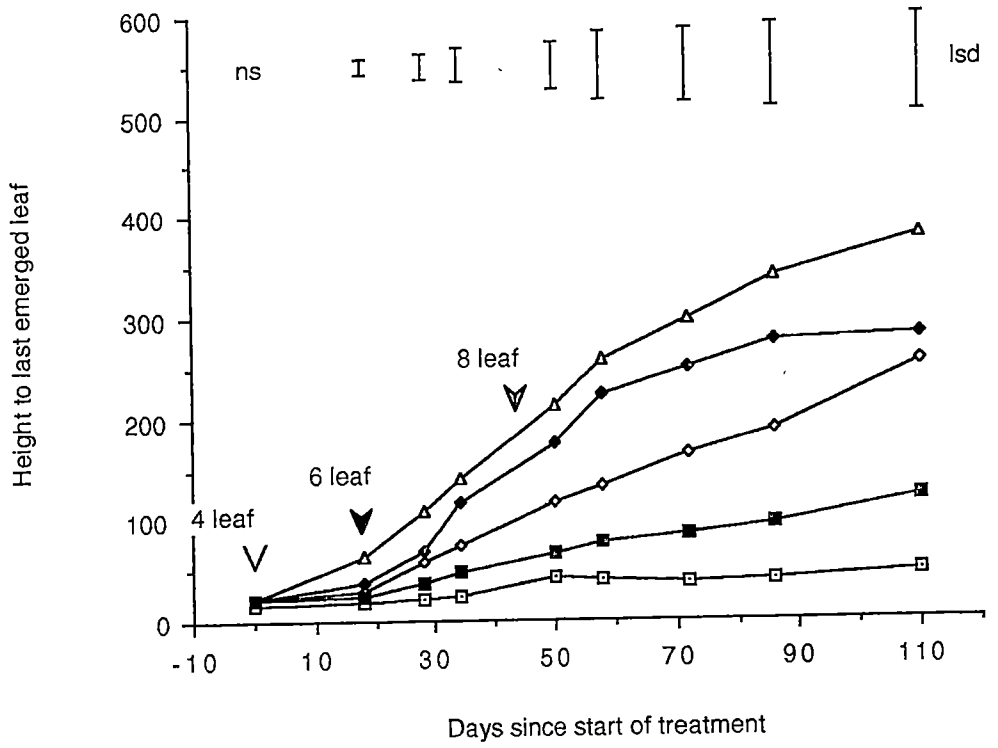


Figure IV.C.5.3.

The effect on elongation, as measured by the height to the last emerged leaf, by the continual presence of a source of exogenous GA_3 while plants subjected to short-days. LSR's, Untreated plants -□- 27.3, application commencing at 2 expanded leaves -◇- 61.8, at 4 expanded leaves -△- 119.6, at 6 expanded leaves -◇- 119.8 and at 8 expanded leaves -■- 41.2.

5.4 Discussion

To achieve a significant response to the application of GA_3 on fennel, 1ml of a 50 or more mg/l solution was required for the mode of application used in this experimentation.

The application of GA_3 to fennel plants at a very early stage of development held under inductive conditions may have resulted in a very high concentration of GA_3 per unit of plant material. Such a high level of GA may have increased the vegetative growth rate of the plant. This may allow earlier perception of the long photoperiod as the juvenile phase of growth would be completed more rapidly and more nodes produced. The net effect would lead to period of more rapid differentiation during initiation, increased branching and increased number of umbels initiated. GA_3 applied prior to the completion of the juvenile phase, 8 expanded leaves, increased exogenous GA_3 levels during the period of initiation producing more rapid elongation and increased umbel initiation. This may be an example of increased flower numbers due to increased vegetative growth, as indicated in Brassica spp. by Thurling and Vijendra Das (1979).

The presence of larger amounts of plant material at the 4 and 6 expanded leaf stages may have resulted in a dilution of the effect of the applied GA_3 resulting in a smaller elongation response. Once the plants reached the stage of initiation, the applied GA_3 was probably no longer active, as indicated by the insignificant affect on umbel number when applied at the 4 expanded leaf stage. Alternatively, GA activity was decreasing as indicated by the affect on umbel number for plants treated at the 6 expanded leaf stage.

GA_3 applied to fennel plants held under short days did not result in umbel initiation. The rate of elongation in response to GA_3 when applied to plants under non-inductive photoperiods was much lower than similar application rates to plants under inductive conditions. Such results indicate that the exogenous applied GA_3 to plants under inductive conditions, may be complementing the endogenous levels of GA's. Alternatively plants held under non-inductive conditions are less receptive to exogenous applications of GA's.

Examples of the manipulation of the elongation and flowering responses in fennel through the application of GA_3, and in combination with changes in photoperiod are presented in Plates IV.C.5.1 and IV.C.5.2. A typical rosette fennel plant produced under short days is shown on the right of Plate IV.C.5.1. The application of GA_3 to fennel plants under short days produced an elongated plant as shown on the left Plate IV.C.5.1. Once all the exogenous applied GA_3 was utilized, reversion to a rosette form of growth occurred on top of the elongated stem. Further vegetative growth will continue unless the plant is subjected to long photoperiods. The result of such treatment can be seen in Plate IV.C.5.2. Initiation of umbels and further elongation occurred, producing the rather odd growth habit.

Plate IV.C.5.1.

An example of the effect of exogenous GA_3 application under short day conditions. Both plants remained under short day conditions but the plant on the left received a total of 1ml of a 20mg per litre solution of GA_3 at the two leaf stage of development. The plant on the right was untreated.

Plate IV.C.5.2.

An example of the manipulation of plant habit by application of GA_3 combined with variations in photoperiod.. GA_3 was applied to the plant whilst under short days. Elongation of the internodes resulted until the exogenous source was utilized. Reversion to a rosette habit followed. By then placing under long days the plant then elongated and floral initiation occurred from the rosette on top of the already elongated main stem.

IV.D Examination of the photosynthetic characteristics of fennel.

IV.D.1 Examination of the effects of temperature and light on photosynthesis of leaves and umbels.

1.1 Introduction

From observations of plants treated with chemical growth retardants, the role of the leaves in supply of assimilates may be minor and the umbels are possibly photosynthetically active units.

The aim of the present study was to investigate the effect of temperature and light intensity on photosynthesis, photorespiration and dark respiration in both the leaves and umbels of fennel.

The supply of photosynthates has been shown to be important for both flower initiation and development, particularly in such crops as oil seed rape. Knowledge of the effects of temperature and light aid in design of future experiments.

1.2 Materials and Methods

The methodology for the measurement of photosynthesis was Infra Red Gas Analysis (IRGA) as this technique allows investigation to be undertaken on attached plant parts. The measurement of photosynthesis, dark respiration, and photorespiration plus oxygen inhibition can be made on the same portion of the plant by illumination, placing in darkness, or supplying with normal CO_2 levels but reduced O_2 levels, respectively.

2.1 Gas exchange system

An open circuit system was used to monitor net CO_2 exchange within a perspex leaf chamber (Figure IV.D.1). The experimental set up was similar to Clark (1980) with some slight modifications to improve humidification and water scavenging.

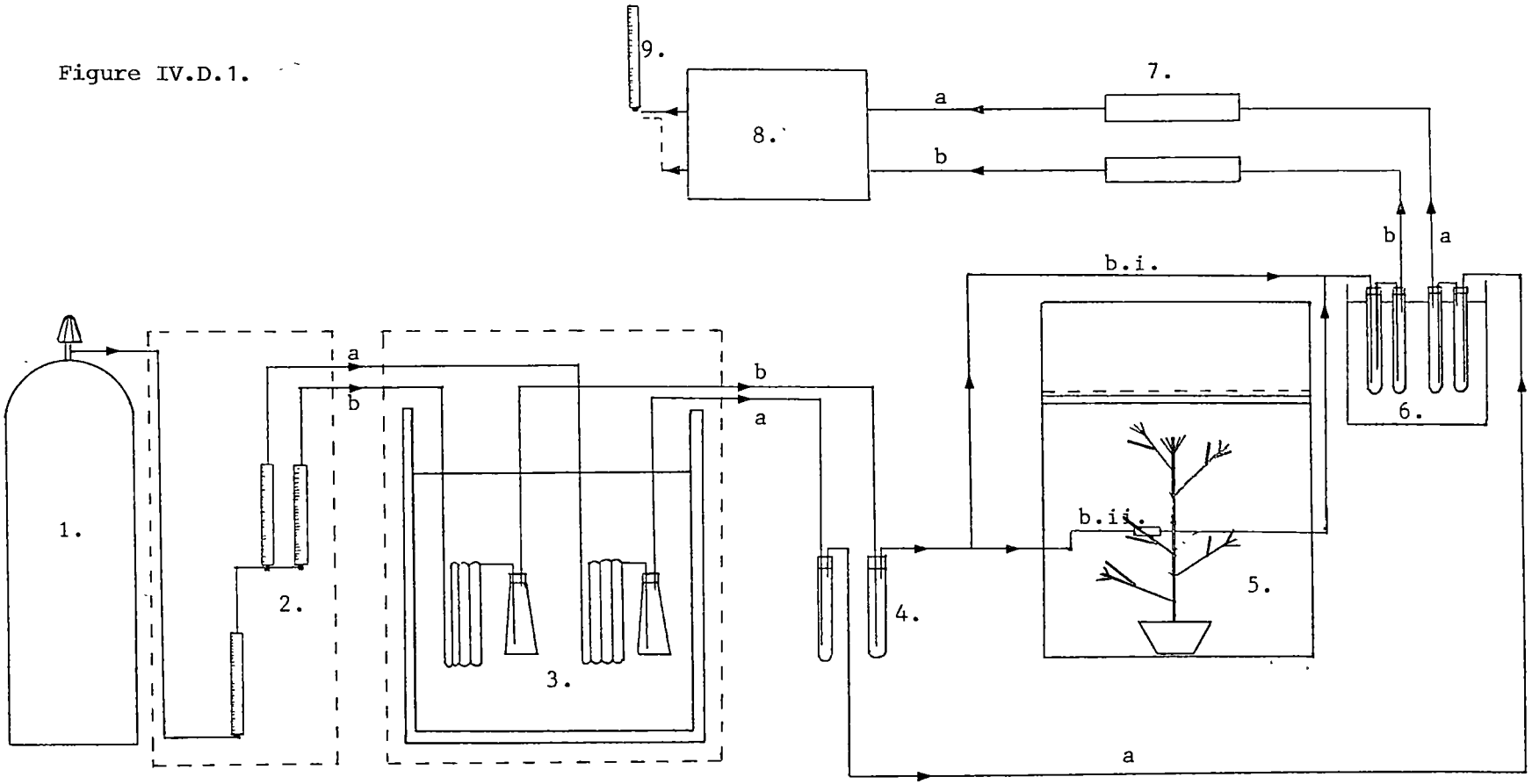
Attached leaves or umbels were placed in the leaf chamber, surrounded by a water jacket to control the temperature within the chamber. Temperature was monitored continuously using a

Figure IV.D.1.

Diagramatic representation of the open circuit CO_2 monitoring system.

1. Gas supply (21% O_2 in N_2, 330 ppm CO_2).
2. Pressure control guages (100-1000ml/min.).
3. Gas temperature control system and humidification system.
4. Tubes to remove excess water.
5. Light conditions
   (i) Lighting Multi metal lamp (M400/BUH, EYE, Japan).
   (ii) Light intensity control (Sharlon shade screens).
   (iii) Water bath.
6. De-humidification system. Test tubes immersed in ice-salt mixture in a vacuum flask.
7. Drying tubes containing drierite.
8. IRGA Model ADC 225-Mk 2.
9. Flow meter (900ml/min.).
   a. Reference line.
   b. i. By-pass line (to allow for calibration and base line correction).
   b.ii. Chamber supply line.

Figure IV.D.1.



copper-constant thermocouple placed on the underside of the plant tissue within the chamber. The chamber was designed to allow maximum mixing of air.

Photosynthetically active lighting was provided by placing the chamber under a Multi-metal lamp (M400/BUH, EYE, Japan) and a 400W mercury vapour lamp. Photon flux density was altered by placing varying thicknesses of Sarlon shade cloth between the light source and the 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 μmole m^{-2} s^{-1}. All measurements of the change in photon flux density were recorded from within the leaf chamber to account for the water jacket and chamber walls.

Several precautions were taken to ensure constant temperature and humidity control over the tissue surface:

(a) Humidification tubes and gas temperature equilibration coils were placed in a water bath maintained at the required temperature of the leaf surface.

(b) To avoid differences between the leaf chamber and the reference air supply due to temperature humidity and CO_2 adsorption by water, both the reference and the sample air supplies were subjected to the same treatment, except that the reference line did not pass through the leaf chamber.

(c) The length of tubing between the humidification system and the leaf chamber was minimised.

(d) As far as possible the room temperature was maintained at the temperature of the leaf chamber.

2.2 Specimen material

Plant material utilized for this series of experiments was clonal material of the variety C25 grown in the glasshouse described in the General Materials and Methods. Daylength was 16 hours at approximately 500 to 1200 μmole m^{-2} s^{-1}.

2.3 Calibration of IRGA and Gas Supply, Calculation of Photosynthetic Rate

The IRGA used during these series of experiments was a Hoddesdon model ADC 225-Mk2 from England. Between each reading the IRGA was allowed to rezero by by-passing the leaf chamber. During this time the leaf chamber was kept supplied with air from an auxillary pump passing through the same temperature and humidification control system as described above.

The change in concentration of CO_2 (ppm) detected by the IRGA was shown on a needle deflection scale, but this did not enable determination of a stable reading unless watched constantly. To overcome this problem a chart recorder was used in its place. The deflection (mm) was accurately converted to change in CO_2 concentration. This was achieved using gas mixtures of known CO_2 concentration varied between reference and sample lines plotted against the chart response (Appendix IV.D.1.1). From this it was possible to convert the chart response to change in CO_2 (ppm):

$$CO_2 \text{ differential} = 0.8587 * \text{Chart response (mm)} + 3.8438$$

Base line correction was achieved by passing air of the same CO_2 concentration through both the reference and sample lines, (i.e. CO_2 differential = 0).

CO_2 concentrations of the gas bottles were checked by using the IRGA in absolute mode to read ppm directly, only gas mixtures in the range of 320 - 360 ppm CO_2 were used.

Conversion of the CO_2 differential (ppm) to net CO_2 exchange (mg CO_2 g^{-1} hr^{-1}) was by the following equation:

$$\text{mg } CO_2 \text{ gm}^{-2} \text{ hr}^{-1} = \frac{CO_2 \text{ (ppm)}}{10^6} \cdot \frac{44 * 1000 \text{ (mg/mole)}}{1} \cdot \frac{0.3 \text{ (l/min)} * 60 \text{ (min)}}{22.4 \text{ (l/mole)}} \cdot \frac{1}{\text{weight (gm)}}$$

2.4 Net CO_2 Exchange Measurements

Net CO_2 exchange was measured at saturating light intensities on mature leaves and umbels over the temperature range of 5 to 35°C at 5°C intervals. The umbels when examined were at a post anthesis stage of development.

Rates of "apparent" photosynthesis and dark respiration on both plant structures were determined by measuring CO_2 exchange in air (21 percent O_2) in the light and dark respectively. The effects of photorespiration was measured on leaves only and determined as enhancement of net CO_2 exchange in 2 percent O_2 at light saturation.

A determination of the light saturation response was carried out on leaves by varying the photon flux density from 100 to 1000 μmole m^{-2} s^{-1}. Optimal temperature, as determined in the above experiment, were used.

The flow rate for both leaves and umbels was 0.3 l per min, this allowed maximum sensitivity but did not allow the plants to reduce the CO_2 levels by more than 5ppm at any time. After changes to temperature or photon flux density the net CO_2 was allowed to stabilize for 15 mins before measurements were recorded, a constant plant response was indicated by a stable rate of CO_2 uptake or efflux.

1.3 Results

1.3.1 Temperature and net CO_2 exchange response

Net CO_2 fixation (apparent photosynthesis) in leaves in 21 percent O_2 reached a maximum at a temperature of 20 to 25°C when exposed to 1100 μmole m^{-2} s^{-1} (Figure IV.D.1.1). This decreased rapidly with increasing or decreasing temperature. Enhancement of net CO_2 fixation in 2 percent O_2 was most pronounced over the same temperature range. Efflux of CO_2 in the dark (dark respiration) increased with increasing temperature. Such a response represented a $\overline{Q_{10}}$ value of approximately 2. The photorespiration component did change significantly with temperature, but the increase was small for the temperature range examined.

True photosynthesis was calculated by eliminating the dark respiration and photo respiration components, assuming dark respiration continues whilst the plant is illuminated, as well as the

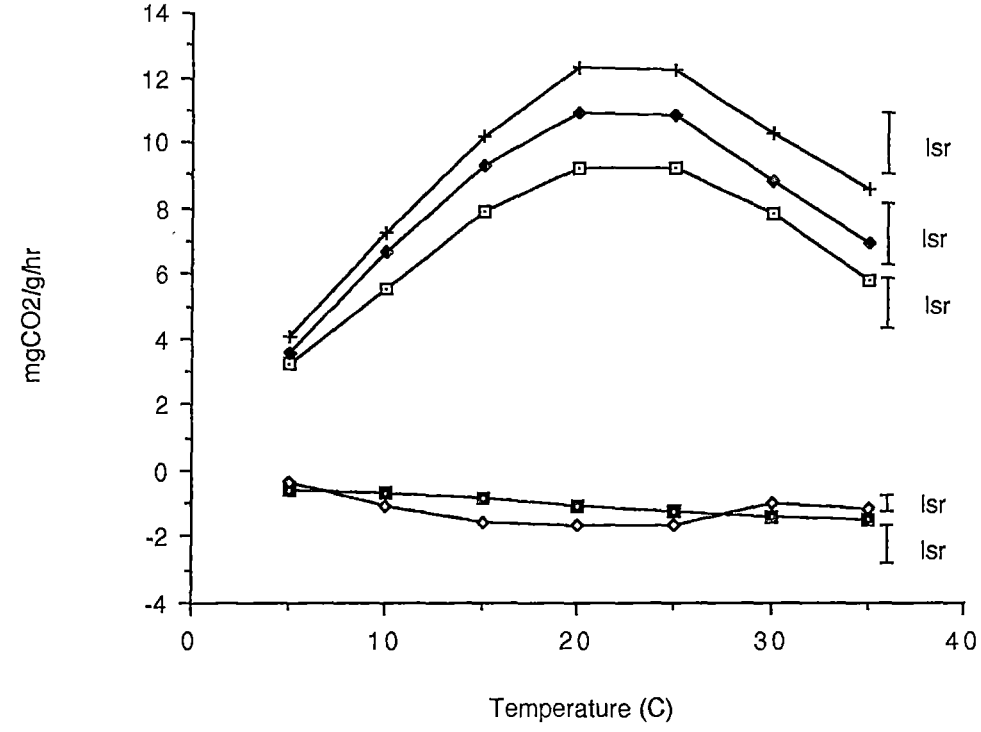
Figure IV.D.1.1.

Net CO_2 exchange characteristics of Fennel leaves

1. -□- 'Apparent' photosynthesis (21% O_2, 330 ppm CO_2, 1100 µmole $m^{-2}s^{-1}$).
2. -■- Dark respiration (21% O_2, 330 ppm CO_2, in the dark)
3. -◇- Enhancement of net CO_2 exchange (2% O_2, 330 ppm CO_2, 1100 µmole $m^{-2}s^{-1}$).
4. -◇- Photorespiration and effect of oxygen inhibition (1-3).
5. -+- 'True' photosynthesis (3-2).

Figure IV.D.1.2.

Net CO_2 exchange characteristics of Fennel umbel (legend as above).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12
10
8
6
4
2
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-2
-4
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10
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30
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Temperature (C)
lsr
lsr
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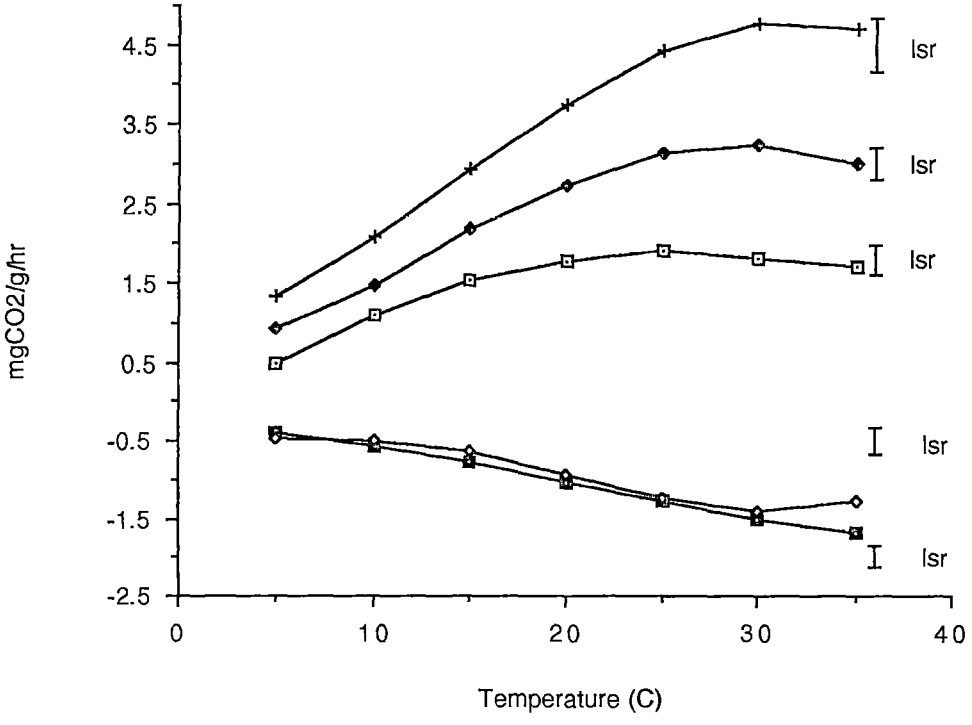
lsr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3.5
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0
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20
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Temperature (C)
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lsr

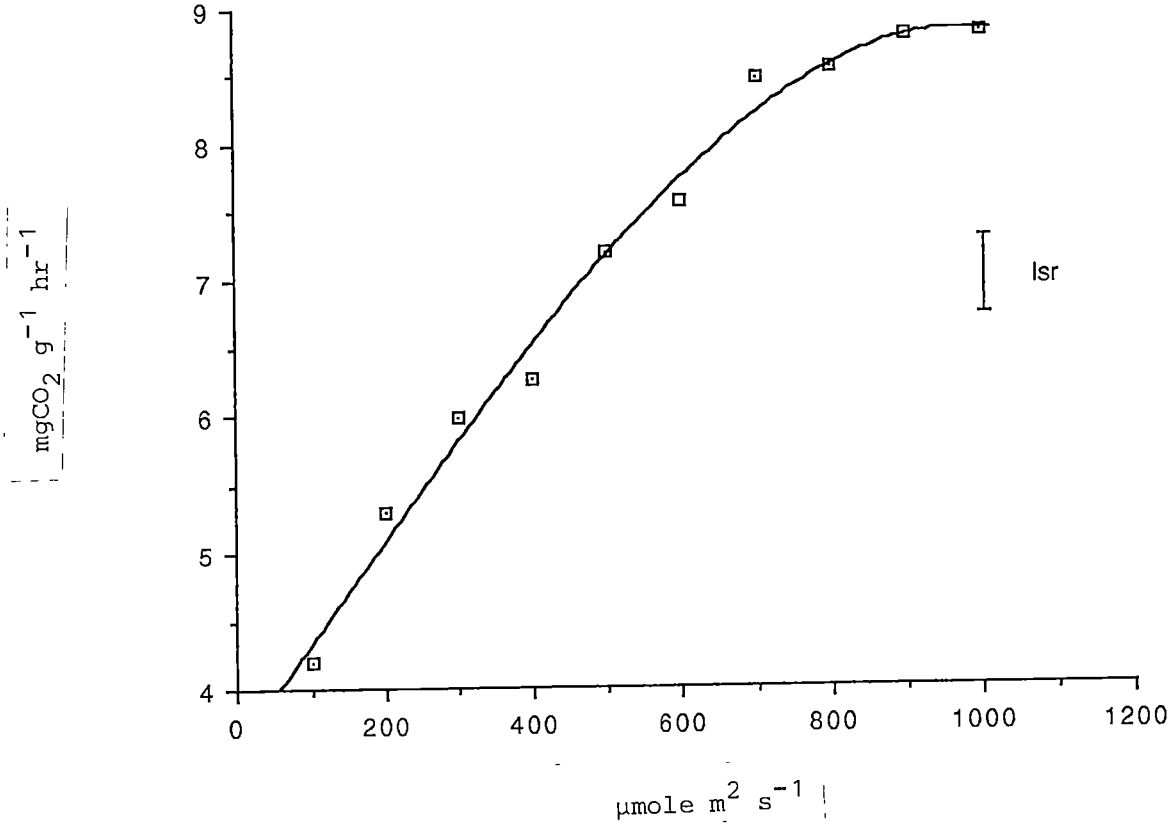


Figure IV.D.1.3.

Change in net CO_2 exchange with increasing photon flux density for fennel leaves at $20^{o}C$.

effect of oxygen inhibition of photosynthesis. The true photosynthesis reached a maximum at 20°C, at temperatures above 25°C a significant decrease was observed.

Examination of umbels under the same conditions revealed that apparent photosynthesis was occurring and reaching a maximum at 25°C, dark respiration again was recorded to have a Q_{10} value of approximately 2 (Figure IV.D.1.2).

The results indicated that once the rate of true photosynthesis reached a maximum at 20°C no significant decrease was observed up to a temperature of 35°C.

1.3.2 Light response and net CO_2 exchange

Increasing the PFD from 100 to 1000 μmole m^{-2} s^{-1} resulted in a significant increase in net CO_2 exchange (Figure IV.D.1.3). Light saturation occurred at 900 μmole m^{-2} s^{-1}.

1.4 Discussion

Net CO_2 exchange is normally expressed on a leaf area basis. However due to the nature of the fennel leaf it was considered difficult to accurately determine the leaf area of the sample placed in the leaf chamber. Similar problems were encountered with the umbel material examined as well.

In this study, the umbel was shown to assimilate CO_2. Both leaves and umbels, achieved maximum net CO_2 exchange levels at the same temperature, 25°C. To enable a comparison of rates of photosynthesis between the two different plant structures, unpublished data on the proportion of the total fresh weight of the plant which was leaf and umbel was used. At the stage of anthesis of the secondary umbels, an average plant of 250gm consisted of 50gm leaves and 62gm umbels. The maximum true photosynthesis recorded for the leaves was 12.3 mg CO_2 g^{-1} hr^{-1}, that is on a per plant basis 615 mg $CO_2 g^{-1}$ hr^{-1}. For the umbels, a maximum rate on CO_2 exchange was 4.75 mg CO_2 g^{-1} hr^{-1}. On a per plant basis this represents a total of 295 g CO_2 hr^{-1}.

The fact that early anthesis umbels were shown to contribute significantly towards net CO_2 indicates that, at a later stage of development, possibly after fertilization, the net CO_2 exchange may

increase. Further work on the source sink relationships between the leaves and the umbels as well as between the umbel orders would be necessary to expand this more fully. Techniques such as removal of source or sink structures and also studies utilizing radio-isotopes, particularly ^{14}C, are satisfactory methods.

The determination that the fennel umbel is photosynthetically active is not surprising as the umbel rays and later seeds all are green, indicating the presence of chlorophyll. The seeds are observed to 'brown' when mature indicating the cessation of the role of chlorophyll in the maturation. Such a change may not be important in the role of production of secondary metabolites which may continue by interconversions from stored photosynthates.

Light saturation for CO_2 fixation in fennel occured at a high level, 900 μmole m^{-2} s^{-1}, in contrast only 450 μmole m^{-2} s^{-1} was necessary for saturation in peppermint (Clark,1980), 400 μmole m^{-2} s^{-1} for saturation in blackcurrants (Kerslake, 1984). This result indicates that fennel may be adapted to high light intensity environments or alternatively to environments were light intensity is not limiting.

IV.D.2 Effect of leaf area reduction on the development of umbels and oil yield and composition of fennel in the field.

2.1 Introduction

In Section IV.C the application of the growth retardant EL500 compressed the habit of the plant to such an extent that only a few expanded leaves were present. The process of umbel initiation and development still proceeded. The resultant plant consisted of a normal floral canopy supported by a very small amount of vegetative material.

Investigations utilizing IRGA demonstrated that the umbels are able to photosynthesize and thus have the capability to provide some of their own assimilates necessary for development.

The aim of this study was to determine the contribution by the leaves towards the overall seed development and oil yield. To determine the importance of leaves, a number of different leaf removal treatments were employed. In some oil seed crops such as oilseed rape the leaf accompanying the developing inflorescence plays an important role in the overall production of assimilates for the developing pods. One method to determine the importance of such leaves is to remove them, and as a comparison to remove all but these leaves. Such treatments were used in this experiment on fennel.

2.2 Materials and Methods

The fennel crop used in this experiment was located in the same area as in Section IV.C.4. This was an area of a commercial crop of fennel (variety C26) that was sown with a precision vegetable drill and located at Plenty in the Derwent Valley.

To ensure 10 plants per m^2 the rows assigned as treatments and also border rows were thinned by hand. The experiment was designed as a randomized complete block consisting of 4 blocks and 5 treatments, as follows:

Treatment Name	Leaf removal treatment
F	100 percent of all leaves
G	50 percent of all leaves
H	all but leaf accompanying the umbel.
I	all leaves accompanying umbels
J	no leaves removed (control)

Treatments were applied to plots 3.2m long and 3 rows wide (1.2m) with a 1m border between each plot. All treatments commenced when the plants were observed to have initiated umbels. The average stage of growth at this time was 9 expanded leaves and 13 nodes present. Throughout the further growth and development of the crop the treatments were maintained until harvest to ensure that no regrowth of leaves confounded the effect of the treatments.

2.2.1 Determination of the effect of defoliation on height and number of rays per umbel

A non destructive measurment of the height to the primary umbel and the number of rays present in the primary, and secondary umbels was recorded on 11th March.

2.2.2 Determination of the effect of defoliation on oil yield and composition

A single harvest of 12 plants per plot for all treatments was taken on the April 4. 6 plants were used for whole plant oil yield determinations. The remaining plants were stripped of umbels to determine the treatment effect on each individual umbel order. The number, fresh weight and oil yield of umbels was recorded. Composition of all oil samples were analysed by the methods detailed in the General Materials and Methods.

2.2.3 Examination of the effect of defoliation on light inteception by Infra Red photography

Photographs of the defoliation treatments were taken using infra-red film on February 18 to examine the portions of the crop which were actively intercepting incident radiation. This time coincided with the period following seed set when umbels were maturing rapidly.

The film used was Kodak High Speed Infra Red film 2481, and the camera a Pentax Spotmatic with a 50mm f4 macro lens, fitted with a red 25A filter. The film was developed in Kodak D19 high contrast developer for 8 minutes and fixed in Kodak rapid fixer for 5 minutes. The photographs were then printed on Ilford Ilfospeed 5.1 M grade 5 paper. The plant appears white against a grey background.

2.3 Results

2.3.1 Effect on oil yield and composition from whole plants

The removal of all leaves and all leaves except the leaves accompanying the umbels significantly decreased the fresh weight of whole plant material present per unit area at time of harvest (Figure IV.D.1.a). These treatments significantly decreased the oil yield, total gms per plant, from approximately 1.6 gms to less than 1.0 gms per plant. Removal of 50 percent or all the leaves accompanying the umbels did not significantly affect the oil yield per plant at time of harvest.

The same effects were recorded in respect to the percent oil yield on either a fresh or dry weight basis. The decrease was only recorded for the treatments, 100 percent or all but the accompanying leaves removed (Figures IV.D.1.b).

The leaf removal treaments applied did not affect the levels of alpha-pinene, alpha-phellandrene, beta-phellandrene, cis-beta-ocimene and fenchone in the oil from whole plants (Table IV.D.2.1). Myrcene and limonene levels decreased in oil from 50 percent defoliated and all but the leaf accompanying treated plants. Estragole and anethole levels were higher in oil from plants receiving greater than 50 percent defoliation.

2.3.2 Effect on umbel number, oil yield and composition from umbels

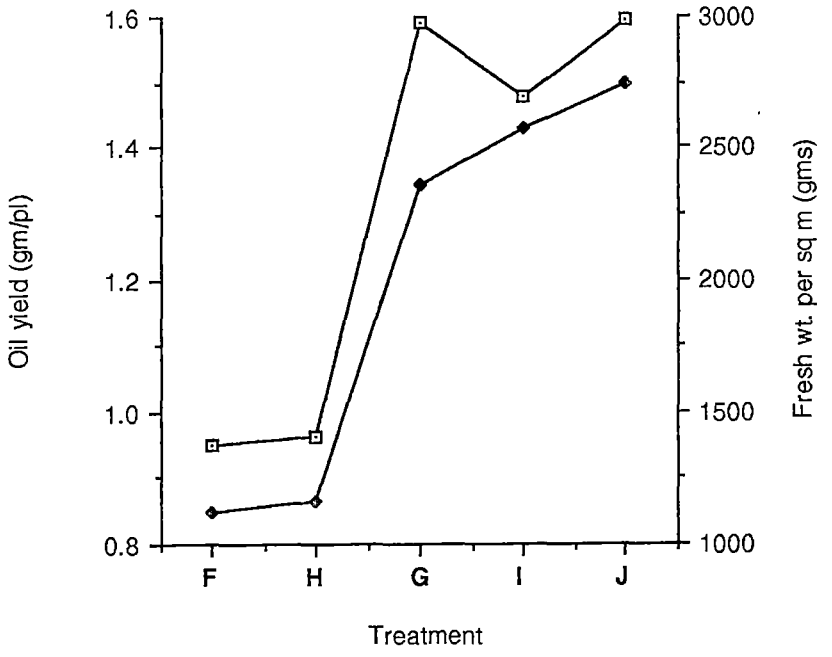
The number of primary and secondary umbels present on each plant was not affected by the defoliation treatments. Removal of all leaves or all leaves except the leaf accompanying the umbel (Figure IV.D.2.2.a) decreased the number of tertiary umbels present at the time of commercial harvest compared to the control plants. These two treatments also significantly decreased the fresh weight of each

Figure IV.D.2.1.a.

Oil yield and fresh weight of whole plants as affected by the various defoliation treatments. LSD's, oil yield (g/plant) ⊡ 0.49, total fresh weight ⊶ 326.4.

Figure IV.D.2.1.b.

Effect of the defoliation treatments on the % oil yield from whole plants, expressed on both a dry and fresh weight basis. LSD's, percent oil yield on a fresh weight basis ⊡ 0.202 and on a dry weight basis ⊶ 0.779.


1.6
1.4
1.2
1.0
0.8
Oil yield (gm/pl)
3000
2500
2000
1500
1000
Fresh wt. per sq m (gms)
F
H
G
I
J
Treatment

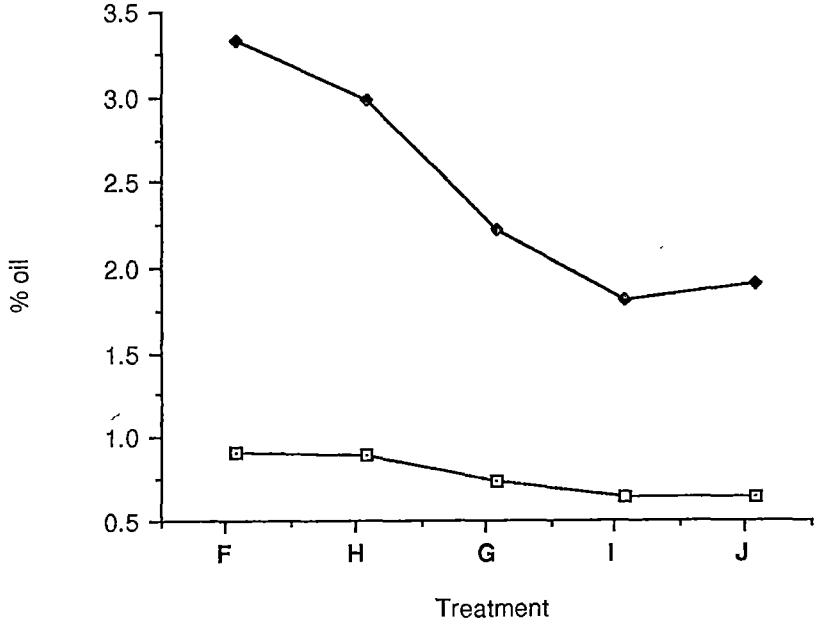

3.5
3.0
2.5
2.0
1.5
1.0
0.5
% oil
F
H
G
I
J
Treatment

Table IV.D.2.1.

Effect of defoliation on the composition of oil from whole plants, mean values for analysis (% total FID response).

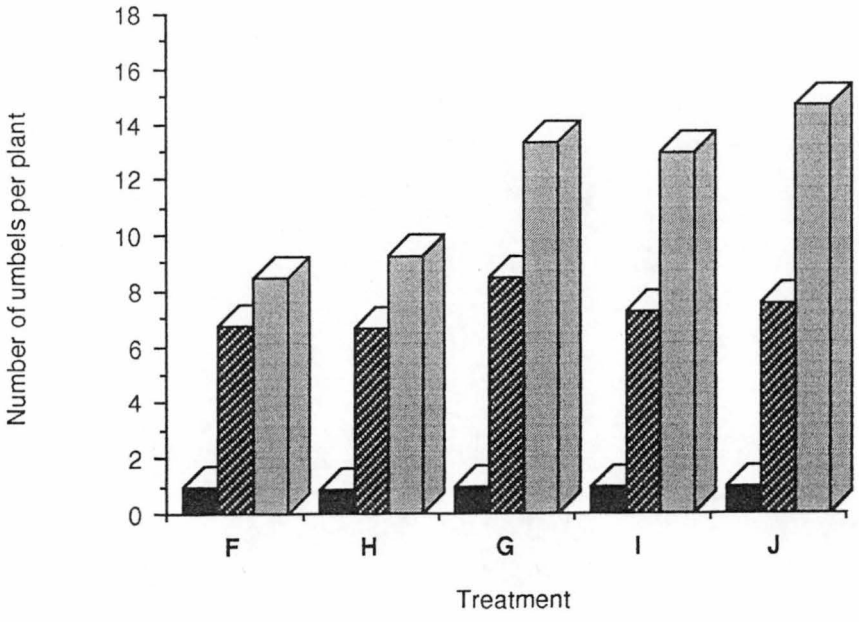
Compound	Leaf removal treatment (acc.= leaf accompanying umbel) Control	Leaf acc.	50%	All but acc.	100%	LSD
Alpha-Pinene	13.01	9.96	10.22	9.80	9.12	ns
Myrcene	1.41	1.29	1.24	1.28	1.44	0.12
Alpha-Phellendrene	6.00	7.06	6.41	5.27	6.18	ns
Limonene	1.82	1.84	1.54	1.71	1.89	0.20
Beta-Phellandrene + Cineole	0.89	1.05	0.86	0.77	0.93	ns
Cis-Beta-Ocimene	0.48	0.51	0.48	0.47	0.47	ns
Fenchone	19.18	18.31	16.70	19.08	18.07	ns
Estragole	2.12	2.20	2.41	2.37	2.31	0.13
Anethole	51.77	54.18	57.41	56.29	56.51	2.90

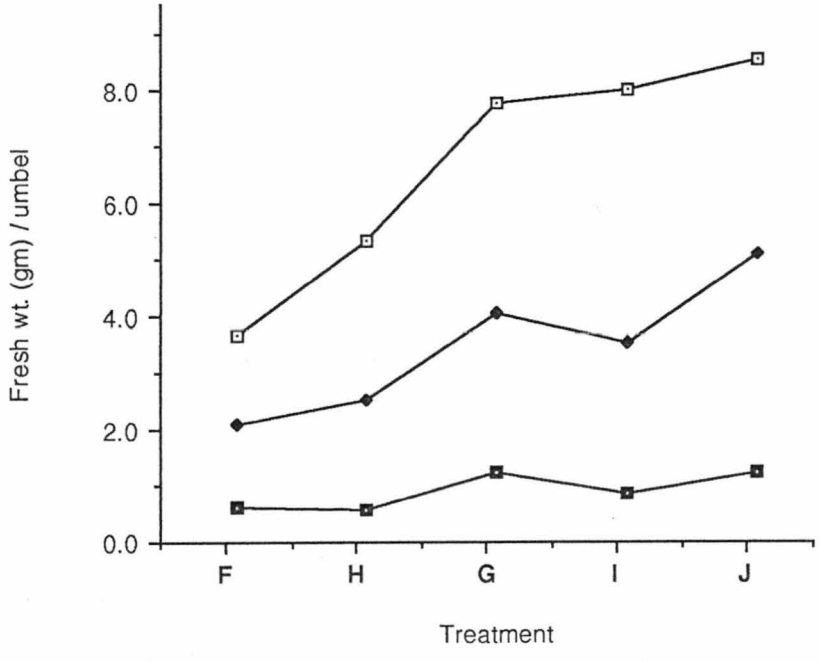
Figure IV.D.2.2.a.

Result of leaf removal treatments on the numbers of primary, secondary and tertiary umbels present at harvest. LSD's, primary umbel ■ ns, secondary umbels ▨ ns and tertiary umbels ▩ 3.22.

Figure IV.D.2.2.b.

The effect of the various defoliation treatments on the fresh weight of the umbels of the primary, secondary and tertiary umbels at harvest. LSD's, primary umbel ⊡ 1.04, secondary umbels ⟡ 0.7 and tertiary umbels ▬ 0.29


Number of umbels per plant
18
16
14
12
10
8
6
4
2
0
F
H
G
I
J
Treatment


Fresh wt. (gm) / umbel
8.0
6.0
4.0
2.0
0.0
F
H
G
I
J
Treatment

primary umbel (Figure IV.D.2.2.b). All leaf removal treatments decreased the fresh weight of the secondary umbels. The secondary umbels of 100 percent defoliated plants weighed 60 percent less than those on untreated plants. The removal of only the leaf accompanying the umbel produced secondary umbels which weighed 47 percent less than the secondaries from untreated plants. The fresh weight of the tertiary umbels was decreased by all leaf removal treatments, 100 percent leaf removal and all but the leaf accompanying the umbel treatments decreased the fresh weight of the tertiary umbels by 60 percent compared to the tertiary umbels of intact plants. Again the removal of the leaf accompanying the umbel significantly decreased the fresh weight of these umbels.

The effect of defoliation on the amount of oil produced by each umbel order was comparable to the changes observed in the fresh weights of the umbels (Figure IV.D.2.2.c). A decrease in oil production by the primary, secondary and tertiary umbels was observed for plants totally defoliated or only left with the leaf accompanying the umbel. However, no significant differences in oil yield were observed for all orders with 50 percent or more leaves remaining.

Cis-beta-ocimene concentration increased in the oil from primary umbels when the leaf accompanying the umbel was removed. No changes in the oil composition were recorded as a result of any other defoliation treatments (Table IV.D.2.2.a). Cis-beta-ocimene levels also increased in the oil from secondary umbels when the accompanying leaves were removed. A decrease in the level of estragole also resulted. No other changes occurred in the oil from the secondary umbels (Table IV.D.2.2.b). This increase in cis-beta-ocimene and decrease in estragole was observed in the oil from the tertiary umbels without accompanying leaves but was not statistically significant. The only significant change observed in the composition of the oil from the tertiary umbels was an increase in alpha-pinene content resulted again from the absence of any accompanying leaves (Table IV.D.2.2.c).

2.3.3 Effect on height

Elongation of the main stem significantly decreased in plants with less than 50 percent of their leaves present. This is shown by the heights to the primary umbel (Table IV.D.2.2.d). This effect was visible within the plots in the field.

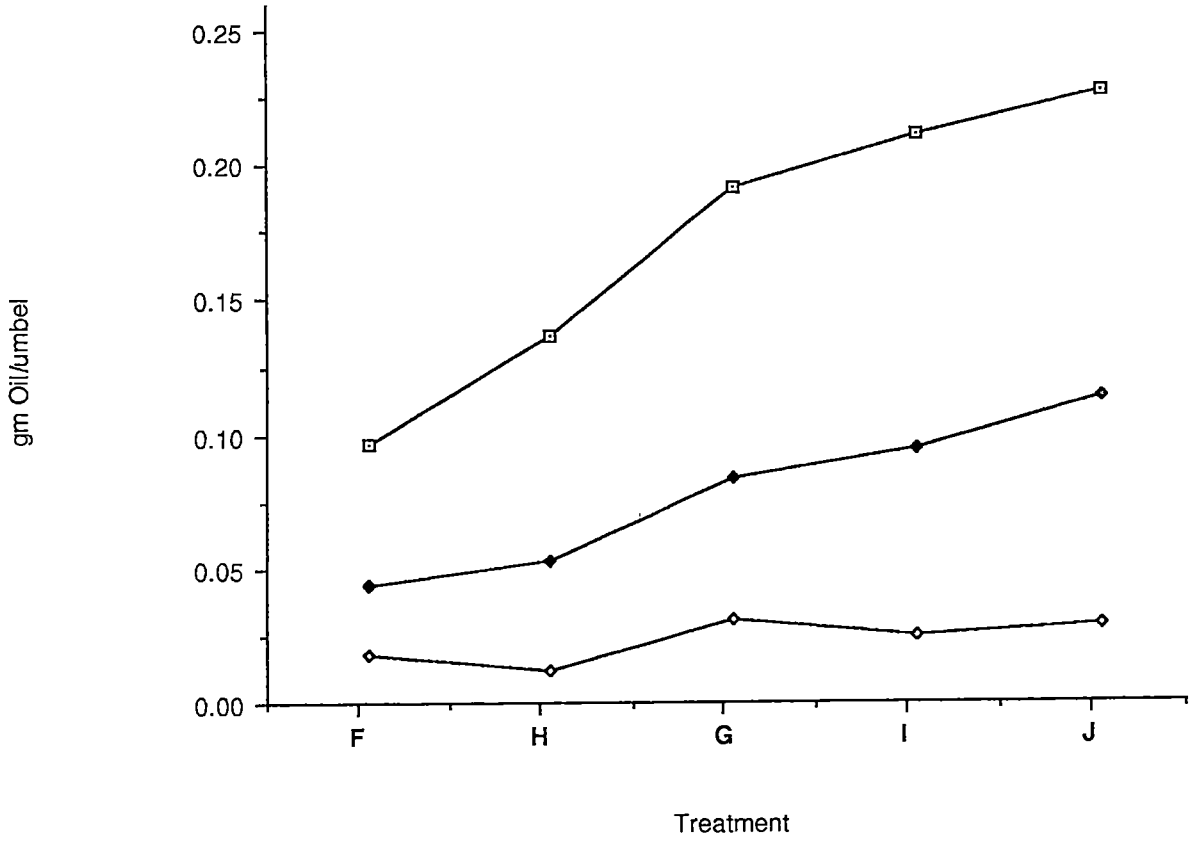


Figure IV.D.2.2.c.

Defoliation of fennel and the effect on the oil yield of the primary, secondary and tertiary umbels. LSD's, primary umbel ⊡ 0.43, secondary umbels ◆ 0.031 and tertiary umbels ◇ 0.01.

Table IV.D.2.2.

a) Effect of defoliation on the composition of oil from primary umbels, mean values for analysis (% total FID response).

Compound	Leaf removal treatment (acc.= leaf accompanying umbel) Control	Leaf acc.	50%	All but acc.	100%	LSD
Alpha-Pinene	3.63	4.82	3.31	4.43	3.56	ns
Myrcene	0.83	0.98	0.85	1.08	0.91	ns
Alpha-Phellendrene	1.18	1.00	1.18	1.18	0.94	ns
Limonene	1.70	1.81	1.74	2.01	1.73	ns
Beta-Phellandrene + Cineole	0.62	1.12	0.58	0.68	0.55	ns
Cis-Beta-Ocimene	0.30	0.50	0.35	0.34	0.29	0.12
Fenchone	25.86	19.23	23.49	24.06	22.79	ns
Estragole	2.68	2.64	2.75	2.62	2.69	ns
Anethole	61.61	66.94	64.26	62.19	65.17	ns

b) Effect of defoliation on the composition of oil from secondary umbels, mean values for analysis (% total FID response).

Compound	Leaf removal treatment (acc.= leaf accompanying umbel) Control	Leaf acc.	50%	All but acc.	100%	LSD
Alpha-Pinene	4.97	7.05	4.73	5.26	4.41	ns
Myrcene	0.98	1.04	0.97	1.17	1.16	ns
Alpha-Phellendrene	1.70	1.64	1.98	1.72	1.54	ns
Limonene	2.05	2.10	1.85	2.09	2.15	ns
Beta-Phellandrene + Cineole	1.03	1.18	0.84	1.05	0.72	ns
Cis-Beta-Ocimene	0.41	0.78	0.38	0.45	0.49	0.21
Fenchone	22.29	18.54	21.83	19.76	22.37	ns
Estragole	2.67	2.37	2.65	2.66	2.62	0.14
Anethole	62.04	61.22	62.04	64.03	61.74	ns

Table IV.D.2.2. con't.

c) Effect of defoliation on the composition of oil from tertiary umbels, mean values for analysis (% total FID response).

Compound	Leaf removal treatment (acc.= leaf accompanying umbel) Control	Leaf acc.	50%	All but acc.	100%	LSD
Alpha-Pinene	5.94	8.46	7.76	6.19	4.56	2.25
Myrcene	1.14	1.52	1.30	1.63	1.15	ns
Alpha-Phellendrene	2.69	2.92	3.15	3.35	2.15	ns
Limonene	2.12	2.39	2.18	2.65	2.11	ns
Beta-Phellandrene + Cineole	0.88	0.94	0.90	1.01	0.92	ns
Cis-Beta-Ocimene	0.52	0.89	0.56	0.86	0.66	ns
Fenchone	19.43	16.26	16.08	17.47	16.96	ns
Estragole	2.59	2.30	2.51	2.42	2.47	ns
Anethole	62.63	61.70	62.60	61.67	66.63	ns

No effect of defoliation on the number of rays per umbel was observed in respect to the primary umbels but a small decrease in the mean number of rays per umbel of the secondary umbels was observed when all leaves were removed (Table IV.D.2.2.d).

Table IV.D.2.2.

d) Effect of defoliation on the height to the primary umbel and the number of rays per umbel.

	Leaf removal treatment (acc.= leaf accompanying umbel)					
	Control	Leaf acc.	50%	All but acc.	100%	LSD
Height of primary (cm)	114.2	109.4	106.3	89.4	80.9	12.4
Numbers of rays						
Primary	18.2	16.9	18.2	17.0	17.9	ns
Highest secondary	19.6	19.2	17.9	18.3	17.3	1.9
Lowest secondary	19.5	20.2	17.3	18.9	17.3	2.1

2.3.4 Infra Red photography

At the time of umbel initiation in the field an infra-red photograph of the canopy indicated the large amount of vegetative material at this point in the plants development. Plate IV.D.2.1 represents the area covered by leaf material from 5 plants.

Photographs of the various treatments within the crop taken during seed maturation are presented in Plates IV.D.2.2 to IV.D.2.5, in each plate 4 plants are present or 1m of row. In all plates the major structures intercepting incident radiation in the plant canopy were the umbels followed by the stem material present. Very little variation in the interception of the incident radiation can be seen between any treatments.

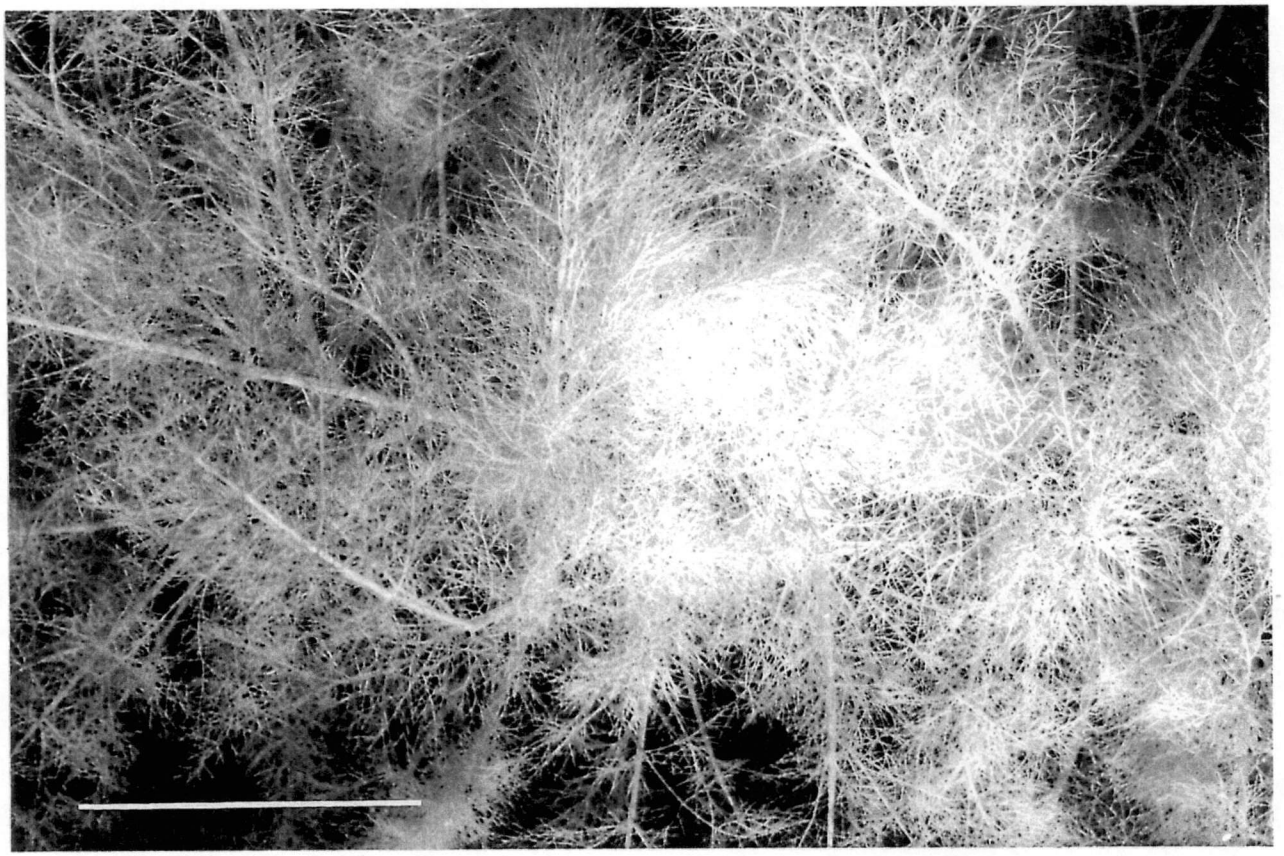


Plate IV.D.2.1.

Infra-red photograph of a typical fennel canopy at the time of floral initiation. (Bar 20cm)

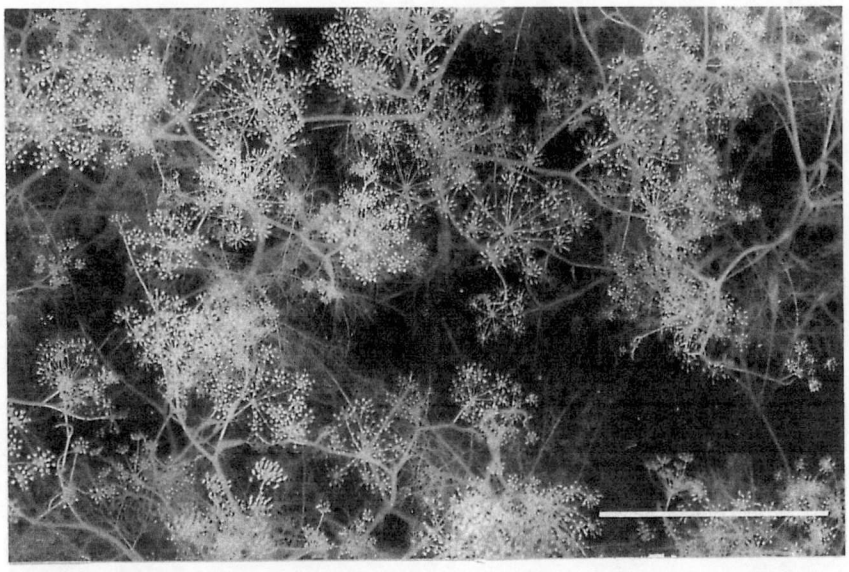


Plate IV.D.2.2.

Infra-red photograph of a control plot taken on February 17. The primary and secondary umbels had set seed and were at the stage of mid seed fill. (Bar 20cm)

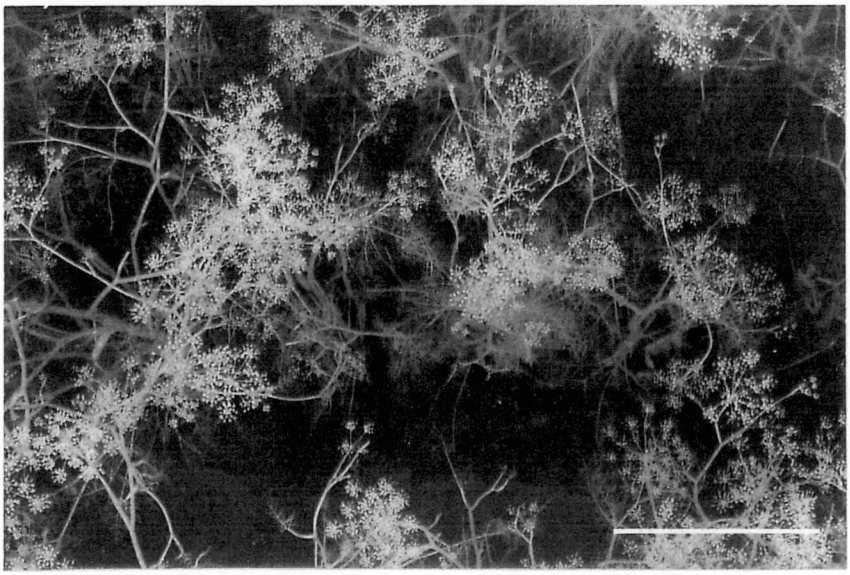


Plate IV.D.2.3.

Infra-red photograph of the 50% defoliated plants. (Bar 20cm)

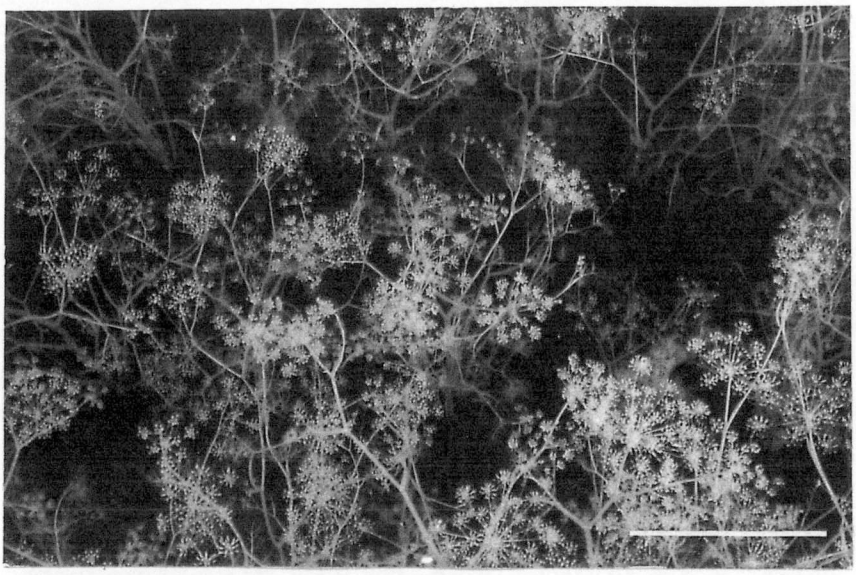


Plate IV.D.2.4.

Infra-red photograph of the canopy of fennel plants, stripped of all leaves except those accompanying the umbels. (Bar 20cm)

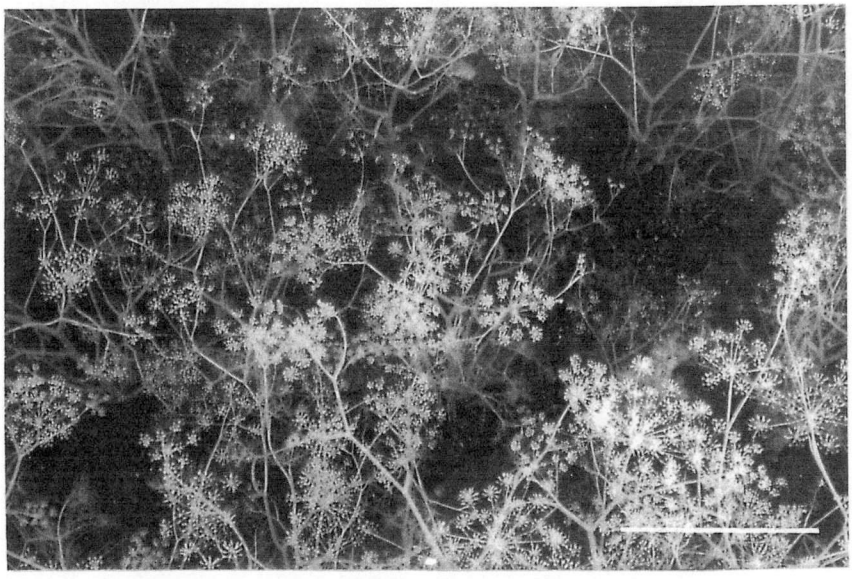


Plate IV.D.2.5.

Infra-red photograph of the canopy remaining after fennel plants were totally defoliated. (Bar 20cm)

2.4 Discussion

The yield of oil from whole plants was not significantly affected by removal of the leaves accompanying the umbels nor by the removal of 50 percent of all leaves from the plant during umbel development. The umbels on partially defoliated plants are able to continue a normal course of development, yielding similar to untreated plants. The removal of all leaves decreased yields substantially, approximately 40 percent. The contribution of the leaf accompanying the umbel towards yield did not significantly increase in comparison to the totally defoliated plants. Also the treatment of removal of the accompanying leaves only, was not significantly different from untreated plants. The role of the accompanying leaf to an umbel appears more important to the composition of the oil from the umbel. These changes were not large when compared to the results from the control plants. But the oil from entire plant samples increased in anethole content when defoliation exceeded 50 percent.

The removal of leaves decreased the number of tertiary umbels present at the time of commercial harvest, but only when severely defoliated. This decrease may have resulted from increased competition within the floral canopy for assimilates. A further explanation is that removal of a large number of leaves exposes the more delicate tertiary umbels which are then more easily damaged by wind and subsequently abort. Certainly the nature of the treatments applied was destructive and some of the decreases in yield may have been a result of mechanical damage or fungal infection. Establishment of Cercosporidium was more rapid within the plots of defoliated plants than the general crop.

Although all treatments were applied post initiation, effects to ray numbers per umbel was detected for the secondary umbels present on severely defoliated plants. Again this may have resulted from increased competition during the early stages of development of these umbels due to lower levels of assimilates present within these defoliated plants.

Although the numbers of secondary umbels was not affected the fresh weight per umbel decreased with increasing defoliation indicating that the leaves play some role, possibly more in the early development of the umbel, prior to anthesis. At later stages in the development of seed on the umbel the role of leaves appears to be

reduced as can be seen from the infra-red photographs of the crop where the leaves are virtually not apparent.

The removal of all leaves after initiation decreased the elongation of the main stem. These treatments either limiting the source of gibberellins within the plant or assimilates thereby decreasing extension growth.

IV.D.3 Autoradiography

3.1 Introduction

The aim of this experiment was to study the distribution pattern of photosynthate throughout the canopy during seed maturation to determine the major sinks and sources of assimilates within the plant. Examination of the assimilate balance, particularly between the umbel orders, will aid in a more complete understanding of the origin of the oil yield from the umbel canopy. The technique of autoradiography was used for this study. Therefore results are purely observational and no quantitative measurements were made.

3.2 Materials and Methods

2.1 Source of Isotope and Generation of $^{14}CO_2$

Sodium bicarbonate ($NaH^{14}CO_3$) was purchased from Amersham International Pty. Ltd. in vials containing 1ml of $NaH^{14}CO_3$, specific activity 58mCi/mmole, radioactive concentration 2mCi/ml. This was diluted with 3.0ml distilled water and adjusted to pH 9.4 with 0.01N NaOH to make a diluted solution of 0.5mCi/ml.

$^{14}CO_2$ was generated with the aid of a Shimshi apparatus (Figure IV.D.3.1). In this the $NaH^{14}CO_3$ was placed in a boiling tube and sealed in the generating cylinder (1), approximately 15ml of 1N H_2SO_4 was added to the funnel (2) and released into the line (5) and sealed. Air then pushed the acid into the generating cylinder (1) until the pressure reached 5 atmospheres and remained at this pressure for 5 mins. The storage cylinder was sealed and connected to the outflow pressure guage and releasing cylinder (10). The generated $^{14}CO_2$ was slowly released from the storage cylinder through output tap (11).

Preliminary trials indicated that a minimum of 5μCi per plant was necessary to achieve detection by autoradiography.

2.2 Method of application of $^{14}CO_2$

The attached plant material to be fed was placed in a cylindrical glass tube 0.3m long and 3cm internal diameter. The tube was clamped in such a way as not to change the normal orientation of the plant

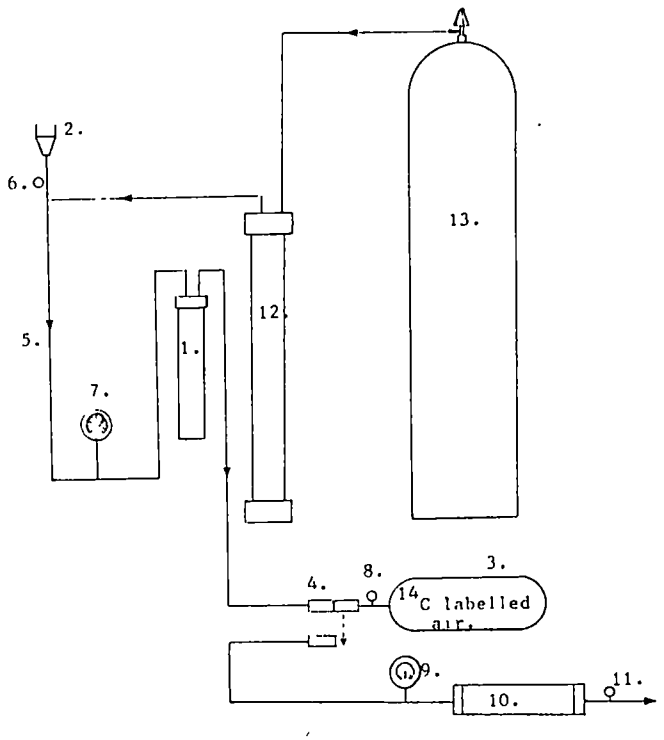


Figure IV.D.3.1.

Diagramatic representation of the Shimshi apparatus.

1. ^{14}C generating cylinder.
2. Separator funnel containing 1N H_2SO_4.
3. ^{14}C storage cylinders.
4. Snap connectors.
5. Air and acid connecting line.
6. Acid line stop tap.
7. Generating pressure guage.
8. Storage cylinder stop tap.
9. Out-flow pressure guage.
10. Releasing cylinder.
11. Control release tap.
12. Acid trap (NaOH pellets)
13. 'G' size air cylinder.

structure. Continual attachment to the plant was afforded by using a split rubber bung placed around the base of the structure and sealed with plasticine then forced into the column and covered with vaseline to ensure a gas tight fit. Four plants were fed at each time. A light intensity of 900μmole m-2 s-1 was available in the glasshouse described in the General Materials and Methods. Plants were allowed to equilibrate at 20°C prior to feeding by passing air from the glasshouse through the glass tube.

20μCi of $^{14}CO_2$ was generated, and flow regulated through the tubes to remain at 0.3 l/min. Once the source of $^{14}CO_2$ exhausted from the storage cylinder of the Shimshi apparatus, flow of unlabelled air resumed. The storage cylinder was flushed with CO_2 and subsequently passed through the tubes to flush any remaining $^{14}CO_2$.

2.3 Treatments

Three experiments were conducted to determine the sources of photosynthates for developing umbels in fennel. In each experiment four plants were fed $^{14}CO_2$ similtaneously. All four plants were carefully selected to ensure that they were all at the same stage of development (primary at mid seed set). One plant was then dissected, as described in the next section, at each of the following times:

4 hours after after feeding $^{14}CO_2$
24 hours after after feeding $^{14}CO_2$
4 days after after feeding $^{14}CO_2$
8 days after after feeding $^{14}CO_2$

2.3.1 Assimilation of $^{14}CO_2$ by the leaves

The first experiment conducted examined the distribution of $^{14}CO_2$ after the leaves, within the vegetative portion of the stem, were fed. The highest vegetative node was fed and the distribution in both the vegetative and floral portions of the canopy examined.

2.3.2 Assimilation of $^{14}CO_2$ by the primary umbel

The second experiment involved feeding the primary umbel and examining the distribution of photosynthates to other umbels and leaves.

2.3.3 Assimilation of $^{14}CO_2$ by the secondary umbel

The third experiment was the same except that this time the most apical secondary umbel was fed $^{14}CO_2$.

In all experiments the following structures were harvested for autoradiography:

Leaf from the highest vegetative node

Next lowest leaf

Primary umbel

Most apical secondary and accompanying tertiary umbels

2.4 Dissection and drying

Plant material selected for autoradiography were excised from the main stem, pressed and dried at 60°C. Leaf material was further dissected by removal of every alternate leaflet from the main stem, in order not to overlay any leaflets when pressed. Prior to pressing umbel material, umbels were dissected leaving a fan of rays in two dimensions. Thick stems at the base of some umbels where also in half longitudinally to allow easy pressing and rapid drying.

Dissection and pressing was necessary to obtain even distribution of the plant material on X-ray film.

2.5 Autoradiography

The plant material was placed on Kodak X-ray film (type X-omat AR) whilst working under a safelight filter (type GBX-2). The film was then laid on a piece of blotting paper and then all placed on a sheet of glass wrapped in aluminium foil. Several pieces of blotting paper were placed on top of the plant material followed by another sheet of foil wrapped glass. Further plant material specimens on X-ray film were placed on top of each other in this manner then the entire stack wrapped in cellotape to prevent slippage of the specimens on the film, and placed in two light proof bags. The entire package was then placed between two pieces of board and clamped in position then left for 8 days at 25°C for exposure.

The film was developed with Kodak liquid GBX developer for 5 mins. at 20°C, rinsed for 30secs. in water then fixed in Kodak X-ray GBX fixer and replenisher. After a final water rinse of 5 mins. in running water the films were air dried at 20°C.

3.3 Results

All experimental results were observational, to interpret these results a scale of darkening of the X-ray film was used, this is shown in Figure IV.D.3.

3.3.1 Leaf $^{14}CO_2$ uptake

$^{14}CO_2$ when fed to the fully expanded leaf on the last vegetative node was readily observed in all portions of the leaf placed in the tube, shade 5. Translocation of photosynthates from the leaf was slow, after 24 hours minimal shading in all other harvested plant material occurred. Shade 3 or 4 was not observed at any time interval in the umbels after feeding. After 8 days a light colouration was recorded in the primary umbel, shade 1, and very light shading, approximately 0.5, in the most apical secondary umbel. 4 days after feeding the leaf at the next lower node exhibited some activity, shade 1, along the midrib but not in the leaflets.

3.3.2 Primary umbel $^{14}CO_2$ uptake

$^{14}CO_2$ was readily utilized by the primary umbel, shade 5 resulting 4 hours after exposure (Plate D.3.1). Developing seed coats, as well as the rays of the umbel all assimilated the labelled CO_2. The autoradiogram indicated a definative cut off point for the $^{14}CO_2$ assimilation. The presence of $^{14}CO_2$ demonstrates the point at which the umbel was placed into the tube and sealed. 24 hours after feeding this cut off point was not easily recognizable (Plate D.3.2). This indicated that some translocation of photosynthates from the umbel occurred, but over a short range.

After 4 days (Plate D.3.3) the activity of ^{14}C had dropped to a shade level of 3, translocation to the stems and umbels on the stems of the most apical secondary and its accompanying tertiary umbels was evident. Activity in the stems was low, shade 1 and less, but the developing seed of both the secondary and tertiary umbels indicated higher activity, shade 2. No further increase in the translocation to other umbels was recorded after 8 days.

At no stage was any activity detected in the vegetative portion of the canopy.

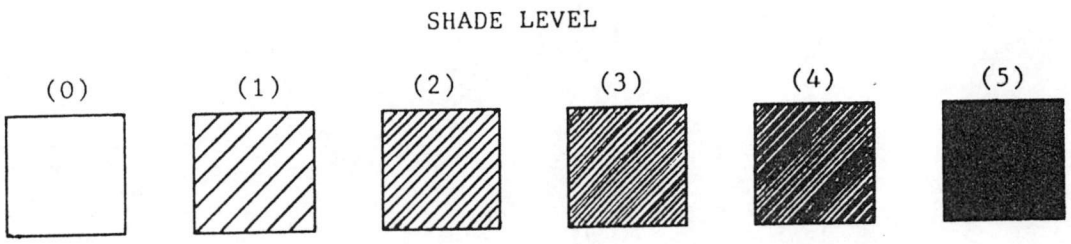


Figure IV.D.3.
Shade intensities used for comparison of 14C distribution within the plant as revealed by autoradiography as developed by Roberts (1989).

Shade (0) indicates no 14C activity, i.e. no change to the x-ray film after development.

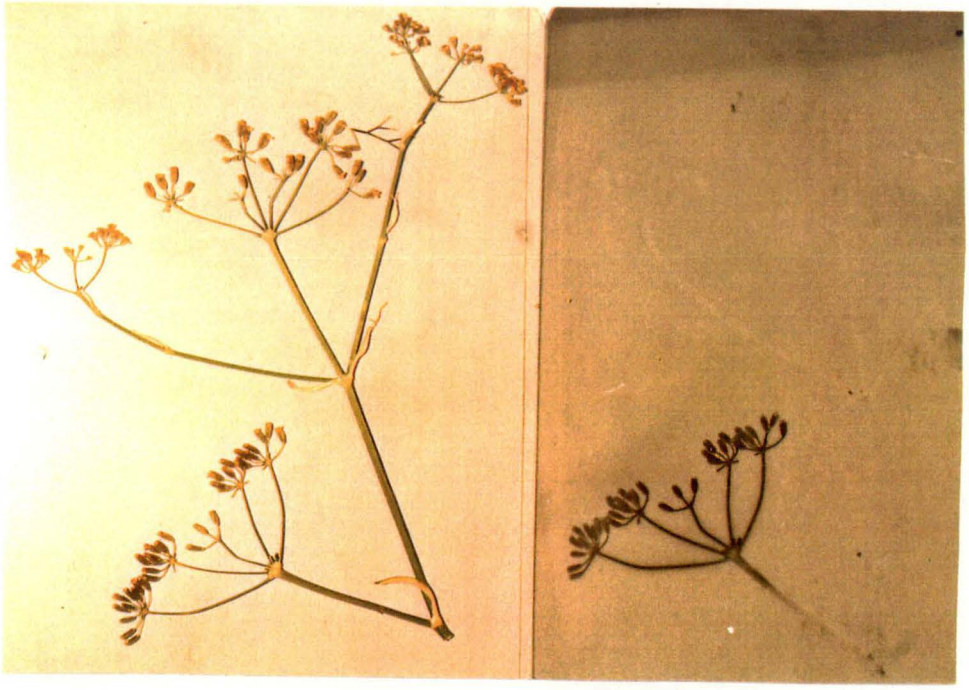
Shade (5) indicates high levels of isotope, i.e. the x-ray film was black upon developing.

Plate IV.D.3.1.

Uptake of $^{14}CO_2$ by the primary umbel 4 hours after feeding. The distinct pattern of the primary umbel is clearly shown by the radiograph.

Plate IV.D.3.2.

24 hours assimilation after feeding of $^{14}CO_2$ to the primary umbel. Movement of $^{14}CO_2$ from the primary umbel was minimal.



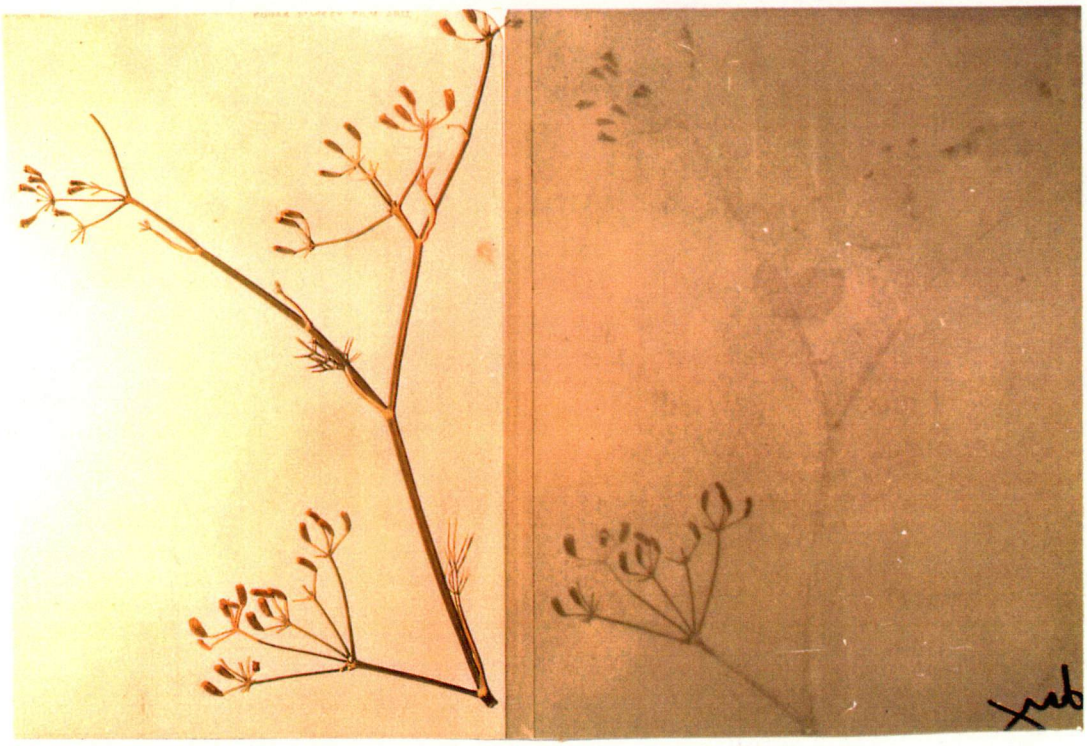


Plate IV.D.3.3.

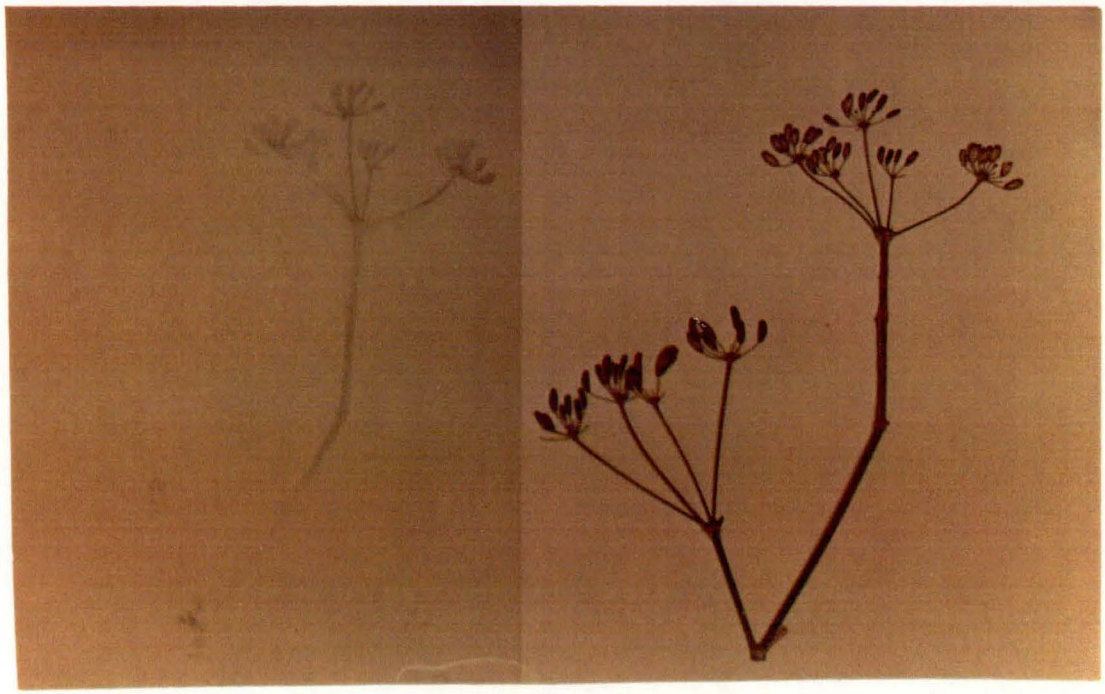
Translocation of $^{14}CO_2$ 4 days after feeding the primary umbel.

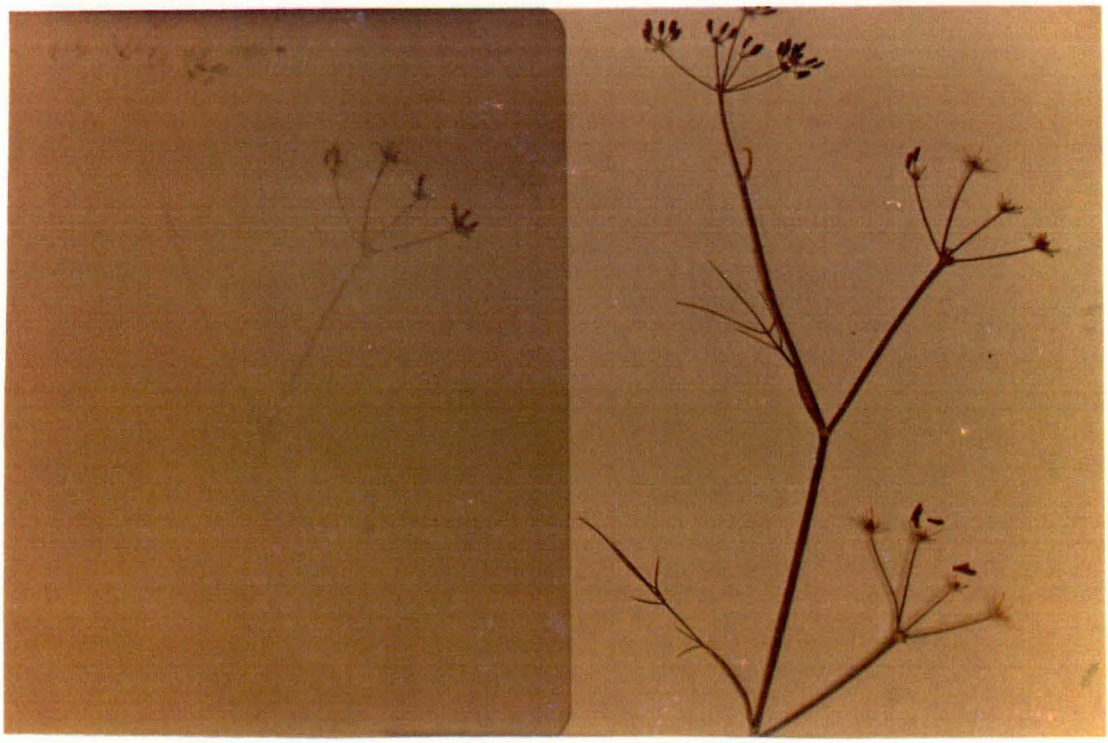
Plate IV.D.3.4.

Uptake and evidence of some translocation 4 hours after $^{14}CO_2$ fed to a secondary umbel.

Plate IV.D.3.5.

Translocation of $^{14}CO_2$ 24 hours after feeding the secondary umbel.





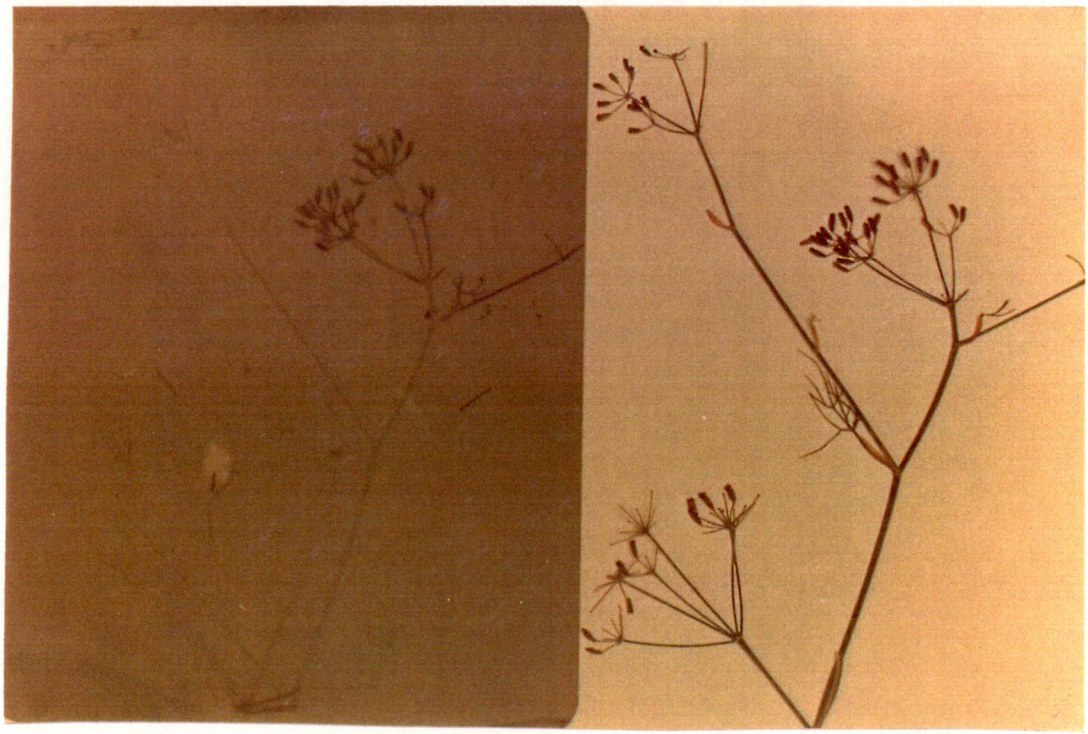


Plate IV.D.3.6.

Distribution of $^{14}CO_2$ to other umbels, 4 days assimilation after feeding $^{14}CO_2$ to the secondary umbel.

3.3.3 Secondary umbel $^{14}CO_2$ uptake

Again the umbels fed readily assimilated $^{14}CO_2$, but the rate of translocation from the secondary umbels was faster than that from the primary umbel. 24 hours after feeding the assimilated ^{14}C had translocated 3 times the distance down the stem towards the primary (Plate D.3.4). After 4 days assimilation activity was observed in the seed of the tertiary umbels, shade 2 (Plate D.3.5). Only slight activity was observed in the primary, less than shade 1.

8 days after feeding, activity in the tertiary and primary umbels was observed (Plate D.3.6). Also at this stage activity was detected in the next lowest secondary umbel and the tertiary umbel accompanying, shade 2 in the umbels and 1 in the stems and rays.

Again at no stage was any activity detected in the leaves below the floral canopy.

3.4 Discussion

The technique of autoradiography allows for rapid determination of the assimilation sites of CO_2 as well as allowing the observation of translocation of photosynthates within the plant. Results from these experiments again indicates that the umbels are able to actively photosynthesize, thereby producing photosynthates for their own utilization. The contribution by the fully expanded leaves on the lower vegetative portion of the plant towards the developing seed was very small, possibly insignificant. These findings support the results of the defoliation experiment, indicating a minor role that the leaves play once umbels have emerged.

Photosynthates are produced by all the various umbel orders within the floral canopy, but the translocation of these assimilates among the various umbel orders may affect the overall yield of each umbel. The rate of translocation from the primary umbel was less than the secondary. The primary more apically dominant. Some translocation was still observed from the primary umbel indicating that the secondaries and the tertiaries are by no means inactive as competitive sinks.

V

GENERAL DISCUSSION

V. GENERAL DISCUSSION

Factors which influence the yield and composition of essential oil from fennel (Foeniculum vulgare Mill.) are discussed. An understanding of the physiology of the plant was necessary to provide a basis for the manipulation of the oil yield components. The full complement of environmental conditions provided in the field cannot be duplicated within the confines of the glasshouse. Only by experimentation in the field will conclusive improvements be made to oil yield and composition of fennel as a crop.

Change in Oil Yield and Composition

The pattern of oil accumulation of a fennel crop was shown to vary between first and second years of production. Oil accumulation by the first year crop began later in the season than the second year crop, but the rate at which the first year crop accumulated oil was greater. The time lag in oil yield profiles reflects the establishment phase of the first year crop. The onset of rapid stem elongation also occurs later for the first year crop suggesting that a particular stage of development must be achieved before floral development and oil accumulation will begin. Plants in their second year of production begin the growing season with a well developed root structure. Such reserves allow for vegetative growth early in the season and a more rapid growth response to Spring conditions.

The second year crop attained a total oil yield, 33 kg per ha less than the first year crop. A possible contributing factor to the low oil production is the change in growth habit of the plant in the second year of production. Once pruned after the first seasons growth, each single plant was observed to initiate some five to six new growing points resulting in a five to sixfold increase in the stem density. In this instance, competition for light, water and nutrients may have occurred producing a decrease in seed set and development.

At harvest, the first and second year crops varied in the contribution made by the different plant structures towards the total oil yield. Although a higher total oil yield was attained by the first year crop, the contribution by the umbels was lower than the corresponding oil yield from umbels of the second year crop. This difference reflects the higher contribution made by the leaves to the total oil yield of the first year crop. Because of differences in the rate of development between the first and second year plants, the foliage remains on the first year crop throughout April whilst senescence of the leaves of the second year crop occurs before this period.

Percentage oil yield from umbels on a fresh weight basis was 2.5 percent for both the first and second year crops. This was in agreement with the oil yield from an Indian variety of bitter fennel determined by Ashraff and Bhatty (1975) but lower than similar determinations performed by Karlsen et al.(1969) in Bulgeria.

Two other factors affecting the change in oil production include the environmental conditions which induce floral evocation, and the change in density as affected by the increase in shoot number in the second year of a crop.

No studies of such changes in oil yield from year to year for this perennial crop were observed in the literature. In addition the number of seasons for which fennel may remain as a viable oil producing crop has not been documented. Experimentation with commercial crops in Tasmania has indicated that four commercially viable harvests may be obtained from a single planting (Clark pers. comms.). Sustainable oil yields may only be maintained through appropriate crop management practices which are based on a knowledge of the oil producing structures within the fennel canopy, the change in oil composition within these structures with time and the physiology of the fennel plant as a crop system.

The results of the study on oil distribution throughout the plant have been used to improve harvest efficiency and distillation costs. Determination of higher percentage oil yield from the umbel material than from the whole plant may greatly affect the stategies employed for harvest of the commercial crops. For example, more efficient utilization of steam for distillation of the crop is achieved by extraction from seed material only.

Less energy is required per unit of oil extracted from seed compared to a herb and seed mix. This has been adopted for commercial production in Tasmania by harvesting the seed directly with conventional grain heading machines. This system replaces the original method of forage harvesting the top half to one third of the canopy of the crop.

As well as improved efficiency in distillation, transportation costs are less due to a reduction in the bulk of material which has to be transported to the distillation facility. This has enabled crops to be grown further away from the distillation facility whilst still retaining an economic return to the grower.

Climatic conditions have been observed to produce seasonal variations in anethole content (Demarest, 1978), resulting in changes of 10 to 15 percent. Any such comparison of oil composition from first and second year crops or between varieties must therefore be performed in the same season. Comparative results between seasons would not be valid, as would a comparison between results obtained in different parts of the world.

In this study, the composition of oil from umbel material differed to that of oil from the whole plant. Higher levels of anethole detected in the oil from umbel material was in agreement with Embong *et al* (1976), but only 10 percent greater not 30 percent as indicated by these workers. The differences detected in anethole content reflect different varieties of *F. vulgare* used in this study compared to the study of Embong *et al*. (1976), and possible climatic variations as indicated above. A corresponding increase in anethole content in the oil from extracted seed compared to forage harvested material has been obtained in commercial extractions.

Although increased oil yield results from forage harvesting the top half of the whole plant higher levels of anethole present in the umbels means that the yield of anethole per hectare remains similar for a direct headed crop. In addition, higher levels of limonene and fenchone observed by Embong *et al*. (1976) were not present in the variety C22.

Similar changes in the composition of the oil from dill umbels (El-Genghaihi and Hornok, 1978) were observed in the oil from fennel umbels. The levels of the terpinenes, alpha-pinene and alpha-phellandrene decreased in the oil from the umbels. In dill, a decrease in the level of limonene also occurred. Such a change was not detected in the oil from the fennel umbel.

In this study, fenchone was the only compound observed to increase substantially during the maturation of the crop, particularly in the oil from umbels of the second year crop. The levels present in both the whole plant and umbel oils were similar for the first year crop. Small increases in anethole content were observed in the oil from umbels of the first year crop. But anethole levels remained constant in the oil from umbels of the second year crop. These results indicate that strategies for harvest may be based solely on oil yield from umbels. The maximum yield of anethole per hectare will then result from harvesting during the period of maximum oil yield from the umbels.

The umbels are the major oil bearing plant stuctures of the fennel plant, contributing 95 percent of the total oil yield. This study examined in detail the accumulation and composition of oil from each of the four major umbel groups present. By combining these results with the umbel numbers, the primary umbel was shown to be the major oil producing umbel. As the umbel order decreased, the oil yield per umbel decreased, indicating some form of competition within the umbel canopy of the fennel plant. The number of tertiary umbels present was the highest, reflecting the branching nature of fennel. The quarternary umbels were expected to be the most prevalent in number, but did not reach the full genetic potential of this variety due to a decline in numbers prior to seed set. Such a decline in numbers of quarternary umbels has also been observed in carrots by Hiller and Kelly (1985). This decrease was a result of seed abortion due to insufficient pollination. Senescence of some of the quarternary umbels occurred after failing to set seed. Similar effects were observed within fennel crops.

Hawthorn et al. (1962) determined that the secondary umbels contributed the most towards total seed weight from carrots. The tertiary umbels were the next major contributors whilst the primary umbel contributed the least.

A mean of 20 umbels per fennel plant were present compared with only 15 in carrots. The mean number of primary and secondary umbels per plant was the same in carrots and fennel, but the number of tertiary umbels were less. The number of quarternary umbels were not recorded by Hawthorn et al. (1962). This umbel order was not considered as a major contributor towards seed yield from carrots. In fennel a maximum of 17 umbels per plant were present, but the number decreased as detailed above. Only a small contribution, 3.8 percent, towards the total oil yield per plant resulted from the quarternary umbels.

Tsvetkov (1970) examined the oil yield from each umbel order in bitter fennel. This work indicated that the primary umbel produced the highest percent oil yield compared to the other umbel orders. The present study indicated that no significant difference occurred between the percent oil yield from the first three umbel orders, but the primary umbel was shown to have the highest oil production on a weight per umbel basis. Such results were expected due to this umbel having the greatest number of rays and number of seeds per ray resulting in the highest number of seeds per umbel. But when the results were expressed as the oil yield per umbel order per plant, the secondary umbels were the major contributors to the overall oil yield. This was a result of the combination of the number of umbels per plant and the oil yield per umbel. The next most significant contributors towards the overall oil yield were the tertiary umbels. Although the oil yield per umbel for the tertiary umbels was much lower than the secondary umbels, the numbers present per plant were higher. No such calculations were performed by Tsvetkov (1970), invalidating much of this works recommendations on harvest date prediction.

At harvest, the secondary and tertiary umbels collectively contributed 84 percent of the total oil yield. In dill, the primary and secondary umbels were the major contributors, namely 94 percent of the total oil yield (Porter et al. 1983). Gray and Steckel (1985) indicated that the variation in the rate of seed maturity increased between umbel orders as plant density increased. A higher plant density may then account for the differences observed for the origin of oil yield from the umbel canopy of dill as determined by Porter et al. (1983). In a high density planting the primary and secondary umbels may account for a high proportion of the total oil yield due to insufficient time for maturation of the higher order umbels.

The changes in oil composition from each umbel order indicates a set oil production pattern, characteristic of a fennel variety. Generally the changes observed were similar in all umbel orders. Any differences observed were the result of the differences in stages of maturity between the umbel orders at any one harvest date. The oil production increased with increasing umbel order indicating the more rapid maturation of the higher umbel orders in the later stages of development as the plant approaches senescence. The oil composition of the first three umbel orders was identical at the completion of the experiment. The composition of the oil from quarternary umbels was also very similar to the other orders. Only minor differences in the levels of most components were recorded.

In addition to the oil yield from umbels, Tsvetkov (1970) examined the oil composition from umbels at harvest. As the umbel order increased the anethole content of the oil increased. The levels of both fenchone and estragole were observed to decrease with increasing umbel order. No statistical analysis was given to support the changes stated. This present study indicated that anethole content did not vary significantly between umbel orders during March and into April. Also, the levels of fenchone detected were lower in the quarternary umbels compared to the other orders. Levels of estragole were less in the higher orders early in maturation, but at harvest, no significant differences between orders were detected.

During maturation, only two compounds were observed to increase in all umbel orders, anethole and estragole. Such changes are very similar to the change in carvone levels observed in dill by Porter et al. (1983) and Hornok (1973). Carvone, like anethole and estragole, is a phenylpropene, a member of the phenylpropenoid group. Carvone content of oil from umbels was observed to increase, particularly with the onset of senescence. Hornok (1973) believed that this increase was related to the developmental stage of the plant. Harborne (1980) indicated that the production of phenylpropenes through shikimate and phenylalanine from the Calvin Cycle was closely related to the production of lignins. The increase in anethole content in the umbels of fennel coincides with the maturation of seed and may be related to the process of lignification of the seed coat.

Floral Initiation

As indicated in the literature review, information regarding the processes of floral initiation in fennel is limited. Detailed examination was required to determine the requirements for initiation.

The present study has demonstrated that fennel behaves as a long day plant, but more importantly, elongation and flowering result from long photoperiods utilizing non-photosynthetically active radiation. From the night break treatments it was apparent initiation was a phytochrome mediated response. This is similar to responses observed in dill (Naylor, 1941). Differentiation of the meristematic zone into floral primordia occurs after some stem elongation. This observation is typical of other members of the Apiaceae (eg. carrots) (Borthwick et al. 1931) (Hiller et al. 1979). After initiation, floral development continued despite further changes to the photoperiodic regime. Fennel requires a critical photoperiod for initiation but is day neutral for flower development. These results place fennel in Group two of Kinet et al. (1985) classification of photoperiodic requirements for complete flowering.

The process of differentiation from vegetative to floral stuctures in fennel may only occur after completion of a juvenile phase of growth. Such insensitivity to inductive conditions during early stages of plant development is well documented in many plants (Bernier et al. (1981) Zeevart, 1969).

Fennel was insensitive to inductive conditions during such a juvenile phase until 8 expanded leaves were present or 12 to 13 nodes. Similar juvenile response in caraway have been observed by Novak (1974), where the change from vegetative to floral development occurred after 13 or 14 nodes were present on the main stem. Similar responses in some cultivars of carrots have also been demonstrated. Floral initiation in carrots in response to low temperatures did not occur until a minimum of 7.5 leaves were present (Atherton et al. 1983).

As already indicated, many of the Apiaceae exhibit such a juvenile response. Fennel was shown in this study to reach a stage of "ripeness to flower" earlier than both caraway and carrots. Dill is reported to have the shortest juvenile period, responding to inductive conditions when one fully expanded leaf is present (Naylor, 1941). The number of nodes present at this stage was not determined by Naylor. In comparison to fennel, when one expanded leaf is present, a total of 3 to 4 nodes have differentiated. The two processes may not necessarily be totally dependent on each other. The number of nodes present indicates the total physiological age of the plant whilst the expanded leaves represent the portion of the plant that is physiologically mature.

One explanation presented for the presence of a juvenile phase is based on the endogenous levels of gibberellins. The juvenile leaves are thought to produce insufficient GA's to promote flowering. For example juvenility in Bryophyllum spp. has been overcome by application of GA_3 (Zeevart, 1969). Although the experiment involving the application of GA_3 to fennel did not result in earlier flowering, the hypothesis that juvenile leaves of fennel are incapaple of providing sufficient GA's may not be completely disregarded. The application of Flurprimidol, accepted as a broad spectrum GA biosynthesis inhibitor, prior to initiation did not inhibit flowering. But flower number was reduced by this GA inhibitor. Additionally, this series of experiments utilized only GA_3 for exogenous application. Other GA's may be involved in the initiation process.

Another explanation for juvenility was proposed by Bernier et al. (1981). Initiation will only result once the ratio of the mature to immature leaves reaches a favourable level. This ratio may be either the number or size depending on the plant in question. In fennel, flowering still occurred when plants were subjected to growth retarding materials which decreased the size of the mature leaves dramatically. Such plants would never have reached a high ratio of size of mature to immature leaves as suggested by Bernier et al. (1981). But the ratio of the number of mature to immature leaves remained similar to an untreated flowering plant.

Examination of the differentiating apex by scanning electron microscopy enabled precise examination of the change in morphology from vegetative to floral within the fennel plant. Once this event is triggered there is rapid differentiation of primordia which forms the primary, secondary and tertiary umbels. Such rapid floral differentiation demonstrated the rapidity of the initiation response once perceived by the plant. The process of differentiation of the first three umbel orders occurred over a period of 5 days. Partial floral expression may only occur during this initial phase of differentiation. Reversion was not observed. Once a plant was induced to flower the PFD only affected the rate of development.

Fennel plants held in non-inductive conditions continued to grow vegetatively, exhibiting the typical compressed internode, rossette habit of overwintering plants. Further vegetative differentiation continued producing as many as 50 nodes after 12 months in these conditions. Without LD's, flowering would not occur. Such results do not agree with some workers, particularly Bernier et al. (1981). Their belief is that plants are steadily progressing towards a reproductive state. Application of GA_3 to such plants promoted stem elongation but did not induce flowering. These results were in agreement with those of Shimada (1959). Shimada concluded that the absence of the correct photoperiodic stimuli could not be overcome by GA_3 application at any stage of development of the plant. This result is in contrast to similar experimentation in dill, where a single foliar spray of GA_3 induced flowering under non-inductive conditions (Wittwer and Bukovac, 1957).

Plant physiologists generally do not agree on a time limitation for stating that a "plant does not flower". Mc Daniel (pers. comms.) has indicated that if a plant can be maintained in a vegetative state for more than one year, then he considers that the plant complies with the statement that it will not flower under the given conditions. Under this definition, fennel can be said not to flower when held under SD conditions.

The required number of inductive phases for initiation was investigated by subjecting plants to varying numbers of long photoperiod ranging from 1 to 25 cycles. A minimum of 10 inductive days were required for initiation of primary inflorescences but to obtain full initiation and emergence of secondary inflorescences 15 days are required.

This period is considerably longer than the 5 to 7 days determined by Mol (1981) for full initiation in Florence fennel. The experiment in this study examining the number of inductive phases required for umbel floral iniation utilized plants with 1 to 2 more leaves than the required minimum to negate the juvenile phase of development. Increased leaf number above the number used may have changed the results. An increase in the leaf area present prior to initiation may enhance the number of umbels produced. In addition, these factors may be important in relation to the number of rays produced per umbel.

Experimentation into fennel by Randhawa *et al.* (1981) indicated that delayed sowing decreased the number of umbels and number of branches per plant present. Similar responses have been observed in Tasmanian fields (Clark *pers. comms.*) The determination of a juvenile phase indicated that fennel must develop to a certain stage of growth before being receptive to inductive conditions. For late sown plants, the daylength would reach inductive lengths before the completion of this phase of growth. Only the minimum number of nodes would be present once the juvenile phase was complete. In contrast, earlier sowing would enable the plant to develop more nodes prior to the long photoperiod, thereby increasing the possible number of sites for transition from vegetative to floral. Increased leaf area may also affect the 'strength' of the response to long photoperiods, increasing the number of higher order umbels or components of the umbel such as the number of rays.

In *Brassica napus* the duration of the vegetative phase prior to anthesis has been shown to be a major determinant of the seed yield. Thurling and Vijendra Das (1979) demonstrated that a decrease in the rate of vegetative growth during stem elongation, adversely affected seed number. Similar responses in the *Apiaceae* may occur, such as exhibited in fennel by Randhawa *et al.* (1981). Other studies involving *Brassica* spp. have indicated that assimilate balance is important at anthesis for the further development of the pods.

Growth Regulators

The responses of other plant species to various plant growth regulators may only be used as indicators of the possible responses which may be obtained when experimenting with fennel. Matthysse and Scott (1980) summarize the problem of conclusions based on other species as follows 'since each species occupies its own ecological niche, one would expect each species to have some unique responses to the environment and these responses are likely to be hormonally mediated'. Only by both endogenous and exogenous techniques under controlled environments can the responses to various environmental changes be examined in detail in a particular species.

In this study exogenous application techniques were used to investigate the effects of various growth retardants on elongation and flowering in fennel. The preliminary study into the effects of plant growth regulators on fennel involved the initial screening of the major effects of growth regulatory substances representative of the five major groups of phytohormones. The use of logarithmic sequence of rates, as recommended by Bernier et al. (1981), enabled a rapid determination of the concentation range in which a substance was active on fennel.

In each experiment the method and site of application was kept constant in order to localize the application. Attention to such detail provided statistically significant data in most experimentations. The only major confounding factor was the change in physiological age of the target tissue. But in many cases such effects may also yield useful information on the change in receptiveness of a plant at different physiological stages.

The pot trial conducted during the 1985 season examined a number of growth retarding chemicals. Effects obtained varied from the complete inhibition of the flowering response resulting in continued vegetative growth, even after 3 months of inductive conditions, to increased tillering resulting in a growth habit consistent with a plant two year or more years old. From this preliminary investigation the growth retardant, flurprimidol (EL500) a GA biosynthesis inhibitor, was found to produce a number of very desirable changes in habit of the fennel plant.

Further preliminary studies using EL 500 showed that the fennel plant could be compressed in habit, by reducing the internode length, without affecting the flowering adversely. Such results indicated that oil yield may also not be adversely affected. Both soil and foliar applications were successful in retarding vegetative growth, reducing internode length and leaf area. This chemical was applied to plants which possessed 6 fully expanded leaves. Consequently the initial treatments were applied before floral initiation.

During further experimentation, a decrease in umbel number resulted when EL500 was applied to fennel plants at an earlier stage of development, 2 or 4 expanded leaves. Suppression of GA levels at this stage appeared to adversely affect the process of floral initiation.

Further investigation of the effects of EL500 were tested in the field. 5 rates of application were utilized ranging from 20 to 160 mg ai/pl. No significant change in the percentage oil yield from the whole plant or any of the umbel orders resulted. The major effect was reduction in the height of the crop. The overall height decreased by 25 percent. The inflorescence bearing area of the canopy was also reduced at time of harvest. The very high rates of EL 500 produced plants with only 3 or 4 leaves, smaller than normal. These plants still managed to flower normally without affecting umbel number or size, and as a result of these, no affect on oil yield was recorded.

Further reductions in height in the field may have resulted by earlier application of EL500. The earliest that EL500 could be applied is at the 6 expanded leaf stage. This is due to the results obtained from the timing of application experiment which demonstrated that earlier uptake of EL500 reduces the yield by lowering umbel numbers. The stage of development at which application of EL500 was performed in the field was at 8 expanded leaves. At this stage, the amount of leaf present was such that, although GA biosynthesis was inhibited, the endogenous levels of GA were probably sufficient to allow further elongation. Such elongation would only cease once endogenous GA levels decreased.

The importance of endogenous GA's was also demonstated in the defoliation experiment. The decrease in stem elongation exhibited by the plants subjected to high levels of defoliation may have resulted from a decrease in endogenous GA's. This decrease in height may also have been due to a reduction in carbohydrate levels due to severe defoliation. But, as node number was the same for all treated plants and only the stem elongation was affected, the response was more indicative of insufficient GA's.

GA application to some Apiaceae under non-inductive conditions has been shown to decrease the time to anthesis of the primary umbel and also to increase the flower yield, eg coriander (Amrutavalli, 1979). In other cases, eg dill, application of GA_3 has had no effect on flowering (Wittwer and Bukovac, 1957). Nieuwhof (1984) demonstrated that under truely non-inductive conditions, carrots could not be induced to flower, at any stage of development, by GA_3 application. This latter point emphasises the importance of examining the timing of application of any growth regulatory substance. In many experimentations into exogenous GA_3 application, the stage of development at the time of application was not considered. This study has shown that, in fennel, the effect of GA_3 application varies depending on the stage of growth at which the plant is treated. Such differing responses may relate to the changes in endogenous GA's within the fennel plant at these various growth stages. The general consensus among plant physiologists is that the changes in the balance of promotory and inhibitory substances initiate the developmental changes of the plant. The application of exogenous GA_3 at a certain stage where endogenous GA's may be at sub-optimal levels, may be sufficient to produce a substantial GA affect. The application of GA_3 to plants during their juvenile phase of growth whilst held under inductive conditions increased vegetative growth. This treatment resulted in increased umbel numbers, again highlighting the importance of vegetative growth prior to initiation.

Assimilate partitioning

The importance of photosynthetic surface early in the development of the fennel plant may be to provide energy requirements for rapid differentiation of the apex during floral differentiation. The infra-red examination of the fennel canopy demonstrated that at the time of floral initiation the leaves were the major metabolically active organs. Increased vegetative growth prior to this phase would increase the leaf area index of the canopy, thereby ensuring that available photosynthates were not limiting during initiation. This would allow for full expression of the genetic potential for maximum number of umbels per plant.

The role of assimilates as an energy source in flower initiation and development is well established. But, control of initiation by assimilates has been shown, especially in respect to many of the environmental factors which influence photosynthesis and/or assimilate supply. The prevention of assimilates from reaching meristems from induced leaves will appear as floral inhibitors, and conversely agents promoting assimilate supply to the meristem will appear as floral promotors. Removal or restriction of competing sinks has been shown to promote flower initiation in many plants (Bernier, 1988). A similar interpretation might be applied to the effects of plant growth regultory substances on flowering. Once the umbels have been initiated, any further environmental or chemical stimuli only affects the development of these umbels. Observations during the experiments into the effects of growth retardants led to the proposal that the umbels themselves were photosynthetic by nature and were able to provide their own carbohydrates. Further investigations measuring the photosynthetic rates of leaves and inflorescences have supported this idea.

To evaluate the contribution of the leaves towards overall seed oil yield an experiment involving the reduction of leaf area by defoliation was conducted on a commercial crop. Leaf removal treatments commenced after initiation and were maintained throughout umbel development. The various levels of defoliation were present for anthesis and seed development of all umbel orders initiated.

From these treatments it was shown that the plant was able to withstand up to 50 percent loss of leaf without any significant effect on the fresh weight and oil yield of primary, secondary and tertiary umbels. Also the leaf subtending the inflorescences was shown to play no significant role in the development of the inflorescence.

Tayo and Morgan (1979) utilized shading and leaf removal techniques to alter assimilate supply in oilseed rape and demonstrated the importance of leaf supplied carbohydrate to the inflorescence from anthesis onwards. The ability of an inflorescence to draw carbohydrate from a source was the major factor. Competition for carbohydrates may be either within the developing flower structure or between flower structures. A shortage of assimilates at any stage prior to anthesis in oil seed rape resulted in fewer pods formed but the plant was able to compensate by increased growth of the pods. Such a model may also apply to the development of the seed in various umbel orders in Apiaceae. The decrease in number of quarternary umbels of severely defoliated plants may have been a direct result of a similar shortage of assimilates during initiation.

Similar experiments by Gray et al. (1962) altered the assimilate balance in carrots by shading and removing various structures within the canopy. The effects on the subsequent seed weight indicated the sink strength of the various umbel orders. The results from autoradiography of fennel indicate a similar degree of sink strength. The major sink was found to be the primary umbel, with the sink strength decreasing with increasing umbel order. Such a profile having been shown in carrots and now fennel indicates a common course of development within these two plants. Such findings are important as the lower order umbels posses a largely unrealized capacity. Maximizing assimilate supply within the plant as an entire system will aid in the further development of these umbels, thereby increasing the yield potential.

This study has provided further understanding of the physiology of fennel enabling changes in management of the plant as a crop system resulting in increased umbel number and oil yield. The application of plant growth regulators has complemented and confirmed the responses to environmental stimuli again enabling improved stategies in the management of fennel for anethole production.

Such studies, in conjunction with assimilate partitioning and defoliation experiments, have highlighted the role of vegetative growth during devlopment. In early stages of growth, leaves play a major role in the determination of the final umbel number which is then followed by a change to a minor role during the development of the umbel and maturation of the seed.

VI

BIBLIOGRAPHY

BIBLIOGRAPHY

ABDALLAH, N., EL-GENGAIHI, S. and SEDRAK, E. (1978). The effect of fertilizer treatment on yield of seed and volatile oil of fennel (Foeniculum vulgare Mill.). Pharmazie 33: 607-608.

ABELES, F.B. (1972). Biosynthesis and mechanism of action of ethylene. Ann. Rev. Plant Physiol. 23: 259-292.

ABOU-ZIED, E.N. (1974). Increase in volatile oil and chemical composition in seeds of caraway and fennel plants induced by succinic acid 2,2-dimethyl-hydrazide. Biol. Plantarum 16(2): 123-126.

ADDO-QUAYE, A.A., SCARISBRICK, D.H. and DANIELS, R.W. (1986). Assimilation and distribution of carbon-14 photosynthate in oil seed rape Brassica napus. Field crops Res. 13(3): 205-216.

AHMED, S.S. and EID, M.N.A. (1975). Effect of gibberellic acid and cycocel on yield of seeds and essential oil of some umbelliferous plants. Egypt J. Hort. 2(2): 227-232.

ALBERT-PULEO, M. (1980). Fennel and anise as estrogenic agents. J. Ethnopharmacol 2(4): 337-344.

ALLEN, E.J., MORGAN, D.G. and RIDGEMAN, W.J. (1971). A physiological analysis of the growth of oilseed rape. J. Agric. Sci. 77: 339-341.

ALONI, B. and PRESSMAN, E. (1980). Interaction with salinity of GA3 induced leaf elongation, petiole pithiness and bolting in celery. Scientia Horticulture 13(2): 135-142.

AMRUTAVALLI. (1979). Gibberellic acid (GA_3) induced enhancement in flowering in Bulgarian coriander (Coriandrum sativum L.) in relation to changes in carbohydrate metabolism. Current Science 48(1): 5-6.

ASHRAF, M. and BHATTY, M.K. (1975). Studies on the essential oils of the Pakistan species of the family Umbelliferae. Part II: Seed oil. Pakistan J. Sci. Ind. Res. 18(5): 236-240.

ATHERTON, J.G., BASHER, E.A. and CUTTING, B. (1983). Control of bolting and flowering in carrot. Acta Hortic. 134: 139-145.

AUSTIN, R.B. and LONGDEN, P.C. (1967). Some effects of seed size and maturity on yield of carrot crops. J. Hort. Sci. 42: 339-353.

AVIV, A., DANTES, E., KROCHMAL, E. and GALUN, E. (1983). Biotransformation of monoterpenes by Mentha cell lines. Planta Medica 47: 7-10.

BADOC, A. (1988). Contribution a l'etude du genre Foeniculum. PhD Thesis. L'Universite des sciences et techniques de Lille Flandres Artois.

BATCH, J.J. (1981). Recent developments in growth regulators for cereal crops. In: Aspects of crop growth. Agronomy Conference, Bournmouth. ADAS ref. book 341.

BEARDER, J.R. (1980). Plant hormones and other growth substances. Their background, structures and occurrence. In, Hormonal regulation of development. I: Encyclopaedia of Plant Physiology. New Series, Vol. 9: 9-112. Ed. MacMillan, J. Springer-Verlag, Berlin.

BEGHTEL, F.E. (1925). The embryogeny of Pastinaca sotina. Am. J. Bot. 12: 327-337.

BELL, R.C. (1971). Breeding systems and floral biology of the Umbelliferae or Evidence for specialization in unspecialed flowers. In: The biology and chemistry of the Umbelliferae. Ed. Heywood, V.H. 93-108

BENDER, L., STIEBELING, B. and Newmann, K.H. (1986). Investigations on photosynthesis and assimilate translocation in Daucus carota L as influenced by a varied phosphorus supply and changes in the endogenous hormonal system following GA3 treatments. Z. Planzeneraetu. Bodenk. 149: 533-540.

BERNIER, G. (1988). The control of floral evocation and morphogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39: 175-219.

BETTS, T.J. (1968). Anethole and fenchone in the developing fruits of Foeniculum vulgare Mill. J. Pharm. Pharmac. 20: 469-472.

BETTS, T.J. (1976). Possible new Western Australian fennel chemovar Aust. J. Pharmaceutical Sciences 5: 78.

BLEASDALE, J.K.A. (1967). The relationship between the weight of a plant part and the total weight as affected by plant density. J. Hort. Sci. 42: 51-58.

BLEASDALE, J.K.A. and THOMPSON, R. (1966). The effects of plant spacing and pattern of plant arrangement on the yield of parsnips J. Hort. Sci. 41: 371-378.

BORTHWICK, H.A. (1931). Carrot seed germination. Proc. Amer. Soc. Hort. Sci. 28: 310-314.

BORTHWICK, H.A., PHILLIPS, M. and ROBBINS, W.W. (1931). Floral development in Daucus carota. American J. Bot. 18: 784-797.

CHAILAKHYAN, M.K.L. (1976). Hormonal regulators of plant flowering. In, Plant Growth Regulators: 258-272. Ed. PILET, P.E. Springer-Verlag, Berlin.

CHAPMAN, J.F., DANIELS, R.W. and SCARISBRICK, D.H. (1984). Field studies on C-14 assimilate fixation and movement in oil seed rape (B. napus). J. Agric. Sci. Camb. 102: 23-31.

CHATURVEDI, S.N. and MURALIA, A.R. (1976). Germination inhibitors in some umbellifer seeds. Ann. Bot. 39: 1125-1129.

CHILCOTE, D.O., YOUNGBERG, H.W. and ALBEKE, D. (1982). Potential for growth retardents in grass seed production. Crop Science Report Ext/CS 42.

CHLADEK, M. (1972). The determination of the start of growth apex differentiation in caraway grown under natural conditions. Bulletin, Vyzkumny Ustav Zelinarsky, Olmouc No.14/15: 33-40.

CHUBEY, B.B. and Dorrell, D.G. (1976). Changes in chemical composition of dill oil during hydrodistillatiion. Can. J. Plant Sci. 56: 619-622.

CLARK, R.C. and MENARY, R.C. (1979). The importance of harvest date and plant density on the yield and quality of Tasmanian peppermint oil. J. Amer. Soc. Hortic. Sci. 104: 702-706.

CLARK, R.J. and MENARY, R.C. (1980). Environmental effects on peppermint (Mentha piperita L.) 1: The effect of daylength, light intensity, night temperature, photorespiration in peppermint with reference to oil composition. Aust. J. Plant Physiol. 7: 693-697.

CLARK, R.J. and MENARY, R.C. (1983). Production of steam distilled essential oil crops. Proc. 9th Inter. Essential Oils Congress - Singapore.

CLELAND, C.F. and ZEEVART, J.A.D. (1970). Gibberellins in relation to flowering and stem elongation in long day plant Silene armeria. Plant Physiol. 46: 392-400.

CROTEAUX, R. and LOOMIS, W.D. (1972). Biosynthesis of mono- and sesquiterpenes in peppermint from (2-^{14}C)-melavonate. In: Encyclopedia of Plant Physiology. Vol.8: Secondary Plant Products. 185-196.

DESMAREST, P. (1978). New aspects of fennel cultivation in France. Acta Hort. 73: 289-295.

DICKS, J.W. (1972). Uptake and distribution of the growth retardant aminozide in relation to control of Cateral shoot elongation in Chrysanthemummori folium. Ann. Appl. Biol. 72: 313.

DICKS, J.W. (1976). Chemical restriction of stem growth in ornamentals, and tobacco. Outlook on Agriculture 9(2): 69-75.

DICKS, J.W. and CHARLES-EDWARDS, D.A. (1973). A quantitative description of inhibition of stem growth in vegetative lateral shoots of Chrysanthemum morifolium by daminozide. Planter (Berlin) 112: 71.

DICKSON, M.H. and PETERSON, C.E. (1958). Hastening greenhouse seed production for carrot breeding. Proc. Am. Soc. Hortic. Sci. 71: 412-415.

DICKSON, M.H. and PETERSON, C.E. (1960). The influence of gibberellins on the flowering of carrots. Canadian J. of Plant Sci. 40: 468-473.

EL-BELLAL, A.S. (1978). Stability of selection response for high essential oil yield in local caraway (Carum carvi L.). Acta Horticulturae 73: 59-64.

EL-BELLA, M. and COUTLIFFE, D.J. (1986). Effect of growth regulators on flowering, number of umbels, seed yield and seed quality of carrot (Daucus carota). Acta Horticulturae 198: 163-170.

EL-GENGAIHI, S. and ABDALLAH, N. (1978). The effect of date of sowing and plant spacing on yield of seed and volatile oil of fennel (Foeniculum vulgare Mill.). Pharmazie 33: 606-607.

EL-GENGAIHI, S.E. and HORNOK, L. (1978). The effect of plant age on content and composition of dill essential oil, Anethum graveolens L. Acta Horticulturae 73: 213-218.

ELY, P.R. and HEYDECKER, W. (1981). Fast germination of parsley seeds. Scientia Horticulturae 15: 127-136.

EMBONG, M.B., HADZIYEV, D. and MOLNAR, S. (1976). Essential oils from spices grown in Alberta dill seed oil, Anethum graveolens (Umbelliferae). Can. Inst. Food Sci. Tech. J. 10(3): 208-214.

EMBONG, M.B., HADZIYEV, D. and MOLNAR, S. (1977). Essential oils from spices grown in Alberta fennel oil, (Foeniculum vulgare var. dulce). Can. J. Plant Sci. 57: 829-837.

ESAU, K. (1965). Anatomy of seed plants. 2nd ed. Wiley. 164-165.

EVELING, D.W. (1984). Examination of the cuticular surfaces of fresh delicate leaves and petals with a scanning electron microscope: A control for artifacts. New Phytologist 96: 229-237.

FREYMAN, S., CHARNETSKI, W.A. and CROOKSTON, R.K. (1973). Role of leaves in the formation of seed in rape. Can. J. Plant Sci. 53: 693-694.

FRAPPELL, B.D. (1973). Plant spacing in onions. J. Hort. Sci. 48: 19-28.

FRITSCH, F.E. and SALISBURY, E. (1955). Plant form and function. Bell and Sons Ltd.

GALLAGHER, J.N. and BRISCOE, P.V. (1978). Radiation adsorption, growth and yield of cereals. J. Agric. Sci. Camb. 91: 47-60.

GARROD, J.F. (1982). The discovery and development of plant growth regulators. In: Plant growth regulator potential and practice. British Plant Growth Regulator Group.

GOODWIN, P.B. (1978). Phytohormones and growth and development of organs of the vegetative plant. In: Letham, D.S., Goodwin P.B. and Higgins, T.J.V. (Ed.) Phytohormones and related compounds - a comprehensive treatise Vol. II.

GRAY, D. and STECKEL, J.R.A. (1985). Variation in flowering time as a factor influencing variability in seedling size in the subsequent carrot (Daucus carota L.) crop. J. Hort. Sci. 60(1): 77-81.

GRAY, D., STECKEL, J.R.A., JONES, S.R. and SENIOR, D. (1986). Correlations between variability in carrot (Daucus carota L.) plant weight and variability in embryo length. J. Hort. Sci. 61(1): 71-80.

GRAY, D., STECKEL, J.R.A. and WARD, J.A. (1986). The effect of cultivar and cultural factors on embryo-sac volume and seed weight in carrot (Daucus carota L.). Annals of Botany 58: 737-744.

GUENTHER, E. (1949). The essential oils. Vol. I and II.

GUPTA, S.C. (1969). The embryology of Coriandrum sativum L. and Foeniculum vulgare Mill. Phytomorphology 14: 530-547.

HAMNER, K.C. and NAYLOR, A.W. (1939) Photoperiodic responses of dill, a very sensitive long day plant. Botanical Gazette 100: 852-861.

HANISOVA, A. and KREKULE, J. (1975). Treatments to shorten the development period of celery (Apium graveolens L.). J. Hort. Sci. 50: 97-104.

HARBORNE, J.B. (1971). Flavonoid and phenylpropanoid patterns in the Umbelliferae. In: The biology and chemistry of the Umbelliferae. (Heywood, V.H. Ed). 301-302.

HARBORNE, J.B. (1980). Plant phenolics. In: Encyclopedia of plant physiology. Vol.8 (Bell, E.A. and Charlwood, B.V. Eds.) Secondary plant products. 335.

HAWTHORN, L.R. (1952). Interrelations of soil moisture, nitrogen, and spacing in carrot seed production. Am. Soc. Hort. Sci. 60: 321-326.

HAWTHORN, L.R., TOOLE, E.R. and TOOLE, V.R. (1962). Yield and viability of carrot seed as affected by position of umbel and time harvest. Proc. Amer. Soc. Hort. Sci. 80: 401-407.

HAYWARD, H.E. (1938). The structure of economic plants. MacMillan Corp. Publishers.

HEBBLETHWAITE, P.D. and BURBRIDGE, A. (1976). The effect of maleic hydrazide and chlorocholine chloride on the growth, seed yield components and seed yield of S23 ryegrass. J. Agric. Sci. Camb. 86: 343-353.

HEDGE and LAMOND. (1972). Umbelliferae. Flora of Turkey 4: 377.

HENRY, B.S. (1982). Composition and characteristics of dill: a review. Perfumer and Flavorist 7: 39-44.

HILLER, L.K. and KELLY, W.C. (1979). The effect of post-vernalization temperature on seedstalk elongation and flowering in carrots. J. Amer. Soc. Hort. Sci. 104(2): 253-257.

HILLER, L.K. and KELLY, W.C. (1985). Daucus carota In: CRC Handbook of flowering. Vol.II: 419-429.

HILLER, L.K., KELLY, W.C. and POWELL, L.E. (1979). Temperature interactions with growth regulators and endogenous gibberellin-like activity during seedstalk elongation in carrots. Plant Physiology 63: 1055-1061.

HOLDSWORTH, M. (1956). The concept of minimum leaf number. J. Exp. Bot. 7: 395-409.

HORE, A. (1979). Improvement of minor (Umbelliferous) spices in India. Economic Botany 33: 290-295.

HORNOK, L. (1973). Investigations of the effects of fractional sowing on the yield and volatile oil content of dill (Anethum graveolens L.) Herba (~~Hungary~~) 12: 37-43.

IMDEN, T. (1950). Studies on the flowerbud differentiation of carrots. Bull. Fac. Agric. Mic. Univ. 1: 14-21.

JACOBSOHN, R. (1978). Research on carrot production 1974-1977. Special publication. Volcanic Center No. 125.

JACOBSOHN, R., SACHS, M. and KEILMAN, Y. (1980). Effect of daminozide and chlor on bolting suppression in carrots. J. Am. Soc. Hort. Sci. 105(16): 801-805.

JANSEN, P.C.M. (1981). Spices, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. Agri. Res. Reb. 20-29.

JONES, M.G. and ZEEVART, J.A.D. (1980). Gibberellins and photo-periodic control of stem elongation in the long day plant Agrostemma githago L. Planta 149: 269-273.

JURICA, H.S. (1922). A morphological study of the Umbelliferae. Bot. Gaz. 74: 292-307.

KADRY, A., TEWFIC, H.A. and HABIB, S.A. (1978). Morphology and distribution of glandular structures in Foeniculum vulgare Mill. Egypt. J. Hort. 5(1): 13-19.

KAPELEV, I.G. (1980). Some results from their introduction of Foeniculum vulgare cultivar Southern in the Nikitski botanical garden, Ukranian-SSR USSR. Rastit. Resur 16(4): 541-546.

KARLSEN, J. BAERHEIM SVENDSEN, A., CHINGOUA, B. and ZOLOTOVITCH, G. (1969). Studies on the fruits of Foeniculum species and their essential oil. Planta Med. 17: 281-293.

KERSLAKE, M.F. (1984). Commercial production of essential oils from blackcurrants. (Ribes nigrum L.) PhD. Thesis Tas. Uni.

KOEDAM, A., SCHEFFER, J.J.C. and SVENDSEN, A.B. (1979). Comparison of isolation procedures for essential oils. 1. Dill (Anethum graveolens L.). Chem. Mikrobiol. Technol. Lebensm. 6: 1-7.

KRAUS, A. and HAMMERSCHMIDT, F.J. (1980). Untersuchungen an Fenchelolen. Dragoco Report 2: 31-40.

KRISHNAMOORTHY, H.N. (1975). Gibberelins and plant growth. Halsted Press. India.

KURATA, H. (1982). Studies on bolting in carrot, Daucus carota L. Technical Bulletin Faculty of Agric. Ogawa University 34(1): 73-77.

LAL, R.N. and SEN, T. (1971). Studies on the essential oils from seeds of Indian varieties of Foeniculum vulgare Miller. Flavor Ind. 2: 544-545.

LANE, H.C., CATHEY, H.M. and EVANS, L.T. (1965). The dependence of flowering in several long-day plants on the spectral composition of light extending the photoperiod. Amer. J. Bot. 52(10): 1006-1014.

LANG, A. (1965). Encyclopedia of plant physiology. Volume XV: Differentiation and development. Part I. Springer-Verlag, Berlin. 1647 pages.

LAWRENCE, B. (1980). New trends in essential oils. Perfumer and Flavorist 5: 6-16.

LEOPOLD, A.C. (1971). Antagonism of some gibberellin actions by substituted pyrimicdine. Plant Phyiol. 48: 537-540.

LEOPOLD, A.C. and KRIEDEMAN, P.E. (1975). Plant growth and development. 2ed. Tata McGraw Hill.

LETHAM, D.S., GOODWIN, P.B. and HIGGINS, T.S.V. (Ed.) (1978). Phytohormones and related compounds - a comprehensive treatise. Vol. 1 and 2. Elsevier and North-Holland Publishers, Amsterdam.

LEVER, B.G., SHEARING, S.J. and BATCH, J.A. (1982). PP333 - A new broad spectrum growth retardant. Proceedings 1982 British Crop Protection Conference - Weeds.

LUCKWILL, L.C. (1976). Growth regulation in top fruit. Outlook on Agriculture 9: 46-51.

MAJOR, D.J., BOLE, J.B. and CHARNETSKI, W.A. (1978). Distribution of photosynthates after 14CO2 assimilation by stems, leaves and pods of rape plants. Can. J. Plant Sci. 58: 783-787.

McDANIEL, G.L. (1986). Comparison of palcobutrazol, flurprimidol and tetcyclins for controlling pointsettia height. Hort. Sci. 21(5): 1161-1163.

MENDHAM, N.J., RUSSELL, J. and BUZZA, G.C. (1984). The contribution of seed survival to yield in new Australian cultivars of oil-seed rape (Brassica napus). J. Agric. Sci. Camb. 103: 303-316.

MENHENELT, R. (1981). Interactions of the growth retardants daminozide and piproctanyl bromide and gibberellins A_1, A_3, A_{4+7}, A_5 and A_{13} in stem extension and inflorescence development in Chrysanthemum morifolium Ramat. Ann. Bot. 47: 359-369.

MENHENELT, R. (1984). Comparison of a new triazole retardant palcobutrazol (PP333) with ancymidol, chlorphonium chloride, daminozide and piproctanyl bromide on stem extension and inflorescence development in Chrysanthemum morifolium ramat. Scientia Horticulturae 24( ): 349-358.

METCALF, C.R. and CHALK, L. (1950). Anatomy of the dicotyledons. Oxford, Clarendon. 1348.

MIGINIAC, E. (1978). Some aspects of regulation of flowering: role of correlative factors in photoperiodic plants. Bot. Mag. Tokyo Special Issue 1: 159-173.

MILLER, P. (1768). The gardeners dictionary. 8th ed. Vol. I.

MOL, C. (1981). Flower formation in Florence fennel. Goenlenen Fruit 37(2): 38-39.

MONTEITH, J.L. (1977). Climate and efficencey of crop production in Britain. Phil. Trans. R. Soc. Lond. 281: 277-294.

MOORE, T.C. (1979). Biochemistry and physiology of plant hormones. Springer, Publisher. New York, Heidelberg, Berlin.

MURNEEK, A.E. (1940). Length of day and temperature effects in Rudbeckia. Bot. Gaz. 102: 269-279.

NAYLOR, A.W. (1941). Effects of some environmental factors on photoperiodic induction of beet and dill. Bot. Gaz. 102: 557-575.

NIEUWHOF, M. (1984). Effect of gibberellic acid on bolting and flowering of carrot (Daucus carota L.). Scientia HO(orticulturae 24: 211-219. Sci. Hort.

NOFAL, M.A. (1982). Volatile flavor compounds from anise, Pimpinella anisum, grown in Egypt. Ain Shams Univ. Fac. Agric. Res. Bull. 0: 1602.

NOVAK, V. (1974). The determination and use of a critical growth stage in caraway. Rostlinna Vyroba 20: 117-128.

PENFOLD, A.R. (1950). Changes in hydrocarbons and oxygenated compounds in Eucalyptus Sp. Perfumery and essential oil record 41: 359-362.

PETERSON, L.E. (1984). A study of fennel oil production in Tasmania. Honours Thesis. Uni. of Tas.

POLOWICK, P.L. and SAWHNEY, V.K. (1986). A scanning electron microscopic study of the initiation and development of floral organs of Brassica napus (cv. wester). Amer. J. Bot. 73: 254-263

PORTER, N.G., SHAW, M.L., SHAW, G.J. and ELLINGHAM, P.J. (1983). Content and composition of dill herb oil in whole plant and different plant parts during crop development. New Zealand J. Ag. Res. 26: 119-127.

PRESSMAN, B. and NEGBI, M. (1980). The effect of daylength on the response of celery to vernilization. J. Exp. Bot. 31: 1291-1296.

PRESSMAN, E. and NEGBI, M. (1987). Interactions of daylength and applied gibberellins on stem growth and leaf production in three varieties of celery. J. Exp. Bot. 38: 968-971.

PRESSMAN, E. and SACHS, M. (1985a). Apium graveolens. In, CRC handbook of flowering. Vol. I: 485-491.

PRESSMAN, E. and SACHS, M. (1985b). Minor vegetables of the family Umbelliferae. In, CRC handbook of flowering. Vol IV: 445-446.

PUTIEVSKY, E. (1978). Yield components of annual Carum carvi L. growing in Israel. Acta Hoticulturae 73: 283-287.

PUTIEVSKY, E. (1983). FEffects of daylength and temperature on growth and yield components of three seed spices. J. Hort. Sci. 58(2): 271-275.

RAEV, R.T.S., BALINOVA-TSVETKOVA, A., ATANASOV, Z. and ZLATEV, S. (1980). Producing high quality essential oil from the above ground part of annual fennel. Rasteniev'd Nanki 17(2): 8-12.

RANDHAWA, G.S., BAINS, D.S. and MAHAJAN, V.P. (1981). Seed and oil yield of fennel as influenced by sowing time and levels of nitrogen. J. Res. Punjab Agric. Univ. 18(3): 244-247.

REID, J.B. and MURFETT, I.C. (1977). Flowering in Pisum: the effect of genotype, plant age, photoperiod and number of inductive cycles. J. Exp. Bot. 28: 811.

ROBERTS, N.J. (1989). Flower bud initiation and development in Boronia megastigma nees. PhD. Thesis. Uni. of Tas.

SAMPSON, M.J., TURNER, J.A. and WILLIAMS, R.H. (1980). Survival versus yield in cereals. The problems and control of apical dominance. Proceedings British Crop Protection Council.

SHIMADA, T. (1959). Effect of gibberellin treatment on the photo-periodically treated fennel plant (Foeniculum vulagare) on the relation between flower differentiation and internode elongation of shoot in long day plant. II. Agric. Bull. Saga. Univ. 9: 1-74.

SLADKY, Z. (1986). The role of growth regulators in the differentiation of flowers and inflorescences. Biologia Plantarum 28(1): 31-37.

SVAB, J. (1978). Problems and results of Foeniculum vulgare cultivation in large scale production in Hungary. Acta Horticulturae 73: 297-301.

TAYO, T.O. and MORGAN, D.G. (1979). Factors influencing flower and pod development in oil-seed rape (Brassica napus L.). J. Agric. Sci., Cambridge 92: 363-373.

THELLUNG, A. (1925). Umbelliferae. In: Hegi, Illustrierte Flora von Mittel-Europa. Ist edition. 5,2.

THOMAS, T.H. (Editor). (1982). Plant growth regulator potential and practice. British Crop Protection Council, Publishers.

TOTH, L. (1967). Untersucchungen uber das atherische Ol von Foeniculum vulgare I. Die zusammensef zung des frucht und wurzelols. Planta Medica 15: 157-172.

TSVETKOV, R. (1970). Study of the fruit quality of some umbelliferous essential oil plants. Plant Medica 18: 350-353.

TUCKER, W.G. and GRAY, D. (1986). The effects of threshing and conditioning carrot seeds harvested at different times on subsequent seed performance. J. Hort. Sci. 61(1): 57-70.

VINCE-PRUE, D. (1975). Photoperiodism in plants. McGraw-Hill, London.

WAREING, P.F. and PHILLIPS, I.D.J. (1981). Growth and differentiation in plants. 3ed. Pergammon Pres.

WITTWER, S.H. (1983). Vegetables. In: Plant growth regulating chemicals. II Nickel, L.G. Ed. 213-227.

WITTWER, S.H. and BUKOVAC, M.J. (1957). Gibberellin effects on temperature and photoperiodic requirements for flowering in some plants. Science 126: 30-31.

ZEEVART, J.A.D. (1962). The juvenile phase of Bryophyllum daigremontanum. Planta 58: 543.

ZEEVART, J.A.D. (1969). The leaf as a site of gibberellin action in flower formation in Bryophyllum daigremontianum. Planta 84: 339-347.

ZEEVART, J.A.D. (1978). Phytohormones and flower formation. In: Phytohormones and related compounds. A comprehensive treatise. Letham, D.S., Goodwin, P.B. and Higgins, T.J.U. Eds. Vol.2: 291-338.

ZEEVART, J.A.D. (1983). Gibberellins and flowering. In: "The biochemistry and physiology of gibberellins." 2: 333-374. Editor, A. Crozier. Publisher Praeger, New York.

VII

APPENDICES

Appendix IV.B.1.1.

Growth room programming of lights and trolley movements for experiment IV.B.

Treatment	Trolley Time Out	Trolley Time In	Hours of Natural Daylight	Lights On	Lights Off
10:14	0730	1730	10	-	-
13:11	0730	1730	10	1730	2030
16:8	0730	1730	10	1730	2330

				1st flash		2nd flash		3rd flash		4th flash	
				On	Off	On	Off	On	Off	On	Off
Night Break	0730	1730	10	1945	2000	2230	2245	0115	0130	0400	0415

Appendix IV.C.4.1.

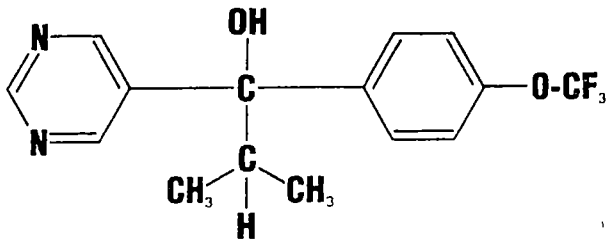
Technical report on EL-500. (Produced by Elanco Pty. Ltd.)

General Information

EL-500 is a foliar and root active plant growth regulator which reduces internode elongation of a broad range of both monocotyledonous and dicotyledonous plants. It has demonstrated desirable growth regulating properties on plants requiring routine maintenance defoliation, pruning, or edging as well as those subject to lodging without phytotoxic effects.

Chemical and Physical Properties

EL-500



$C_{15}H_{15}F_3N_2O_2$ flurprimidol* M.W. 312.29

α-(1-Methylethyl)-α-[4-(trifluoromethoxy)phenyl]-5-pyrimidine-methanol

CUTLESS™ is the Elanco Products Company (A Division of Eli Lilly and Company) registered trademark for flurprimidol

*The American National Standards Institute has accepted flurprimidol as the common (generic) name for the compound

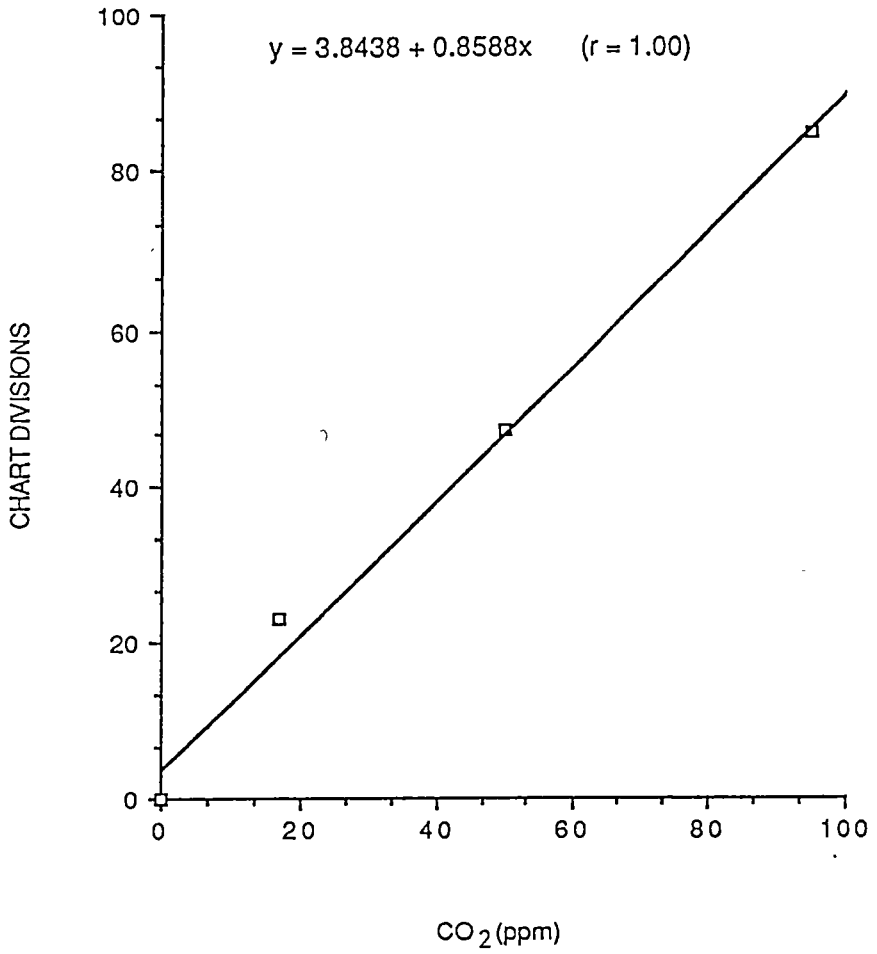
Pure flurprimidol is a white odorless crystalline solid which melts at 94-96°C. It is soluble in the organic solvents, acetone, chloroform, dichloromethane, methanol, and cyclohexanone, but only slightly soluble in hexane and heavy aromatic naphtha. The solubility in water is 120-140 ppm at 25°C at pH 4, 7, and 10. The n-octanol/water partition coefficient is log K = 2.97. The chemical is susceptible to photolysis in laboratory studies with a half-life in aqueous solution of approximately three hours whereas aqueous solutions held in the dark at 25°C showed no degradation of flurprimidol. This suggests that hydrolysis is not a significant mode of degradation in the environment.

Soil Behavior

Flurprimidol was found to have an adsorption coefficient of 1.7 when evaluated in the laboratory using a sandy loam soil. This indicates that flurprimidol is only weakly adsorbed to soil while the desorption data indicate that most of the adsorbed flurprimidol is readily desorped back into solution. Both factors increase the potential for movement of flurprimidol in soil.

Laboratory leaching studies with aged soil and ^{14}C labeled flurprimidol indicate that under severe leaching conditions, flurprimidol and/or metabolites are susceptible to leaching. The same sandy loam soil used to determine the adsorption coefficient was employed in these experiments. Exposure of a 30 cm (12 inch) column to 45 consecutive days of leaching with one-half surface inch of water applied per day resulted in an accumulation of 7.3% flurprimidol and/or metabolites in the leachate. Flurprimidol and/or metabolites remaining within the column after 45 days were equally distributed from top to bottom.

Preliminary data from field soil monitoring studies with flurprimidol indicate that in areas of rainfall greater than 30 inches per year flurprimidol has a soil half-life of less than six months. Yearly reapplication in these studies shows no likelihood of residual accumulation of flurprimidol. Similar studies in semi-arid areas (10-15 inches annual rainfall) where the treated site received supplemental irrigation typical of turfgrass cultural practices also indicate a soil half-life of less than six months.



Appendix IV.D.1.1.

Calibration curve of chart recorder response (mm) against change in CO_2 concentration.