COMMERCIAL PRODUCTION OF

ESSENTIAL OILS FROM BLACKCURRANTS

(*RIBES NIGRUM L.*)

by

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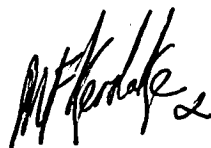
APRIL, 1984

But those who trust in the Lord for

help will find their strength renewed.

They will rise on wings like eagles;

they will run and not get weary;

they will walk and not grow weak.

Isaiah 40:31

This thesis does not contain any material which has been

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SUMMARY

The aim of the research programme was to investigate factors influencing the production of extracts from blackcurrant buds. The results indicate that a small, but viable production unit could be established within the framework of a broader essential oils industry in Tasmania.

Various agronomic factors which influence the growth of black-currant crops were examined in a systematic fan design. Investigations for a source of bud material revealed that blackcurrant plants pruned each winter to ground level, provide the maximum bud yields. In this system, the blackcurrant bush will remain vegetative. The importance of maintaining a healthy balance between plant vigour and bud yield needs to be appreciated, as the annual pruning regimen can potentially subject the plant to physiological stress and reduce bud yields in subsequent years.

The form of the bud yield-plant density response is asymptotic as determined by non-linear regression analysis. Further, investigation of the relationship of total cane fresh weight and shoot numbers per plant to planting density revealed that plant size is decreased at high densities. Growth depression is observed, with respect to shoot length at both high and low planting densities. This is discussed in terms of competition for resources, in particular light. Basal cane girth is shown to be related to yield parameters and is suggested as being a

reliable estimator of plant productivity.

Two canopy types are distinguished over the range of planting densities examined. The continuous, uniform canopy at high densities intercepts light more efficiently than the discontinuous, clumped canopy observed at low planting densities. The continuous canopy meets criteria laid down for an ideal canopy. In particular, it reaches maximum size quickly, before incident radiation reaches its summer peak, as well as being easy to maintain at maturity.

The compositional and organoleptic methods used on a number of varieties in this assessment reveal White Bud as the preferred variety. This analysis confirms the relationships established by Todd in his identification key, based on phenotypic features.

Scanning electron microscopy identified, late November - early December, as the period of most rapid increase in oil gland size. Gas chromatographic methods reveal that the rate of oil synthesis increases in early to mid January, corresponding to a period of increased photo-synthate availability as leaf growth slowed.

Investigation of oil quality during bud burst, under both glass-house and field conditions, showed that the strength of the catty note increases as the buds break from dormancy. This raises questions con-cerning the complexity of biosynthetic changes that are occurring, particularly the relationship between terpene synthesis and abscisic acid.

Components in blackcurrant bud oil were analysed by liquid chroma-tography on silica gel or florisil columns using a series of different polarity solvents. The catty note was not eluted using these techniques, however a reversed phase procedure employing High Performance Liquid Chromatography was successful in separating this compound. Compositional data and identification were obtained by gas chromatographic and mass

spectrometry methods. One hundred and twenty-three components were detected, of which sixty-six were positively identified, and good quality mass spectra presented for fifty-seven unknowns. Some twenty-three components reported have not been previously identified in black-currant bud or fruit oils.

Gas chromatographic effluent odours were associated with the corresponding peaks to determine their individual contribution to the overall aroma complex. Five regions of the aroma profile were shown to be important to the blackcurrant bud aroma, but the individual components were not identified.

Various extraction solvents were investigated, and petroleum ether was shown to produce an extract resembling the French product. The superiority of liquid carbon dioxide extracts was demonstrated, and these hold much promise for future commercial operations.

Both harvesting techniques examined were effective in removing buds. However, the mechanical harvester is better adapted for commercial operation due to lower labour inputs. This prototype consisted of a set of rotating brush rollers which act to lift the bud and break the petiole. Other rollers control the speed at which the cane passes these brushes. In contrast, the chemical method utilizes sprays of ethephon to cause abscission layer formation in the bud petiole.

An economic analysis was undertaken, examining the effect of price, planting density and harvesting method on the internal rate of return of capital invested. A mechanical harvesting operation is preferable, returning a higher profit margin than manual harvesting, since the latter requires high labour inputs. Lower planting densities, than those con-sidered desirable for agronomic reasons, are more profitable due to reduced establishment costs.

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CHAPTER I

INTRODUCTION

Many plant materials are known to contain natural mixtures of terpene oils; these oils are distinguished by their volatility and odour or "essence". Such oils are often given the general term essential oils in practise, but a more strict definition of the term essential oil is "the odoriferous products obtained by steam distillation of plant matter of defined botanical origin or by expression from the pericarp of citrus fruits and separated from the aqueous phase by physical methods; by extension, other products obtained by other methods of fractional distillation, or yielded during their preparation under subsidiary treatments such as filtration, centrifugation, rectification or treatment by absorbants for the selective concentration or elimination of certain constituents" (International Organisation for Standardization).

Tasmania, at present, has a demonstrated advantage for the production of flavour and fragrance products extracted by such methods, as shown by the excellence of its lavender, hop, parsley and peppermint products. The island appears to be ideally suited to the production of essential oils having a temperate climate, high latitude, cheap productive land and available irrigation water.

Essential oils have a wide variety of uses in the flavour and fragrance industry; they are blended with foods to enhance flavours, added to soaps and other household items to improve fragrant qualities of jealously guarded, as perfumes, to excite and stimulate our senses. Blackcurrant bud oil has a variety of uses; it is especially suited for scenting lipsticks, and is also highly prized for the excellent nuances it provides in high grade perfume creations. The classic use

of the oil is to reinforce and modify natural or artificial black-
currant flavour.

At present, the bud material is obtained from prunings as a
by-product of the blackcurrant fruit industry in France and England.
Blackcurrants are an important crop in Tasmania with a reputation for
fruit with excellent flavour and colour.   High labour costs led to
a contraction of the industry during the 1960s and early 1970s.
However, the advent of mechanical harvesting techniques has brought
about an increase in production areas, which now exceed 300 hectares.
By developing a ready market for blackcurrant fruit, in the long-term,
the industry had the potential to develop major small fruit exports.
In 1982/83 marketing problems have been caused by several factors;
a failure of processors to effectively market to the consumer; reliance
on traditional products and traditional overseas markets; and a failure
to deal adequately with subsidized European and New Zealand fruit
dumped on Australian markets; all of which have led to depressed prices
and uncertainty amongst growers.   The ability of the industry to
recover will depend on aggressive and new marketing strategies.

While Tasmania has the potential to develop a smallfruit export
industry, it is hoped with proper research and development, the pro-
duction of essential oils from the blackcurrant can also be considered
a profitable enterprise.   The oil is currently in short supply and
has the advantage of being a low volume, highly priced product which
offsets Tasmania's major trade disadvantage - its distance from world
markets.

This research project - the development of a commercial quality
blackcurrant concrete - has the following objectives:

(i)   Assessment of oil yield per hectare in relation to
        cultivars, cane maturity and cultural techniques -
        including planting density and special pruning systems.

(ii)   Mechanical and/or chemical harvesting of buds.

(iii)   Examination of extraction procedures and identific-
        ation of quality components - involving sensory
        evaluation carried out in conjunction with fragrance
        and flavour houses.

(iv)   Morphological and physiological aspects of oil
        accumulation, with particular emphasis on yield and
        quality of oil.

CHAPTER II

LITERATURE REVIEW

1. INTRODUCTION

1.1 The Blackcurrant Plant (*Ribes nigrum* L.)

The Blackcurrant is a deciduous shrub, growing up to two metres in height; the fruit occurring as a racemose inflorescence, with small pulpy berries about 5 mm in diameter. Blackcurrants bear best on one-year-old wood with spurs developing on older wood, but these usually become non-productive after only two or three seasons. A vigorous plant, composed of wood no more than three-years-old, is desirable and all older wood should be pruned out at ground level. On average, approximately one third of the bush would be pruned out each year.

Most of the blackcurrants being grown in Tasmania have been bred in England. The present standard variety, White Bud, appears to be a local selection of Baldwin, the main English variety. White Bud is reported to be less damaged by frost than other varieties, with well positioned fruit to aid sunlight penetration and harvesting (Tas. Dept. Agric. 1978).

Essential oils have been prepared from various parts of the blackcurrant plant. The buds appear to be the most lucrative source containing about 0.2-0.4% oil (Latrasse and Lantin 1977), as compared to the leaves - 0.017% oil (Anderson et al. 1963), and the fruit - 0.0009% oil (Anderson and von Sydow 1964).

1.2 Cultural Characteristics

The blackcurrant thrives on a wide range of soil types from river silts to medium clays; poorly drained soils should be avoided. Although often grown in reasonably acid soils (pH 5-6), the plant prefers a soil near neutral (pH 7). It is shallow-rooted and needs to be well-watered during the summer months, especially on sands and light silty soils (Tas. Dept. Agric. 1978).

Propagation is best achieved by using wood cuttings taken in April-May before leaf fall; however, cuttings taken throughout the winter grow well. During the winter, pruning, application of fertilizer and weed control need to be carried out; and in addition, the interrow sod mowed.

Bud burst occurs in mid-September with rapid extension growth. By the third week of October all the flowers are pollinated and considerable top growth has occurred. The flowers are susceptible to frost damage, from the early 'grape' stage until the last flower on the fruiting truss has set and the fruit has begun to swell, thus frost at flowering or fruit set can cause complete crop failure. Blackcurrant bushes also need to be well protected from the wind; hot northerly winds can shrivel a crop in one day while constant exposure to the prevailing cool westerlies, as in Tasmania, will stunt plant growth and result in poor crops. At the end of October the first signs of fruit drop occur. It is estimated some 50% of flowers produced do not set fruit due to wind and frost (Wilson pers. comm.[*]).

Irrigation is essential from October up to harvesting in late December. After fruit harvest, an application of ammonium nitrate and supplementary irrigation are also recommended as it has been shown (Wilson and Jones 1980) post harvest moisture stress can reduce yields

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[*] S.J. Wilson, Plant Research Officer, Department of Agriculture, New Town, Tasmania.

by up to 50% in the following year.

1.3 Botanical Relationships

The blackcurrant is a member of the order Rosales and the family Grossulariaceae, which consists of a single genus *Ribes*, with some one hundred and thirty species in temperate and alpine regions (Porter 1967). Those represented in Tasmania are:

Ribes nigrum	blackcurrant
Ribes grossularia	gooseberry
Ribes sativum	)
	)white and red currants
Ribes rubrum	)
Ribes sanguineum	flowering currant.

An authorative key to established blackcurrant varieties was constructed by Todd (1962), containing some thirty-three described varieties; these are all *Ribes nigrum* subspecies *europaeum* (Knight pers. comm.[*]). Table 1.3.1 contains a listing of some important cultivars of *europaeum* and their origin.

The majority of blackcurrant varieties bred and grown in Russia are derived from *R. nigrum sibiricum,* either selections from wild forms of *sibiricum* or from crosses with *R. nigrum europaeum*. There are also several Russian cultivars derived from *R. dikuscha;* for example Primorskie, Chempion, Golubka, Cascade; and cultivars with *europaeum, sibiricum* and *dikuscha* ancestors (Knight pers. comm.).

Related species have been used in breeding blackcurrants in Europe and elsewhere, but only relatively recently. Consort, Coronet and Crusader, which were bred in Canada are *R. nigrum* × *R. ussuriense* F_1 hybrids (Knight pers. comm.). The Ben Lomond and Ben Nevis cultivars have Consort, and consequently *R. ussuriense,* in their ancestry:

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[*] V. Knight, East Malling Research Station, Kent, England.

TABLE 1.3.1 Origin of some *Ribes nigrum europaeum* cultivars

Cultivar	Parentage	Year Raised	Year Introduced	Country	Reference
Baldwin	unknown	before 1820	-	unknown	1
Blacksmith	Baldwin × Victoria	-	1916	Britain	1
Boskoop Giant	unknown	c. 1885	1895	Holland	1
Carters Champion	unknown	before 1882	1882	Britain	2
French Black	unknown	before 1850	-	unknown	1
Goliath	Victoria o.p.	before 1920	-	Britain	1
Grahams No. 1	White Bud o.p.	1950	-	Australia	3
Hatton Black	Boskoop Giant × ? Carters Champion	1912	-	Britain	1
Kerry	ex. Black Naples	-	-	Canada	1
Lees Prolific	unknown	1860	-	Britain	1
Magnus	ex. Black Naples	-	-	Canada	1
Seabrooks Black	French Black o.p.	before 1885	1913	Britain	1
Super C	White Bud o.p.	before 1950	-	Australia	3
Victoria	unknown	-	-	Britain	2
White Bud	ex. Baldwin	-	-	Australia	4

o.p. = open pollinated
References: 1. Knight, V.H. (pers. comm.) 2. Hatton (1919) 3. Wilson, S.J.[*] (pers. comm.)
4. Wilson, S.J. and Jones, K.M. (1983)

[*] S.J. Wilson, Department of Agriculture, New Town, Tasmania.

both are (Consort × Magnus) × (Brodtorp × Janslunda) (Anderson and
Jennings 1966).

Malling Jet is a first backcross from *R. bracteosum,* and is the
only named cultivar with this species in its ancestry (Keep *et al.*
1976). Much of the material currently under selection at East Malling
Research Station, England, is derived from *R. bracteosum, R. diskuscha*
and/or *R. grossularia* (Knight 1983). An extensive breeding program
at the Scottish Crop Research Institute is also concentrating on
developing hybrids derived from the frost and cold tolerant Nordic,
Canadian and Russian wild ecotypes (Anderson pers. comm.[*]). In Scotland
species related to *R. nigrum* being used in breeding are *R. dikuscha,*
R. ussuriense and *R. bracteosum* and, less commonly, more distant spec-
ies such as *R. grossularia, R. diverisatum, R. sanguineum* and *R. niveum*
(Anderson pers. comm.).

1.4 Secretory Structures

Glandular secretory systems, despite their structural diversity,
are broadly classified on the basis of their function into either
lipophilic or hydrophilic types. Lipophilic glands, which are poorly
understood, include glands which secrete terpenes (essential oils and
resins), waxes and fats (Schnepf 1974). The simplest cases of sec-
retion are carried on by ordinary unspecialized epidermal cells to
produce the so-called glandular surfaces. Modification of epidermal
cells can occur to produce sharply defined secretory areas called
glandular spots (Haberlandt 1928).

Glandular trichomes are diverse in form and structure. A
glandular hair consists of a basal cell, a uniseriate stalk one or
several cells long, and a head of one or several secretory cells.

[*] M.M. Anderson, Scottish Crop Research Institute, Ivergowrie,
Dundee, Scotland.

The cell wall around the secretory cells is differentiated into a cuticle, cuticle layer, a petic layer and a cellulotic layer (Fahn 1974).

The secretory glands which occur in the blackcurrant *Ribes nigrum* are known as glandular scales.   These glandular scales possess more or less well developed stalks, but are characterised by the fact that the secreting elements are arranged in the form of a flattened scale, or in some cases, of an almost basin-shaped cell plate (Figure 1.4.1).   Other examples of the glandular scale are the well-known lupulin glands of hops *Humulus lupulus* (Menary and Doe 1983), the glandular trichomes of the genus *Thymus* (Figure 1.4.2), *Cannabis sativa* L. (Hammond and Mahlberg 1973, Dayanandan and Kaufman 1976) and peppermint *Mentha piperita* L. (Clark and Menary 1982).

FIGURE 1.4.1 (from Haberlandt 1928)
Young (a) and adult (b) glandular scales from a leaf of *Ribes nigrum*; z - secretory cells; v - glandular cavity formed by distension of the cuticle
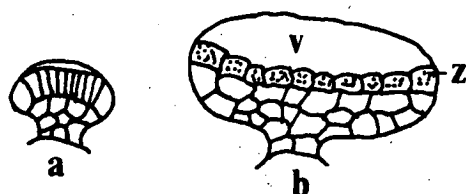
FIGURE 1.4.2 (from Fahn 1974)
Cross-section of the leaf of *Thymus capitatus* showing a secretory gland
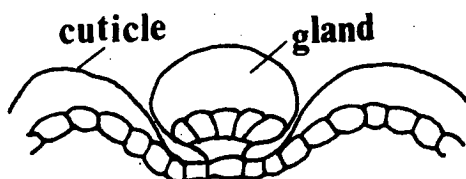
2. ESSENTIAL OILS FROM THE BLACKCURRANT

2.1 Harvesting

Harvesting the buds by hand is a laborious and time-consuming task; for example Thomas (1979) states that it takes 4 hours labour to pick a kilogram of bud material.   The odd shapes and sizes of prunings from fruit plantations will inhibit the speed of picking. Peter *et al.* (1980) state that the relatively high price of the black-currant absolute is readily explained by the high labour intensity required for the harvest of buds.   They note, as it takes a skilled worker about five hours to cut buds properly off the canes, a kilogram of absolute requires 200 hours cutting time.   There is a need to develop a mechanical or chemical method of harvesting the buds due to the high cost of labour and a low return, $20 Australian, per kilogram of bud material.

The use of chemical growth regulators has become increasingly important for many horticultural crops due to the development of once over mechanical harvesting with its associated requirements for even-ness of maturity and ease of fruit removal.   Ethephon, an example of such a chemical, at present is widely used for accelerating tomato ripening, initiation of flowering in pineapples and for advancement of peach and apple maturity.

Ethephon (tradename Ethrel) is the compound 2-chloroethylphos-phonic acid, which decomposes spontaneously in aqueous solution and in tissues to yield ethylene, a natural plant hormone (Moore 1979).   The nature of the chemical changes that occur to release ethylene are depicted in Figure 2.1.1.   Ethylene is known to regulate cell differ-entiation, in particular to trigger formation of an abcission zone in leaf or fruit petioles.

FIGURE 2.1.1 Ethephon decomposition (from Moore 1979)

$$Cl-CH_2-CH_2-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^- \ + \ H_2O \ (or \ OH^-)$$

$$\Downarrow$$

$$Cl-CH_2-CH_2-\overset{\overset{O^-}{|}}{\underset{\underset{H}{\underset{|}{O}} \ O^-}{P^+}}-O^-$$

$$\Downarrow$$

$$Cl^-+CH_2=CH_2 \ + \ H_2PO_4^- \ (or \ HPO_4^{2-})$$
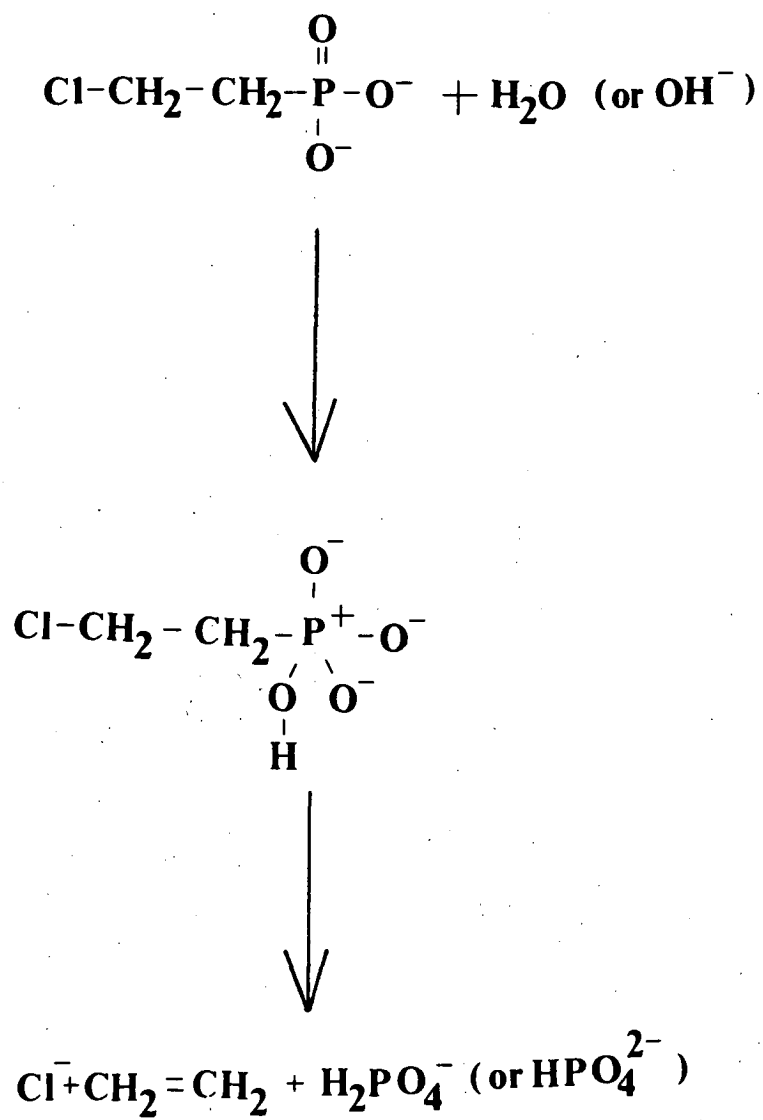
Hedberg and Goodwin (1980) noted that the natural ease of grape-berry removal varied between cultivars, and seemed to be related to the ratio of berry weight to berry/pedicel contact area. Ethephon aided grapeberry removal and was most effective in the evenings. These researchers demonstrated that absorption is mainly cuticular rather than stomatal. This less important role for stomatal entry by Ethephon is in agreement with Schonherr and Bukovac (1972), who doubt that much foliar applied chemical enters through the stomatal pores under commercial conditions.

Further, Nir and Lavee (1981) reported that uptake of C^{14} labelled Ethephon was only 19-26% of that applied to grapevine cultivars, and suggested that the presence of complete layers of cuticle and waxes on mature tissues may play an important role in restricting the rate of penetration of Ethephon into these tissues. Gentle peeling of the cuticle, from the stem of young *Hevea brasiliensis* seedlings, has been shown to increase the uptake of Ethephon ten-fold (Audley *et al.* 1976). Nir and Lavee (1981) also demonstrated that most of the labelled Ethephon they applied remained at the application site for many hours. Similarly, only slight translocation of Ethephon has been reported in other species i.e.: - apple and cherries (Edgerton and Hatch 1972)

- walnut (Martin *et al.* 1972)

- peach (Abdel-Gawad and Martin 1973; Lavee and

Martin 1974).

The rate of Ethephon decomposition has been reported to increase with increasing vapour pressure, at a constant temperature and pH, up to an optimum. The optimum vapour pressure for decomposition approx-imately doubles for each 10°C rise in temperature (Klein *et al.* 1979). From these studies it was concluded that failure to induce olive, *Olea europea* L., fruit abcission under certain environmental conditions

can be readily attributed to rapid breakdown of Ethephon at elevated temperatures and low relative humidities.

Olien and Bukovac (1978) also demonstrated that temperature had a pronounced effect on ethylene evolution, both from Ethephon treated leaves of sour cherry and Ethephon in buffered solution. These workers noted that the temperature dependence of Ethephon degradation was un-affected by pH over the range pH 3 to 7. A slow rate of decomposition for Ethephon, a dibasic acid, was reported by Biddle et al. (1978) up to a pH value of 4.5, where Ethephon is almost completely in the mono-anion form. At higher pH values the decomposition proceeds at an apprec-iable rate, particularly as the pH increases from 6 to 8 - the region in which the acid is converted from the monoanion to the dianion form.

The addition of urea or potassium iodide to a solution of Ethephon is known to cause leaf abcission to occur more rapidly in sprayed decid-uous trees (De Wilde 1971). Biddle et al. (1978) showed that this reported biological effect is not due to an increase in ethylene pro-duction, as the addition of urea or potassium iodide to a buffered Ethephon solution did not affect the rate of acid decomposition. Poovaiah and Leopold (1976) demonstrated conclusively that NH_4^+ and, to a lesser degree, K^+ increased the permeability of root and leaf tissues thus suggesting that the addition of urea or potassium iodide acts to increase the rate of uptake of Ethephon by tissues. Further-more, Poovaiah (1979) prevented the effects of Ethephon on membrane leakage without altering the rate of ethylene evolution by the addition of divalent (Ca^{++}, Mg^{++}) and trivalent (La^{+++}) cations. The monovalent cations, K^+ and Na^+, reduced leakage somewhat, but NH_4^+ was without effect in relieving the Ethephon effect.

A number of workers have successfully used Ethephon to aid the harvest of blackcurrant berries (Zandke 1977; Pankova et al. 1979 and Sandke 1980), but there are no reports in the literature investigating

the use of abcission chemicals on fruit bud attachment. From the

discussion above it can be seen that for commercial operations hand

harvesting of bud material is becoming uneconomic. The use of Ethephon

is widespread in the harvesting of many crops and its development to

harvest buds or the design of a mechanical picker will be necessary to

ensure an adequate supply of cassis concrete in the market place at

reasonable cost.

2.2 Extraction Procedures

2.2.1 Blackcurrant Buds

The buds were extracted by Glichitch and Igolen (1937) with cold

benzene to yield 2.4 - 3.0%, by weight, of a semicrystalline dark green

concrete possessing a very strong aroma. An essential oil, 0.4 - 0.5%

yield, was obtained by passing steam over the concrete. By similar

methods Chiris (1937), reported obtaining a 6% yield of an almost

colourless oil.

Latrasse (1968 and 1969) macerated the blackcurrant buds for six

days in benzene. The solution was then concentrated on a rotary evap-

oration at 40°C under reduced pressure. Latrasse gathered two fractions

at -78°C using ethanol-dry ice traps. The light fraction was obtained

by heating the residue at 100°C at 3 mmHg and the heavy fraction by

heating with a naked flame at 3 mmHg; the total yield of oil was 0.07%

by weight.

Pentane has also been used to extract oil from blackcurrant buds

as reported by Tucknott and Williams (1970) and Fridman (1971).

The former provided no further details, but the latter workers prepared

water infusions of the buds and berries, which were subsequently extracted

with pentane to obtain the aromatic fraction.

In an examination of the autoxidation of the monoterpene fraction of the blackcurrant bud essential oil, Latrasse and Demaizieres (1971), prepared samples by steam distillation of bud material to obtain a lemon yellow oil with an intense odour (yield 0.5%). They noted on exposure to air, there was a slow polymerisation to give an odourless resin. Separation of this oil into monoterpene hydrocarbon and heavy fractions was carried out by fractional distillation under vacuum. A later report by Williams and Tucknott (1973b), stated that blackcurrant buds were homogenised under approximately three times their weight of methanol and steeped in this solvent to reduce enzyme action. The methanolic solution was extracted with pentane to yield extracts organoleptically superior to those obtained using pentane alone.

In a more detailed contribution Williams (1972) extracted buds, from a mixture of blackcurrant cultivars, with pentane, ether or methanol in a specially designed extractor. Cold water was circulated around the buds to keep them at a low temperature during extraction and heat supplied to the side arm returning the solvent to the flask, rather than the flask itself. In this way prolonged heating of the bud material during extraction was prevented. Williams concentrated, extract solutions prepared by this method and that of Williams and Tucknott (1973b), recorded above, on fractionating columns packed with Fenski helicies. The extracts produced were dark green in colour, waxy and possessed a strong blackcurrant aroma. Some of the waxes were removed by cold extraction with ether at -20°C, followed by centrifugation. The volatile portion of the oil was then obtained by high vacuum distillation (10^{-3} mmHg) with cold traps cooled by liquid nitrogen.

The French researchers, Latrasse and Lantin (1974), in an examination of compositional differences between varieties, extracted the essential oil by macerating 5 or 10 g of buds in 100 mℓ of a 70% water

ethanol mixture (70% w/v). The water vapour is removed leaving a
clear ethanol distillate. Later these same workers (Latrasse and
Lantin 1976 and 1977), macerated 0.5 g of bud material in pure carbon
tetrachloride. This suspension was refluxed for ten minutes, then
cooled and the bud material filtered out; the remaining solution was
then used for gas chromatographic analysis.

2.2.2 *Blackcurrant Fruit and Leaves*

Andersson, Bosvik and von Sydow (1963) extracted the oil of
blackcurrant leaves by homogenising the leaf material with water and
distilling the solution at atmospheric pressure. Distillates from 27
successive runs were pooled and re-extracted with two portions of
diethyl ether. The ether fraction was dried over anhydrous sodium
sulphate, filtered and concentrated to yield 0.017% of oil.

In their examination of blackcurrant fruit aromas Andersson and
von Sydow (1964) mashed the fruit and then extracted this fruit pulp
with redistilled pentane. The solvent was evaporated and the residue
washed with sodium bicarbonate to remove acidic compounds. The residue
was then steam distilled at atmospheric pressure and the resulting dis-
tillate extracted with diethyl ether, dried, filtered and concentrated
to yield 0.0009% by weight of oil.

The fruit aroma has also been investigated by Spanyar *et al.*
(1964 and 1965) who subjected 1500 ml of fruit juice or the equivalent
in fresh fruit pulp to steam distillation to obtain 300 ml of distillate
in two cold traps. The aqueous distillate was saturated with sodium
chloride, then extracted four times with 30 ml of ether-pentane (2:1).
The solvent fraction was dried over anhydrous sodium sulphate then
reduced in volume by distillation under vacuum.

To investigate the lower boiling point compounds in blackcurrant

fruit, Andersson and von Sydow (1966a) mashed a fruit sample with sodium fluoride to supress fermentation and treated the mixture with depectinizing enzyme for 24 hours. The juice was extracted by a hydraulic press and separated from the bulk of the water and non-volatiles by a flash stripper distillation unit. The distillate was collected in three cold traps, recombined and concentrated further using a glass column packed with helices. The distillate was collected in a similar series of cold traps, then re-extracted with ethyl chloride, in a liquid liquid extractor, before concentration by evaporation of excess solvent.

In Finland Kussi et al. (1966) also analysed the lower boiling point components of blackcurrants using a fruit mash. The aroma was collected by running a slow stream of nitrogen through the mash at room temperature and trapping the volatiles on two cold traps. To analyse the higher boiling point compounds the mash was subjected to high vacuum distillation, with the condensate, collected in a cold trap, re-extracted using ethyl ether in a liquid liquid extractor. This distillate was then concentrated using a rotary vacuum evaporator.

The British workers, Nursten and Williams (1969a and b; Williams 1966) examined both a commercial steam distillate and fresh Baldwin blackcurrants. The commercial blackcurrant distillate was extracted with peroxide-free ether, dried over anhydrous sodium sulphate and concentrated in a microdistillation apparatus. The fresh fruit were crushed, pressed through muslin, the juice diluted with distilled water and filtered again before distillation. The distillate collected in the cold traps was purified by extraction with ether, dried and the solvent evaporated to obtain an oil.

To investigate the effect of heat on the aroma of blackcurrants von Sydow and Karlsson (1971a and b; Karlsson-Ekstrom and von Sydow

1973), prepared a mash by homogenization of a fruit sample with the same volume of distilled water. Sodium fluoride was added to supress fermentation and pectinolytic enzymes used to avoid gelatination. The mash was then heated to different temperature regimes and the headspace volatiles concentrated in a cold trap prior to injection onto a gas chromatographic column.

Recently Latrasse, Rigaud and Sarris (1982) investigated the principal aroma of blackcurrant fruit. A juice was prepared by boiling the fruit to obtain a puree which was cooled and pressed. The juice was distilled under reduced pressure at 40°C and the condensate collected in a series of three traps, the first cooled by water, the second by an ethanol/dry ice mixture and the third liquid nitrogen. The condensate, from the first two traps, was extracted with dichloromethane. The solvent fractions were combined, sodium sulphate added and the mixture concentrated before re-extraction with 2 mℓ of hexane. These French workers also prepared hydroalcoholic infusions by macerating the fruit in an ethanol/water (50% w/v) mixture and leaving them in sealed jars for three months. Each 400 mℓ infusion was then diluted with 950 mℓ of water, before extraction with 100 mℓ of Freon II in a liquid liquid extractor. Sodium sulphate was then added to the Freon fraction and the solution reduced to a 1 mℓ volume by distillation.

2.3 Identification

2.3.1 Blackcurrant Buds

The early workers Glichtich and Igolen (1937) used classical techniques of fractionation and chemical derivatisation to examine the blackcurrant bud oil. They record the buds as having a rather weak but agreeable odour that could not be attributed to any particular

chemical consituents.   The essential oil was subjected to chemical

tests which indicated that it was free from nitrogenous substances,

aldehydes and ketones; as well the following physical and chemical

consituents were determined (Table 2.3.1).

TABLE 2.3.1   Physical and chemical constants of blackcurrant
bud oil

	Glichitch & Igolen (1937)		Chiris (1937)	Schimmel & Co.
Density	D^{15}	0.879	0.8994	0.8741
Optical Rotation	D^{25}	+1°35'	D^{20} +3°20'	D^{20} +2°30'
Refractive Index	n^{20}_D	1.4870	1.4930	1.48585
Acid Index	I.A.	1.12	1.96	0
Ester Index	I.E.	7	11.2	5.6

Glichitch and Igolen, by fractionation and derivatisation identif-

ied beta-pinene, sabinene, d-caryophyllene and cadinene as being among

the components of the oil.   Approximately 85% of the oil was composed

of terpene hydrocarbons; other components were 6% terpenic alcohols

(including sabinol and terpineols), 0.25% of a mixture of phenols

(comprising phenol and beta-napthol), 0.7% of acetic acid and 0.5% of

combined higher acids.

Chiris (1937) extracted an almost colourless oil, having a black-

currant aroma with a 'styrol like' note.   Chiris also mentions that the

House of Schimmel and Company reported obtaining an oil (yield 0.75%)

from blackcurrant buds; this essence was judged to contain p-cymene by

its odour.   The physical and chemical constants of both oils are recorded

in Table 2.3.1 for comparison.

Modern analytical techniques were used by Latrasse (1968 and 1969)

to examine a rich terpenic essential oil extracted from the buds.
Analyses were carried out isothermally on two gas chromatographic
columns, the apolar Silicone SE52 and the polar Reoplex 400. The
compounds appearing on the chromatograms were characterized by their
KOVATS indices and are listed in Table 2.3.2. By similar methods,
Fridman (1971) examined a water/pentane extract of blackcurrant
buds and reported (Table 2.3.2) a composition different to that of
Latrasse. These researchers reported limonene (23.91%), as the most
abundant monoterpene; whereas in Latrasse's investigations myrcene
(34%) and caryophyllene (21.2%) were present in larger amounts than
limonene (10.9%).

TABLE 2.3.2 Composition of blackcurrant bud oil

Component	Percentage Composition	
	Latrasse (1968 & 1969)	Fridman (1971)
beta-pinene	1.0	6.66
myrcene	34.0	3.08
delta-3-carene	2.5	2.81
limonene	10.9	23.91
p-cymene and phellandrene	4.2	-
linalool	2.0	1.78
geraniol	1.3	6.51
caryophyllene	21.2	6.32
alpha-terpineol	-	3.4

The French continued investigations into blackcurrant bud oil
when Latrasse and Demaizieres (1971) examined the auto-oxidation of
the monoterpene fraction. They report that this fraction is less stable

than the heavy fraction and note that five compounds in particular;
alpha-phellandrene, delta-3-carene, beta-phellandrene, beta myrcene
and an unidentified component, are readily oxidised. These workers
reported a composition (Table 2.3.3) based on identification by infra-
red spectroscopy and KOVATS retention indices.

TABLE 2.3.3 Composition of blackcurrant bud oil

Component	Percentage Composition (Latrasse & Demaizieres 1971)
alpha-pinene	4.0
styrene	1.0
camphene	0.3
beta-myrcene	0.3
delta-3-carene	35
alpha-phellandrene	0.1
limonene	10.0
beta-phellandrene	11.0
p-cymene	1.4

Williams and Tucknott (1973b) using gas chromatography techniques
on a pentane extract of buds from mixed blackcurrant cultivars and a
single cultivar (Baldwin) have revealed over seventy components.
Evidence, based largely on mass spectral information, has indicated the
presence of 23 hydrocarbons, 5 alcohols and 4 esters, as listed in
Table 2.3.4. These workers observed that beta pinene was the major
component in the extracts obtained from mixed cultivars, unlike a com-
mercial extract or that obtained from the single cultivar, where delta-
3-carene was the major component. They also determined that estimates
of limonene were found to vary with the degree of oxidation that occurred

during the extraction procedure.

TABLE 2.3.4   Components identified in blackcurrant bud oil
(from Williams and Tucknott (1973b)

Hydrocarbons	Hydrocarbons
a methylbutene	alpha-terpinene
cyclohexene	gamma-terpinene
benzene	terpinolene
toluene	delta-cadinene
ethyl benzene	caryophyllene
camphene	beta-elemene
car-3-ene	
	Alcohols
p-cymene	
limonene	citronellol
p-methylisopropenyl benzene	linalool
myrcene	4,6-menthadien-8-ol
cis-ocimene	alpha-terpineol
alpha-phellandrene	
	Esters
beta-phellandrene	
alpha-pinene	citronellyl acetate
beta-pinene	bornyl acetate
sabinene	ethyl oleate
	methyl palmitate

An important contribution was made by Williams (1972) in a more detailed report of that noted above by Williams and Tucknott (1973b). In extracts from mixed cultivars, delta-3-carene (15%), beta-pinene (24%) and terpinoline (9%) were major components and limonene (0.8%) was of secondary importance only. Williams (1972) noted the wide differences in quantitative percentages of compounds reported by Latrasse (1969, Fridman

(1971) and his own work; he suggested they may well be varietal in origin.

Latrasse and Lantin in renewed investigations (1974, 1976 and 1977) demonstrated that the composition of the essential oil is a discriminative feature characteristic of each cultivar. These researchers identified six monoterpene phenotypes based on the percentage composition of five common monoterpene hydrocarbons; sabinene, delta-3-carene, limonene, beta-phellandrene and terpinolene. Seven sesquiterpene phenotypes were also determined, based on beta-caryophyllene, alpha-humulene, alpha-elemene and four unidentified components.

Lewis et al. (1980) identified pulegone and an unnamed compound, of molecular weight 186, to be present in blackcurrant oil for the first time. The unnamed compound was noted to have mass spectral and gas chromatographic characteristics similar to those of 8-mercapto-p-menth-3-one, which is responsible for a catty note in Buchu oil.

More recently at the 8th International Congress of Essential Oils, Peter et al. (1980) attempted to complete the characterisation of the volatile portion of the concrete by cochromatographic techniques. This offered 2 methyl 2 mercapto n-butyl pentan-4-one* as a possibility for causing the catty note of blackcurrants; this however was not confirmed by any other technique. These workers examined both the non-saponifable fraction, where they reported a number of sterols (Table 2.3.5), and the acid fraction (principally Hardwickic and 0 acids). Peter and his co-workers noted that the monoterpene group comprised 50-60% of the volatile fraction; this group was completely identified (Table 2.3.5), confirming the results of Latrasse and Lantin (1977). The heavier fraction was dominated by beta-caryophyllene and terpinene-4-ol as reported by Williams (1972).

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* this chemical not mentioned in manuscript but presented as part of verbal presentation.

TABLE 2.3.5 Consituents of blackcurrant bud absolute
             (from Peter *et al.* (1980))

Sterols	%	Monoterpenes	Sesquiterpenes
campesterol	1.9	alpha-pinene	octene-3-ol
stigmasterol	2.1	beta-pinene	alpha-copaene
beta sitosterol	88.4	sabinene	beta-elemene
delta-5-avenasterol	1.0	delta-3-carene	caryophyllene
delta-7-stigmasterol	0.5	myrcene	unknown X
delta-7-avenasterol	6.1	alpha-terpinene	terpinene-4-ol
		limonene	alpha humulene
		beta-phellandrene	citronellyl acetate
		cis-ocimene	unknown Y
		gamma-terpinene	unknown Z
		trans-ocimene	unknown V
		octanone-3	
		p-cymene	
		terpinolene	
		unknown	

2.3.2 *Blackcurrant Fruit and Leaves*

The composition of the essential oil of blackcurrant leaves was examined by Andersson *et al.* (1963). They used gas chromatography, infra-red spectrometry and mass spectrometry to identify the following components (Table 2.3.6). It is interesting to compare these results with those of Latrasse (1969).

Ten common components have been identified. Latrasse proposes the high levels of myrcene 34%, limonene 10.9%, and caryophyllene 21.2% observed in the bud essence as compared with the leaf oil (0.6, 3.3 and 16.8% respectively), are due to different stages of metabolism.

TABLE 2.3.6 Composition of oil from blackcurrant leaves
(from Andersson, Bosvik and von Sydow (1963))

Component	Percent Component	Component	Percent Component
alpha-pinene	*1.5	1-methyl-4-isopropyl	1.3
myrcene	*0.6	benzene	
oct-1-en-3-ol	6.3	linalool	3.6
delta-3-carene	*19.2	terpinen-4-ol	0.6
p-cymene	*1.5	methyl salicylate	1.6
m-cymene	1.5	geraniol	*6.0
limonene	*3.3	citronellyl acetate	*0.9
beta-ocimene	1.6	caryophyllene	*16.8
beta-phellandrene	*2.4	humulene	2.0

* Components common with Latrasse (1969) refer: Table 2.3.2

Andersson and von Sydow (1964 and 1966a) in Sweden investigated the fruit of Brodtorp blackcurrants and used retention data on two gas chromatography columns, a 10% DC 200 silicone column and a 10% SAIB + 5% Quadrol column, together with infra-red spectroscopy and mass spectrometry data for identification. A list of components identified and their relative abundance are contained in Tables 2.3.7 and 2.3.8.

Spanyar et al. (1964 and 1965) investigated the headspace of blackcurrant fruit pulp and reported the presence of ethanol, butyl alcohol, amyl alcohol, ethyl acetate, butyl acetate and ethyl caprylate. The British researchers, Nursten and Williams, examined both a commercial steam distillate (1969a) and fresh Baldwin blackcurrants (1969b), relying on retention data on three gas chromatography columns and infra-red spectroscopy for identification. Investigation of the commercial distillate revealed over 150 components. The twenty components listed in Table 2.3.9 were positively identified; and the thirty-four in Table 2.3.10

TABLE 2.3.7 Higher boiling point compounds in blackcurrant fruit
(from Andersson and von Sydow (1964))

Hydrocarbons	Percentage Composition	Alcohols	Percentage Composition
m-Cymene	0.4	Citronellol	1.4
p-Cymene	0.9	alpha-Terpineol	0.5
Myrcene	1.4	Terpinen-4-ol	3.3
cis-beta-Ocimene	2.6	p-cymen-8-ol	2.9
trans-beta-Ocimene	2.9	cis-hex-3-en-1-ol	0.1
gamma-Terpinene	0.7	oct-1-en-3-ol	0.3
beta-Phellandrene	5.3		
Terpinolene	4.9	Esters	
Limonene	3.8		
Car-3-ene	25.9	Citronellyl acetate	2.5
alpha-Pinene	7.0	Methyl benzoate	0.1
Camphene	0.6	Ethyl benzoate	0.2
Caryophyllene	11.6	Methyl salicylate	0.5
Humulene	0.2		

Carbonyls

Benzaldehyde   0.1

TABLE 2.3.8 Lower boiling point compounds in blackcurrant fruit
(from Andersson and von Sydow (1966a))

Alcohols	Carbonyls
methanol	acetaldehyde
ethanol	hexanal
propanol	acetone
2-methyl propanol	2-butanone
butanol	2,3 butandione
3-methyl butanol	
pentanol	Esters
hexanol	
2-butanol	methyl acetate
2-pentanol	ethyl acetate
2-methyl-3-buten-2-ol	butyl acetate
3-methyl-2-buten-1-ol	ethyl butyrate
1-penten-3-ol	pentanal
2-methyl butanol	

Hydrocarbons

Styrene

Miscellaneous

1,8 Cineole

tentatively identified. Examination of an essence prepared from fresh Baldwin fruit revealed the presence of 24 components (Table 2.3.11).

The results of Nursten and Williams, from fresh blackcurrants (1969b) and a commercial distillate (1969a), differ from those of Andersson et al. (1964, 1966a and b). For example, Andersson and von Sydow (1964), found that car-3-ene and caryophyllene constituted a large proportion of the less volatile components whereas Nursten and Williams found these to be present in minor quantities only. On the other hand alpha terpinene was not detected by the Swedish workers.

Differences between the Swedish and British work could be explained by varietal differences, differences in soil and climate in which the bushes were grown, time of harvest and method of storage. However, Nursten and Williams (1969b) consider method of extraction is the most probable cause, particularly in the case of the terpenes.

Andersson and von Sydow (1964) showed terpenes to be present in the high boiling point fraction prepared using n-pentane extraction followed by steam distillation. In their more recent paper (1966a) the Swedes used an extraction process similar to that of Nursten and Williams (1969b) but no mention is made of terpene hydrocarbons.

Nursten and Williams (1969b) found difficulty in explaining why certain terpene hydrocarbons were detected in their work. They suggested that since compounds they identified, such as phellandrenes and cymenes, could be easily formed by rearrangement and car-3-ene and caryophyllene (identified as major components by the Swedes), could not, the Swedish methods of extraction caused fewer unwanted chemical reactions.

Latrasse (1969) also examined an essence of fresh blackcurrant fruit and reported the presence of a number of aldehydes, esters and alcohols (Table 2.3.12). More recently Latrasse, Rigaud and Sarris

TABLE 2.3.9 Components positively identified in a commercial black-
currant distillate
(from Nursten and Williams (1969a))

Hydrocarbons	Alcohols	
alpha-terpinene	methanol	2 methyl but-3-en-2-ol
beta-phellandrene	ethanol	n-hexanol
gamma-terpinene	n-propanol	cis-hex-2-en-1-ol
p-methyliso-propenylbenzene	n-butanol	trans-hex-2-en-1-ol
	isobutanol	terpinen-4-ol
Carbonyls	isopentanol	
2-hexenal	**Esters**	
Miscellaneous	ethyl n-butyrate	
1,8 cineole	methyl n-hexanoate	
	methyl benzoate	

TABLE 2.3.10 Components tentatively identified in a commercial black-
currant distillate
(from Nursten and Williams (1969a))

Hydrocarbons	Carbonyls	Alcohols
alpha-pinene	ether	pent-1-en-3-ol
camphene	acetaldehyde	2-pentanol
myrcene	2-butanone	linalool
sabinene	3-methylbutan-2-one	2 ethyl butanol
alpha-phellandrene	benzaldehyde	2-hexenal
limonene	butanal	oct-1-en-3-ol
p-cymene		citronellol
beta-ocimene		4-octanol
		geraniol
Esters		p-cymen-8-ol
methyl acetate		pent-4-en-ol
isopropyl acetate		
methyl n-butyrate		
isobutyl acetate		
isopentyl acetate		
n-butyl acetate		
n-pentyl acetate		
methyl salicylate		
ethyl-n-hexanoate		

TABLE 2.3.11 Components identified in fresh Baldwin blackcurrants (from Nursten and Williams (1969b))

Hydrocarbons	Carbonyls
toluene	trans-2-hexenal
myrcene	2-hexanone
alpha-phellandrene	
gamma-terpinene	Alcohols
caryophyllene	
	ethanol
Esters	n-butanol
	iso-butanol
methyl acetate	n-hexanol
ethyl acetate	trans-hex-2-en-1-ol
isopentyl acetate	2-ethylhexan-1-ol
ethyl n-butyrate	terpinen-4-ol
methyl n-butyrate	alpha-terpineol
methyl-n-hexanoate	
ethyl n-hexanoate	Other
methyl n-benzoate	
	1,8 cineole

TABLE 2.3.12 Components identified in blackcurrant fruit (from Latrasse (1969))

Esters	Aldehydes	
butyl formate	ethanal	
ethyl formate	pentanal	
ethyl acetate		
ethyl valeriante	Alcohols	
isoamyl butyrate		
	methanol	terpinene-4-ol
	ethanol	alpha-terpineol
	isobutanol	citronellol
		p-cymen-8-ol

TABLE 2.3.13   Components recently identified in blackcurrant fruit
(from Latrasse, Rigaud and Sarris (1982))

Alcohols

fenchyl alcohol
limonene-4-ol
cis and trans piperitol
ledol
oct-2-ene-1-ol
isopropyl-4-cyclohexanol
hexan-3-ol
3 methyl hexan-2-ol
heptanol
nonanol
cumin alcohol
cyclohexanol
phenyl ethanol
furfuryl alcohol

Esters

neryl acetate
geranyl acetate
4-acetoxy-1,8-p-menthadiene
cis methyl jasmonate

Various

methoxybenzyl pyrazine
anhydride of 2,3 dimethylmallic
   acid
carvacrol
o-cresol
phenol
allyl phenol
vanilline

Carbonyl Compounds

alpha-ionone
cumin aldehyde
1-dimethyl-4-cyclohexen-3-yl
methyl ketone
3-cyclocitral
benzaldehyde
oct-1-ene-3-one
hept-2-ene-1-al
2-octenal
2-nonenal
2-decenal
heptadien-2-4-al
nonadienal
camphor
damascenone
tiglaldehyde
umbellulone
carvone
piperitone

Lactones

gamma-nonalactone

(1982) reported on the aroma of the berries.   They prepared an
extensive list of compounds previously reported in blackcurrants as
well as identifying new compounds (Table 2.3.13) by retention indices
on gas chromatography columns or mass spectral data.

2.4 Organoleptic Evaluation

In spite of modern analytical instruments the Fragrance and Flavour industry depends upon the perfumer and his assessment of odour quality as the final arbiter of fragrance value.

2.4.1 *Blackcurrant Buds*

In an important contribution Williams (1972) undertook the first reported organoleptic assessment of blackcurrant bud oil. The majority of odour comments were characteristic of what one would expect from terpenes, being musty, pinelike or reminiscent of turpentine in the lower boiling point region, and spicy aromatic in the higher boiling point region.   In this study, using a Carbowax 20 m column, Williams stated that no particular region could be associated with the 'catty' blackcurrant aroma, although some peaks in the terpene hydrocarbon region did have a minty character.   Other peaks had green and cucumber aromas, both of which Williams considered could contribute to the 'catty' note of the buds.   One of the latter high boiling point regions could be associated with the heavy, sweet smell of commercial blackcurrant flavours.   Using a non-polar SE30 packed column, Williams (1972) was able to detect the 'catty' blackcurrant aroma and associate it with relatively low boiling components, mainly monoterpene hydrocarbons.   Since the aroma could not be detected at all on the Carbowax 20 m column Williams suggested that the odour may be due to a compound with a low threshold level which is absorbed on the column at low concentrations.

Various sulphur containing compounds with similar odours have been suggested as possibilities for the 'catty' constituent. These include compounds reported from Buchu oil by von Sundt *et al.* (1971) and Kaiser *et al.* (1975). A catty note from Buchu was identified by von Sundt

et al. (1971) and associated with the compound (+) menth-on-8-thiol (Figure 2.4.1). They also reported a synthesis of menth-on-8-thiol based on pulegone. Kaiser *et al.* (1975) recorded the full fruity character of typical of blackcurrants and associated it with the same compound. In addition, Lewis *et al.* (1980) identified pulegone and a compound of molecular weight 186 with mass spectral and chromatographic characteristics similar to those of p-menth-on-8-thiol in black-currant bud oil. However, these workers made no mention of any aroma associated with this compound.
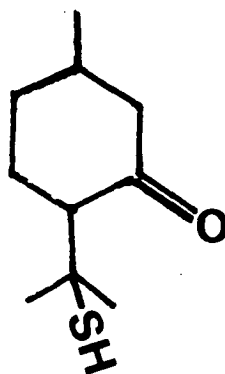
FIGURE 2.4.1 p-menth-on-8-thiol

Peter *et al.* (1980) state that the monoterpenes present in the volatile portion of the cassis absolute cannot account for the typical and potent aroma of blackcurrants. Further, these researchers found that the monoterpene fraction, when isolated by liquid chromatography lacked the characteristic odour completely. They detected this note clearly in the more polar and extremely complex mixture eluted after beta-caryophyllene.

2.4.2 *Blackcurrant Fruit*

The fruit aroma has undergone a more extensive assessment than that obtained from blackcurrant buds. Andersson and von Sydow (1966a)

noted that a concentrate of low boiling volatiles from blackcurrant

juice had a very strong odour without any resemblance to that of the

fresh fruit. A powerful 'green' odour note was observed, possibly

due to the presence of cis-3-hexen-1-ol. In earlier work, Andersson

and von Sydow (1964) reported that the odour of an oil containing only

high boiling point components (greater than 150°C) was reminiscent of

fresh blackcurrant fruit, although some of the odour 'top notes' were

apparently missing.

Nursten and Williams (1969b) reported that when 98 compounds

they identified in blackcurrants were recombined their odour seemed to

be overwhelmed by the 'green' note of the hexenols and octenol. How-

ever, the addition of these compounds to a commercial blackcurrant

essence gave a fresher blackcurrant note.

In the early seventies, von Sydow and Karlsson (1971b) developed

an odour quality assessment technique to examine the effect of heating

on blackcurrant fruit aroma. Odour qualities reported to increase on

heating were those that contributed undesirable odours to fresh fruit;

for example 'cooked aroma', 'sharp', 'sickly' and 'burnt'. Odours

reported to decrease on heating are generally those more desirable in

fresh fruit; for example 'floral', 'green' and 'fruity'. No mention

was made of a 'catty' aroma. Later work (Karlsson-Ekstrom and von

Sydow 1973) attempted to associate the observed aroma changes with

particular components. Data presented in this study showed that the

unpleasant odour qualities are positively correlated with compounds

observed to increase on heating (dimethyl sulphide and some aliphatic

aldehydes: ethanal, propanal, 2 methyl propanal, and 2-methyl butanal);

and negatively correlated with compounds observed to decrease on heat-

ing (mainly terpenes: delta-3-carene, caryophyllene, beta-phellandrene,

beta-pinene, sabinene, cis-beta-ocimene, trans-beta-ocimene and terpinolene).

More recently Latrasse *et al.* (1982) have reported an extensive analysis of the main and secondary aromas of blackcurrant berries. They report detecting the twenty elementary odours listed in Table 2.4.1, which are characterised into two groups. The first, located in the light fraction, contains the six compounds which are necessary to obtain the characteristic blackcurrant aroma. If any one of these is omitted, the aroma mixture is incomplete, and one is unable to recreate the blackcurrant aroma. The second group contains many floral notes which affect overall aromatic quality.

TABLE 2.4.1 Principal and secondary odour notes of blackcurrant fruit
(from Latrasse *et al.* (1982)

Principal Odour	Secondary Odour
1. butter (diacetyl)	7. shells of green peas (methoxy iso-propyl pyrazine)
2. fruit (ethyl butyrate)	8. floral (linalool)
3. Cats urine (not identified)	9. Wine cork
4. Mushroom	10. Faint odour (terpine-4-ol)
5. Mushroom	11. floral (methyl acetophenone)
6. Balsam (eucalyptol)	12. faint odour (alpha terpineol)
	13. Roots (limonen-4-ol)
	14. dung (phenolic compound)
	15. floral (citronellol)
	16. floral (geraniol)
	17. jam (damascenone)
	18. lactone odour
	19. jam
	20. conifer odour (warm vapour) (composed of MW220)

2.5 Varietal Differences

Andersson and von Sydow (1966b) presented data on the essential
oil extracted from the fruit of six botanical varieties of blackcurrants.
The varieties were: Brodtorp, Silvergieters Zwarte, Wellington XXX,
Cotswold Cross, and two hybrids, Wellington XXX x Brodtorp and Cotswold
Cross x Brodtorp.

Differences in oil content were found to occur, Cotswold Cross
having roughly three times as much essential oil as the other varieties
(Table 2.5.1).   Silvergieters Zwarte and Wellington XXX which are of
similar botanical origin were found to possess monoterpene fractions
of almost identical composition (Table 2.5.1).   In comparison both
Brodtorp and Cotswold Cross contain much more caryophyllene. Brodtorp
is characterised by low concentrations of gamma-terpinene and terpinen-
4-ol.   Cotswold Cross has relatively high concentrations of these
compounds and a low concentration of delta-3-carene (Andersson and von
Sydow 1966b).

These workers also noted that these characteristic features can
be easily traced in the hybrids investigated.   For example, Wellington
XXX x Brodtorp has a high content of gamma-terpinene and terpinen-4-ol
from Wellington XXX and of caryophyllene from Brodtorp.

Latrasse and Lantin (1974) examined eighteen varieties of black-
currants and classified them into three distinct families based on
their monoterpene hydrocarbon composition.   Sabinene, delta-3-carene,
beta-phellandrene and terpinolene were found to be the discriminatory
components.   Phenotype A is defined by a high sabinene content only
(75%).   Phenotype B has sabinene (58%) and two other major components,
delta-3-carene (20.7%) and terpinolene (10.1%); Phenotype C has delta-
3-carene (35.9%) as the principal component with other components
beta-phellandrene (20.6%) and terpinolene (15.6%).   The classification

TABLE 2.5.1 Quantitative data for some major components in the essential oil of six varieties of blackcurrants
(from Andersson and von Sydow 1966)

Variety	ppm essential oil in fruit	% terpenes in oil					
		delta-3-carene	gamma-terpinene	terpino-lene	terpinen-4-ol	citronellyl acetate	caryo-phyllene
Brodtorp	13	18	0.6	5.7	1.1	1.5	12
Wellington XXX	10	14.5	3.5	5.0	12	1.2	4.9
Silvergieters Zwarte	11.5	12.5	2.9	4.3	10	1.2	5.1
Cotswold Cross	31	<2.0	7.4	1.5	26	1.3	10.5
Wellington XXX x Brodtorp	10.5	8.6	3.7	3.4	14	1.9	12
Cotswold Cross x Brodtorp	12	<1.5	3.5	0.6	13	1.0	13

of varieties is shown in Table 2.5.2. Pheno-type C, which includes

the traditional French varieties Noir de Bourgogne and Royal de Naples,

was considered organoleptically superior to either phenotype B (includes

Baldwin) or phenotype A.

A further examination of the following varieties; Black Reward,

Brodtorp, Golubka, Noir de Bourgogne, P9-8-38 (Consort x Consort),

Rosenthal, Silvergieter and their hybrids was undertaken by Latrasse

and Lantin (1976 and 1977). These workers identified three new mono-

terpene phenotypes as well as six principal sesquiterpene phenotypes.

They tabulated the composition of six monoterpene phenotypes, including

three previously identified in the earlier paper (1974). This data is

reproduced as Table 2.5.3.

TABLE 2.5.2 Classification of varieties by phenotypes
(from Latrasse and Lantin (1974))

Phenotype A	Phenotype B	Phenotype B
sabinene (75%)	sabinene (58%)	delta-3-carene (35.9%)
	terpinolene (10.1%)	beta-phellandrene (20.6%)
	delta-3-carene (20.7%)	terpinolene (15.6%)
Mendip Cross	Silvergieter	Noir de Bourgogne
Golubka	Rosenthal	Royal de Naples
Tor Cross	Baldwin	Brodtorp
Cotswold Cross	Wellington XXX	
Malvern Cross	Goliath	
M 59-3	Victoria	
	Consort	
	Tenah 8	
	Davidson's 8	

TABLE 2.5.3 Composition of monoterpene phenotypes
(from Latrasse and Lantin 1976 and 1977)

		Major constituents (%)				
		sabinene	delta-3-carene	limonene	beta-phellandrene	terpinolene
Phenotypes	A	100				
	B	60.8	27.5			7.6
	C		57.5	8.1	25.2	14.0
	D		72.5			19.7
	E			25.0	71.5	
	F	73.6		7.4	18.9	

Latrasse and Lantin (1976 and 1977) identified seven major sesquiterpene peaks by their retention volume relative to beta-caryophyllene, a common oil component. The composition of the six sesquiterpene phenotypes is contained in Table 2.5.4. These workers proposed an hypothesis whereby three major genes are accepted as controlling monoterpene synthesis. These genes are:

T_1 - sabinene

T_2 - delta-3-carene and terpindene

T_3 - beta-pinene, limonene and beta-phellandrene.

Under this hypothesis, monoterpene phenotype A for example would have the genotype: $T_1T_1t_2t_2t_3t_3$. They further proposed that the synthesis of the sesquiterpene, beta-caryophyllene, is under independent genetic control, whereas the components 2,4 and 3,4 linked with 7 could be dependent on a single allele pair.

Recently Latrasse *et al.* (1982) in an extensive examination of the principal aroma of blackcurrant fruit, reported that extracts of

TABLE 2.5.4 Composition of sesquiterpene phenotypes
(from Latrasse and Lantin 1976 and 1977)

component[2]	Major Constituents (%)						
	1	2	3	4	5	6	7
retention time	0	1.14	1.20	1.25	1.37	1.78	1.91
I			23.8	76.2			
II_1	59.2					41.7	
II_2	39.4					60.6	
III_1	53.7		10.7		33.5		2.0
III_2	31.0		10.5				2.0
IV	60.6	15.1		15.1			9.0
V	42.1		3.6	23.1	28.4		2.7
VI	57.9	18.1		9.4		10.1	4.3

(left side label: Phenotypes)

Note 1 - retention times are relative to beta-caryophyllene

Note 2 - 1: beta-caryophyllene,   3: alpha-Humulene,

6: alpha-Elemene,   2,4,5,7, not identified.

Noir de Bourgogne and Royal de Naples are richer in aroma than other varieties studied.   The components and odours associated with them are reported in Table 2.5.5.   There is an abundance of terpinen-4-ol in the extracts from Cotswold Cross, Malvern Cross and Davidson's Eight. These varieties are offspring from crosses with Baldwin as a common parent, suggesting that abundance of terpinen-4-ol is an hereditary character (Latrasse *et al.* 1982).   Noir de Bourgogne and Royal de Naples are rich in diacetyl, ethyl butyrate and eucalyptol, components important to blackcurrant aroma.

TABLE 2.5.5 Amounts of some components in hydroalcoholic infusions prepared from various blackcurrant varieties (from Latrasse et al. 1982)

Principal Odour	Compound	Variety									
		Noir de Bourgogne	Royal de Naples	Tenah 4	Cotswold Cross	Giant Boskoop	Malvern Cross	Tasma	Golubka	Wellington XXX	Davidson's Light
butter	diacetyl	3.7	1.5	1.1	0.3	0.9	0.0	0.0	0.0	0.0	tr
fruit	ethyl butyrate	8.6	8.7	5.2	4.6	7.3	0.6	0.4	0.8	7.1	0.2
cats urine	not identified	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Balsam	eucalyptol	2.4	3.0	1.0	0.2	0.3	0.3	0.2	0.0	0.4	0.2
Secondary Odour											
green pea	methoxyisopropyl pyrazine	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
floral	linalool	0.6	0.6	1.0	0.3	0.3	0.4	0.2	0.2	0.0	0.2
floral	methylacetophenone	1.2	nd	nd	nd	nd	nd	nd	nd	nd	nd
roots	limonen-4-ol*	1.0	0.07	1.0	0.04	0.4	0.07	0.7	0.3	0.5	0.4
salicylic ester	methyl salicylate	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
rose	geraniol	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
jam	damascenone	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
conifer	not identified	17.0	12.0	1.7	0.4	1.8	2.8	0.0	0.0	0.0	0.0
	terpinen-4-ol*	3.5	18.0	3.5	87.5	21.0	64.0	7.3	17.5	37.7	67.0
	alpha terpineol*	2.2	5.2	0.7	2.8	0.3	1.8	1.9	0.8	0.7	2.8

* Limonen-4-ol and alpha terpineol levels were by GC on CW20M.
The compounds underlined appear to be varietal characteristics
n.d. no amount

2.6 Commercial Significance of Blackcurrant Oil

Thomas (1979) estimates the annual world production of Cassis Absolute (the blackcurrant Bud Oil), is estimated to be in the order of 200-600 kilos, and is currently priced at $1000 Australian per kilo c.i.f. Europe and U.S.A.

The principal production source of the Absolute is Grasse (France), and the principle sources of bud material are the blackcurrant fruit plantations of France and England.

The world's leading producer, accounting for perhaps 80% of production, is Cammili Albert and Laloue (CAL) of Grasse, a subsidiary of Pfizer. CAL Cassis Absolute sets the industry standard for quality (Thomas 1979).

No reliable information is available on the main markets for Cassis Absolute according to Thomas (1979), but it is likely that it follows the geographic distribution of the Fragrance and Flavour (F/F) industry but skewed more to American and Western European markets.

Cassis Absolute seems to be rarely, if ever, traded by dealers as it seems most of the business has been developed directly between CAL and other F/F houses through the Pfizer selling network (Thomas 1979).

Cassis Absolute has been on the market for about 20 years and for most of that time it has generally been in short supply. Thomas (1979) quotes CAL as saying that demand is growing strongly and can be maintained at about 20% per annum if the price can be kept at present levels in real terms.

CAL (1979) report the classic use of Cassis Absolute is to reinforce and modify natural or artificial Blackcurrant flavour, but more recently it has found applications in fragrances where remarkable

results are observed.

Dumont (1941) refers to the rarer essential oils and their uses in perfumery, noting that the oil of the blackcurrant buds is specially suited for scenting of lipsticks. Dumont also records the oil as giving excellent nuances in Chypre, Fougere, Ambre, Lierre and perfumes with an oriental scent after the manner of Crepe de Chine and Goya. A local retailer, St. Cloud Perfumery, quotes Crepe de Chine at $8.50 per 1/8 fluid ounce and considers the perfume to be in the medium to high price bracket (pers. comm.).

Dumont (1941) states, "where price plays no part, the extract bud oil is also to be recommended for soap perfume oils, as truely remarkable effects can be secured with it."

J. and E. Sozio, a French company, estimates in 1983 the market to be 500-1000 kg of absolute per year in the perfumery industry, with use in flavouring being several orders of magnitude larger (pers. comm.).

Pernod-Ricard purchase buds from farmers in France and extract directly with ethanol. This alcoholic extract is incorporated directly into the French Liquer "Cassis de Dijon" (pers. comm.).

3. ENVIRONMENTAL EFFECTS ON CROP GROWTH

3.1 Planting Density

In studying the effect of plant density on the yield of economically important parts of plants, it is essential to differentiate between the effects of increasing the number of plants per unit area, with plants arranged in a rectangular and even manner, and the effect of changing the pattern in which a given number of plants per unit area are arranged (Bleasdale[*] pers. comm.).

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[*] J.K.A. Bleasdale, National Vegetable Research Station, Wellesbourne, Warwickshire, England.

The most important and obvious effect of increasing the plant density whilst retaining, say, a square pattern of plant arrangement, is initially to increase the yield per unit area. This increase is, at first, directly proportional to the increase in population, but as the plants increasingly 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 further increase in yield (Bleasdale pers. comm.).

There are many reports in the literature describing these asymptotic relationships (Figure 3.1.1) for plant parts of commercial interest; Bleasdale (1967a) for carrots and radishes; Nichols et al. (1973) for tomatoes and Frappell (1973) for onions. With some crops the yield rises to a maximum with increasing plant density and then declines at higher densities (Frappell 1979). This form of relationship is known as parabolic (Figure 3.1.2) and is also well reported in the literature; Bleasdale and Thompson (1966) using parsnips; Frappell (1968) using red beet and Nichols (1974) using sweetcorn.
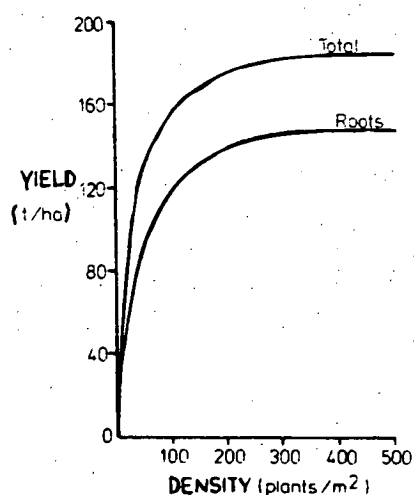
FIGURE 3.1.1 Yield-density relationship for total yield and root yield of carrots (from Frappell 1979)
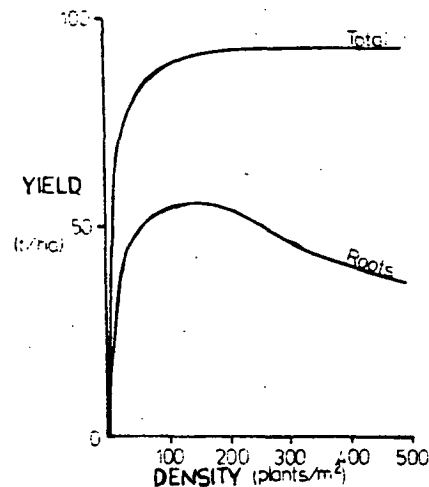
FIGURE 3.1.2 Yield-density relationship for total yield and root
yield of red beet
(from Frappell 1979)

At the outset, it is clear that the density-dependant effects on

yield are due to competition between adjacent plants for the necessary

natural resources. The basic assumption is that a plant located at a

given site is constrained to draw nutrients only from its immediate

vicinity. This 'influence zone' may be larger than the size of the

actual plant and would have an irregular shape both on the surface and

into the ground. It is not a hypothetical region and can be mapped

by tracer experiments (Pant 1979).

While it is generally accepted that the yield-density relationship

for total biological yield is asymptotic, it should be recognized that

the relationship for a plant part may be asymptotic or parabolic, and

that the latter form may range from near asymptotic to steeply parabolic

(Frappell 1979). When using a yield-density relationship to modify

a production system for a particular objective, then it is important

that the form of the relationship be established for the crop in

question. In practical terms, if the objective is to achieve maximum

yields of the desired quality per unit area, then there is an optimum

spacing for a crop which will provide sufficient plants to cover the

ground surface as quickly as possible with leaves, yet few enough plants to permit each to develop the required quality characteristics (Frappell 1979).

The mathematical function that has been most commonly used for the analysis of plant density experiments is the reciprocal equation proposed by Bleasdale (1966):

(1) $\quad \dfrac{1}{W}^{\theta} = \alpha + \beta\rho$

where W is the weight per plant, ρ is the plant density and α, β and θ are parameters of the model. When $\theta=1$, an asymptotic relationship is described and when $\theta<1$, the relationship is parabolic.

Equation (1) is a simplified version of the equation proposed earlier by Bleasdale and Nelder (1960):

(2) $\quad \dfrac{1}{W}^{\theta} = \alpha + \beta\rho^{\phi}$

which is very similar except that it introduces another parameter ϕ.

A further equation proposed at the same time is that of Holliday (1960a and b):

(3) $\quad \dfrac{1}{W} = \alpha + \beta\rho + \gamma\rho^{2}$

where the symbols have a similar meaning, with the exception of γ replacing θ as the third parameter of this model. With this equation the form of the relationship is asymptotic when $\gamma=0$ and parabolic when $\gamma>0$.

When the yield-density relationship is asymptotic, then all of these relationships become identical:

(4) $\quad \dfrac{1}{W} = \alpha + \beta\rho$

Such an equation is based on a linear relationship between the reciprocal of the yield per plant, and density (Figure 3.1.3). These equations
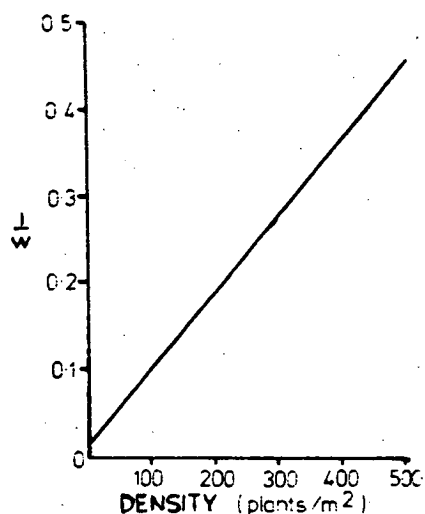
FIGURE 3.1.3 Relationship between the reciprocal of total dry
weight per plant and density
(from Frappell 1979)

have been comprehensively reviewed by Willey and Heath (1969) who

conclude to describe yield-density relationships realistically it is

desirable to use those equations, such as (1), which have a better

biological foundation and have proved the most satisfactory in practice.

Gillis and Ratkowsky (1978) compared models (1) and (3) concluding

that, although both models described the yield-density relationship

equally well in practice, equation (3) had better statistical properties

than equation (1) when fitted using least squares. For example, the

estimators of the parameter of equation (3) were less biased and closer

to being normally distributed than those of equation (1). Further dis-

cussion of the statistical properties of least squares estimators, with

reference to yield-density models appears in Ratkowsky (1983).

When using the asymptotic relationship it is possible to give a

simple biological interpretation to the parameters α and β. As density

tends towards zero, the value of weight per plant tends to $\frac{1}{\alpha}$, which is

considered to be a measure of the genetic potential of a crop in a

particular environment. On the other hand, as density tends towards

infinity, the yield per unit area approaches the asymptotic value of $\frac{1}{\beta}$

which is considered to be a measure of the potential of the environment. For the parabolic relationship it is more difficult to give a biological interpretation to these two parameters (Frappell 1979).

In order to obtain the data required to establish the form of the yield-density relationship it is necessary to study the effect on yield of a large range of plant densities. Conventional randomized block designs involve carrying out experiments of enormous size, in which more than half the plants would be guards. Such large experiments are not statistically desirable and often not practical with the resources available; particularly with numbers of perennial plants required (Bleasdale 1967b).

Crops planted in rows which radiate from a point, with the distance between plants along a radius approximately equal to the distance between radii at that point, enable a large range of plant densities to be grown in a small area. Further, guard plants would only be needed around the outer edge of a group of plants arranged in this systematic manner (Bleasdale 1967b). Nelder (1962) developed a series of designs for spacing experiments based on these principles, using grids which could be defined by the intersection of sets of parallel or concurrent straight lines and arcs of concentric circles. Bleasdale (1967b) provides an expanded discussion and presentation of all steps necessary for calculating the dimensions of these designs. The fan design described by Nelder (1962) and Bleasdale (1967b) has been used to study yield-density relationships of intercropped sorghum and soybeans (Wahua and Miller 1978) as well as the effect of spacing on blackcurrant fruit yields (Nes 1979).

3.2 Light Interception and Utilization

Studies of light interception provide the scientific basis for the practical management of orchard canopies, i.e. for the choice of tree size, number per hectare and arrangement and pruning, so as to

optimize the production of assimilate and its conversion into economic yield (Jackson 1980a). Two distinct objectives are involved: the first is to find ways of maximizing light interception by the trees, as light energy falling on the grass in the alleyways is obviously not producing fruits; the second is to optimize light distribution within the canopy and interception of light by different parts of the canopy, so as to maximize the efficiency of light utilization in photosynthesis, fruit bud formation and development (Jackson 1980a).

The dry matter yields of many crops appear to be directly proportional to their interception of radiant energy; e.g. maize (Duncan et al. 1973), cereals (Gallagher and Biscoe 1978) and sugar beet (Monteith 1977). Recent studies (Palmer and Jackson 1977; Jackson 1978) indicate that the same holds true for both dry matter and fruit (economic) yield of apple orchards at least when comparing young orchards of the same rootstock/scion combination managed in a consistent way but growing at a range of densities.

In annual crops, the greatest loss of light interception occurs at the beginning and end of the season. Such crops frequently intercept virtually all available light at full canopy (Sceicz 1974). They may, however, be slow to attain this because of delayed leaf emergence and slow leaf growth in spring (Sibma 1977); while in the autumn senesence of leaves may reduce interception while conditions are still suitable for growth (Jackson 1980a).

Orchard crops on the other hand tend to attain their maximum leaf area by mid-summer but intercept only a relatively low proportion of available radiant energy over their lifetime (Jackson 1980b). The trees in a five year old orchard may intercept only 30% of available light at full leaf, while the corresponding figure for mature orchards seldom exceeds 70% (Jackson 1975). This obviously puts a low upper limit to dry matter production (Monteith 1977).

Spacing trials have shown fruit yield of apples to be a linear function of orchard light interception, up to a value of at least 60% (Jackson 1978), but if spacing is close enough for efficient light interception soon after planting the orchard may subsequently become too dense at maturity (Verheij 1972). This poses a problem as fruit bud production, fruit retention, growth and colour development are all reduced by shade to a greater extent than is vegetative growth as shown by smaller increases in shoot length and girth increments under shade conditions (Jackson 1980b; Jackson and Palmer 1977). The latter is closely related to the increment in dry weight of vegetative parts of an apple tree (Moore 1978).

In studying the photosynthetic efficiency of apple trees Avery (1975) concluded that 80% of full photosynthesis could be obtained at between 10 and 40% of full sunlight. The data of Sirois and Cooper (1964; cited in Lasko and Seeley 1978) indicated that the rate of photosynthesis of apple trees is reduced to only 70% of its maximum bright light value when irradiance is 25% of full sunlight. Barden (1977) showed the net photosynthetic rate of shade leaves was 70% of sun leaves at saturation under 80% shade. The density of canopy which might be ideal for dry matter production is thus well in excess of that which can produce good quality fruits (Jackson 1980b).

The ideal is clearly a canopy which is shallow or open enough to produce good quality fruits throughout, without excess depth needing expensive management, such as pruning. And one that is arranged so nearly all the light is intercepted and does not provide energy to grow grass in alleyways. Such a canopy should reach its maximum size soon after planting and be easy to maintain at maturity (Jackson 1980b). Light interception by such a canopy is determined by the amount and arrangement of the leaves, fruits and branches within the tree crown, the tree shape and size, spacing, row orientation and the angular

distribution of light from the sun and sky (Palmer 1981). Comparative
field evaluation of all possible canopy shapes and arrangements would
be inordinately slow and expensive: a modelling approach to provide
even a preliminary sieve is needed (Jackson 1980b).

In annual crops much information about the size of the photosyn-
thetic system has been obtained by measuring leaf area index (LAI)
(Montieth 1977). LAI data have been used in conjunction with light
interception records to analyse the basis of canopy productivity (Loomis
et al. 1971; Montieth 1977). A general equation (1) for the penetration
of light down the canopy has been developed:

$$(1) \qquad \frac{I_L}{I_o} = e^{-KL}$$

where I_L = light penetrating canopy,

$\quad\quad I_o$ = incident light energy

$\quad\quad L$ = LAI of canopy

$\quad\quad K$ = the extinction coefficient for visible radiation.

Light intensity, therefore, declines logarithmically with LAI from the
top of the canopy and total interception is consequently a logarithmic
function of LAI (Jackson 1980a).

These classical light interception models, where transmission of
light by the canopy is experimentally related to LAI, are clearly inapprop-
riate because of the non-random distribution of leaves in orchards
(Monsi et al. 1973). The way in which the foliage is clumped in terms
of tree height, tree thickness, and between tree spacing, determines the
pattern of cast shadows (Jackson 1980b). The dimensions and arrangement
of continuous hedgerows would affect light interception if they were
opaque, and so effectively establish limits to the light interception
by hedgerow orchards of any given geometry (Jackson and Palmer 1972).

Two models have been developed (Charles-Edwards and Thorpe 1976; Palmer 1977) to calculate the transmission of direct-beam radiation through hedgerows, taking into account their leaf area densities and, in the latter case, the distribution of fruits and branches as well. These computer models require the overall canopy geometry to be defined in relatively simple mathematical terms.

More recently Jackson and Palmer (1979) described a simple general equation (2) for light interception by any discontinuous canopy:

$$(2) \quad \frac{I}{I_o} = T_F + (1 - T_F)e^{-KL^1}$$

where $\frac{I}{I_o}$ is the average fraction of the incident light reaching the orchard floor

T_F is the transmission due to the overall form of the canopy (i.e. the fraction of light which would reach the ground if the trees were solid)

K is the measured within tree light extinction coefficient

and L^1 is $LAI/(1 - T_F)$.

For simple shapes T_F is calculated directly from the data; for example, it is one minus the fractional interception as calculated by Jackson and Palmer (1972). For more complex shapes T_F is calculated from measurements of interception made on non-transmitting scale models as outlined by Jackson (1980b).

This equation has been found (Jackson and Palmer 1979) to give a good estimate of light interception by hedgerow orchards if K was assumed to be 0.6, which has been shown by Jackson (1978) to be an average value for apple. For other tree crops or, indeed, for apple orchards of different types, it would be more desirable to determine K directly using actual orchard measurements of $\frac{I}{I_o}$ and LAI in conjunction

with a physical scale model of the orchard and equation (2) (Jackson 1980b).

The model represented by equation (2) (Jackson and Palmer 1979) has been further developed in order to be able to calculate the way in which changing canopy characteristics will change the total volume of canopy in which the irradiance is at, or above, any specified level; and to calculate the area of leaves within such a volume (Jackson and Palmer 1981). The following equations (3) and (4) are those used to calculate leaf area (L_1) and canopy volume (CV_1) in zones external to any chosen contour of mean irradiance:

(3) $\quad L_1 = [(\ln^I)/(-K)](1-T_f)$

(4) $\quad CV_1 = L_1/(\text{leaf area density } m^2m^{-3})$

The transmission equations (1 to 4) so far defined can be expressed in interception form so as to link with the widely used concept of Fractional Interception (F). The term $(1-T_f)$ can be redefined as F_{max} i.e. the fractional interception by non-transmitting 'trees' or 'hedgerows' of the same shape and arrangement as real ones (Jackson 1981; Palmer 1981). In which case equation (1) becomes:

(5) $\quad F = F_{max} - F_{max}e^{-KL}$

The computer modelling of light interception by hedgerow trees has produced the following conclusions. If the leaf area index is low (<1) then within quite wide limits, tree size and spacing have an effect on light interception. At higher leaf area indices, tree size and arrangement become significant factors if there are conventional wide alleyways. The closer an orchard approximates to a continuous canopy of leaves the less important the tree size and arrangement become (Palmer 1981).

3.3 Photosynthate Resources

3.3.1 Reserve Carbohydrate

In temperate fruit trees and bushes carbohydrates, which make
up the major part of the food reserves, are stored throughout the
leafless period and are distributed through all the living cells of
the plant. The perennial tissues include living parenchymatous tissues
among the non-living lignified elements so the whole structure serves
for storage (Priestly 1981). Reserves are needing during renewed
growth in spring, but not all those accumulated by the previous leaf
fall remain until then. Losses may appear to be larger from roots
because they have a higher proportion of living cells than other tissues.
Normally, in apple, stems lose the same proportion of their reserve as
roots; in blackcurrant, roots lose a greater proportion of their reserves
than other regions (Priestly 1981).

In blackcurrants winter dormancy is broken by exposure of buds
to low temperature during autumn and winter, and normal development then
ensures when minimal environmental conditions for growth occur in the
spring (Wright 1975). Apple buds have been shown to have similar
requirements (Thompson et al. 1975). Carbohydrate losses during winter
dormancy would be expected to increase with increases in temperature.
Priestly (1981) showed this effect with apple rootstock cultivars by
achieving greater losses in dry weight after, as well as during, a
raised temperature treatment in the dormancy period. Total residue
weight in the stem region remained constant; consistent with it represent-
ing structural material. However, total amounts both of carbohydrate
and residue were less at the end of the dormancy period compared to the
beginning. This is interpreted as a loss of structure from root
extremities which behave as sacrificial organs supplying the truely

perennial parts (Priestly 1981).

Late summer water stress in blackcurrants has been shown (Wilson and Jones 1980) to reduce fruit set and reserve carbohydrates, which is consistent with the view expressed by Priestly (1971) that early spring growth is dependent on accumulated reserves. Hardy (1981) demonstrated losses up to 30% of potential yield through unseen damage caused by blackcurrant borer moth. The pith is rich in starch (Wilson and Jones 1980), and the larvae feed on the pith, especially in autumn and spring (Miller 1981). Hardy (1981) attributed reduced fruit set to removal of the pith by the borer moth larvae, which causes depletion of available carbohydrates at crucial times, during the initiation and development of flower primordia.

Starch is rapidly changed to sugars and is a highly mobile and accessible storage reserve. Wilson (pers. comm.*) reports that qualitative assessment of iodine stained stem sections indicates solubilization of stem starch in early dormancy, possibly as a freeze protection mechanism. Shoot starch levels then stay low and fairly constant until the flowering period when similar observations suggest recommencement of stem starch deposition shortly after fruit set.

Wilson and Jones (1980) recorded impaired starch accumulation, and earlier but reduced total spring bud burst following imposed summer/autumn water stress. Wilson (pers. comm.) advises that the stressed (low reserve) plants, although they developed leaves earlier, appeared to have reduced leaf area at each bud compared with the untreated controls; indicating that initial leaf expansion is dependent on carbohydrate reserves.

3.3.2 *Photosynthate Effect on Oil Composition*

In the photosynthate model proposed by Burbott and Loomis (1967) and revised by Clark and Menary (1980a), the balance between production

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and utilization of photosynthate is an important determinant of pepper-
mint oil composition.   Within this model, the balance between daytime
accumulation of photosynthate and night-time utilization of photosynthate
determines monoterpene composition.   Factors favouring the maintenance
of high levels of photosynthate (i.e. long days, high photon flux
density, low night temperatures), favoured high concentrations of cineole
and menthone (desirable peppermint oil components), and low concentrations
of pulegone and menthofuran (undesirable components) (Clark and Menary
1980a).

Assuming that increased carbon dioxide fixation and increased
carbon dioxide evolution by the plant reflect increased production and
increased utilization of photosynthate by the plant, respectively, then
factors contributing to changes in 'apparent' photosynthesis are important
determinants of oil composition (Clark and Menary 1980b).   'Apparent'
photosynthesis can be considered to have three components: 'true' photo-
synthesis, photorespiration and dark respiration.   It is apparent an
increase in night temperature would increase dark respiration, shifting
the balance of photosynthate towards utilization, resulting in increased
menthofuran (Burbott and Loomis 1967; Clark and Menary 1980b).   Increas-
ing the daytime temperature to that required for maximal rates of 'appar-
ent' photosynthesis will shift the balance towards production of photo-
synthate.   On the other hand, increasing day temperatures above the
threshold required for maximal 'apparent' photosynthesis will lead to
an increase in dark respiration and an even greater increase in photo-
respiration, once again shifting the balance to utilization (Clark and
Menary 1980b).

Catabolism of essential oil components during times of photo-
synthate deficiency does not seem unreasonable, as such compounds rep-
resent a considerable amount of potential metabolic energy (Loomis and

Croteau 1973). During maturation of mint oil grown in the Yakima Valley, there is a large loss of menthone which accompanies metabolic maturation of the oil. This loss in menthone cannot be accounted for by the increase in menthol or other oil constituents, nor can it be rationalized as due to evaporation; thus, a catabolic process is implied (Croteau and Martinkus 1979).

3.3.3 Carbon 14 Tracer Studies

Carbon compounds within a plant may be conveniently labelled with radioactive [14]carbon if single leaves or groups of leaves are allowed to photosynthesize metered doses of [14]CO_2 (Priestly 1973). This method leads to a better understanding of the partitioning of carbohydrates into the various regions of consumption in the plant and has been widely used: for example, the turnover of carbohydrates in apple (Kandiah 1979a and b); the effect of supplementary doses of nitrogen on apple (Priestly et al. 1976a and b); and the distribution of photosynthetic assimilates in orange (Guy et al. 1981).

The most striking finding to emerge from the numerous in vivo tracer studies on monoterpene biosynthesis is the almost universally poor incorporation of exogenous labelled substrates. Such low incorporations have been attributed to poor uptake of precursor, to competition for precursor by other biosynthetic or degradative pathways and, most significantly, to comparmentation of monoterpene biosynthesis at sites that are isolated and energy deficient (Loomis and Croteau 1973 and 1980; Charlwood and Banthorpe 1978). A number of important observations have come from these studies, however, not the least of which is evidence for rapid metabolic turnover of monoterpenes in plants. Another curious finding is the preferential labelling of the monoterpene portion derived from isopentenyl pyrophosphate even with [14]CO_2 as the precursor (Loomis and Croteau 1980).

3.4 <u>Dormancy</u>

From studies of endogenous growth inhibitors, it has been proposed that the natural onset of winter dormancy in buds of woody species is induced by an inhibitor synthesized in the leaves and translocated to meristematic regions (Phillips and Wareing 1958). The synthesis of this inhibitor, later identified as abscisic acid, is thought to be under photoperiodic control although this has been questioned (Lenton et al. 1972).

El-Antably et al. (1967) demonstrated that abscisic acid from leaves of *Betula pubescens* plants, grown under short day conditions, caused blackcurrant seedlings, grown under long day conditions, to cease growth; also resting buds complete with scales set as if they were caused to go dormant by photoperiodic induction. Wareing (1969) put forward the concept that the annual cycle of bud growth and dormancy is regulated by a balance between endogenous growth inhibitors and gibberellic acid. Moore (1979) states it is probable that induction of dormancy, in at least some cases, is brought about by high abscisic acid and low gibberellic acid levels, whereas the converse is true for the emergence from dormancy.

Tinklin and Schwabe (1970) have determined the seasonal fluctuations of free inhibitor content of blackcurrant buds. They have shown there is a maximal activity in late autumn, followed by a continuing decline during winter, with a minimum reached in early spring. These authors found that bud break could be induced by the removal of bud scales during the period when the shoots had attained complete winter dormancy. Thus concluding that winter dormancy was due to the formation of an inhibitor produced by the leaves and accumulated in the bud scales. Kuzina (1970) using relatively crude extracts of blackcurrant buds, reported a growth inhibitor which increases with the approach of autumn irrespective of

day length, and declines on breaking dormancy.

Wright (1975) showed that high levels of free abscisic acid were found in blackcurrant buds during early winter dormancy. Free abscisic acid then falls rapidly and bound abscisic acid increases throughout the dormant period - suggesting a change from the free to the bound form. After bud burst free abscisic acid levels increase (Figure 3.4.1). By dissecting a bud during winter dormancy Wright demonstrated that some 62% of the free abscisic acid was found to reside in the inner part of the bud (leaf and flower primordia), and only 12% in the bracts. Free abscisic acid (ABA) levels in vigorously growing shoots were found to average 24±8.9 µg equivalents of ABA per kg fresh weight of tissue (Wright 1975). Further, the level of free ABA was found to be higher during the mid-winter period (80 µg), although this figure is relatively low when compared to the autumn peak (580 µg), but nonetheless sufficiently high to maintain dormancy, particularly if allowance is made for the distribution of abscisic acid within the bud.

In blackcurrants winter dormancy is broken by exposure to low temperature during autumn and winter; normal development then ensues when minimal environmental conditions for growth occur in the spring. Hoyle (1960) reports blackcurrants have a chilling requirement of 12-15 weeks at 2-7°C. He found little effect of daylength when the chilling requirement was satisfied; however, when the plant was not completely chilled more buds were observed to burst in long day than short day conditions. These observations have been confirmed by Thomas and Wilkinson (1964), who found a chilling requirement of 12-15 weeks at or below 7°C. El-Antably (1965), however, demonstrated a shorter chilling requirement, 10-12 weeks chilling at 2°C, by showing 70% bud break after transfer to warm (20°C) long day conditions.

During growth bud swell is probably under the control of gibberellins and cytokinins, with some help from the gradual fall in free abscisic
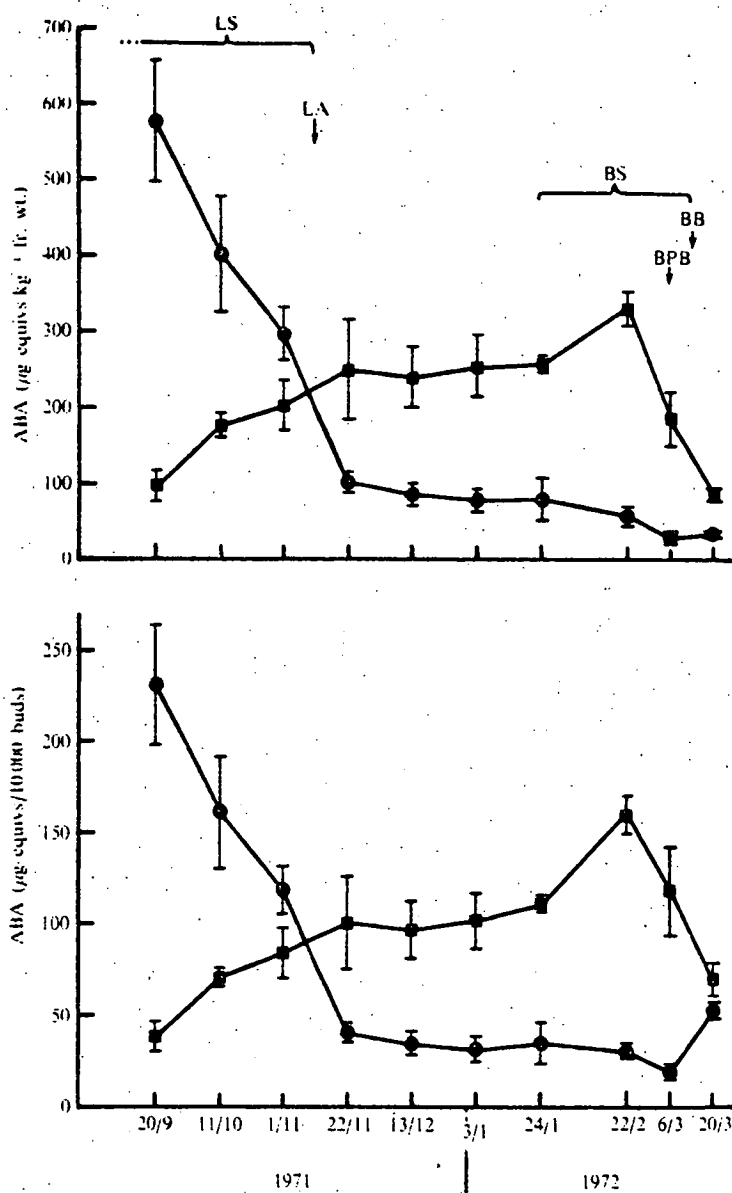
Seasonal fluctuations of free and bound ABA levels in blackcurrant buds. LS = senes-
cence of leaves subtending buds; LA = leaf abscission; BS = bud-swelling phase; BPB = buds
on the point of bursting; BB = bud burst. ● = free ABA; ■ = bound ABA. The vertical lines
represent the 2 s.e. limits.

FIGURE 3.4.1 Seasonal changes in abscisic acid
(from Wright 1975)

acid level up to the time of bud burst (Wright 1975). The high level

of bound abscisic acid in swelling buds may lead to a feedback reaction

slowing down the conversion of free abscisic acid to the bound form.

In this way, abscisic acid may act as a brake preventing the young bud

growing too vigorously until the external environment is favourable.

4. MODERN ANALYTICAL TECHNIQUES IN THE FLAVOUR FIELD

4.1 Liquid Solid Column Chromatography

The separation of flavour volatiles into fractions consisting of

broad chemical classes by means of liquid solid chromatography on silica

gel is a very useful means of achieving preliminary fractionation of

flavour isolates.

The most common method of fractionation used is the separation

of hydrocarbons from oxygenated terpenoids as described by Kirchner

and Miller (1952). Hydrocarbons are separated from the total oil by

column chromatography on silica gel by elution with hexane; however,

due to large variations in the relative percentages of different com-

pounds present in such mixtures, problems arise in their gas chroma-

tographic separation and identification. Scheffer et al. (1975)

developed a pre-fractionation technique to overcome these problems.

They eluted the monoterpene hydrocarbon fraction from a silica gel

column with pentane, and collected it as a number of small fractions

for gas chromatographic analysis. To prevent possible acid catalysed

reactions (Scheffer et al. 1976a) the silica gel was acid washed,

neutralised and wetted to a specific water content for improved

separation of terpenes. A later development for separation of

naturally occurring oxygen containing monoterpenes (Scheffer et al.

1977) used a gradient elution series of ethyl ether in pentane.

Isomerization processes could be avoided by using purified and deactivated silica gel. These methods have been used successfully to examine the essential oils of *Abies alba* (Scheffer *et al.* 1976b) and *Alpina galanga* (Scheffer *et al.* 1981).

Murray and Stanley (1968) developed a simple dry column technique for fractionation of complex flavour mixtures by liquid chromatography on silica gel using low boiling solvents at 1°C. This method was later scaled down to a microfractionation technique (Murray *et al.* 1972). The silica gel absorbent is placed in a flexible polytetra-fluroethylene (PTFE) column and sample is applied at the bottom of the column; this sample is then developed vertically upwards with dichloromethane until the solvent just reaches the top. Different fractions are then recovered by slicing the column into sections and eluting the volatiles with a small volume of diethyl ether directly into transfer traps which contain column packings for GC-MS analysis. This method concentrates the minor components of sensory importance found in the original sample.

Another absorbent, Florisil, has been investigated and confirmed as a suitable absorbent for the column chromatography of labile terpenoids that undergo chemical changes on other absorbents, i.e. silica or alumina (Ayling 1976). Ayling demonstrated that when activated for at least 5 hours at 130°C Florisil could be most effectively used to separate hydrocarbons from oxygenated components. Its efficiency is not impeded when compounds that are difficult to separate are present, i.e. cineole.

4.2 Gas Liquid Chromatography

A principle concern of the flavour chemist is the choice of column to be used for a particular separation, and the operational

parameters which will allow optimal column performance (Merritt and Robertson 1982). It is now clearly established that in terms of separation efficiencies, speed of analysis, sensitivity and cost open tubular or capillary columns are vastly superior to their packed column counterparts (Jennings 1980a and b). Open tubular columns, while of smaller bore than packed columns, are usually of greater length with low dead volume resulting in higher resolution (Merritt and Robertson 1982). In addition, the recent developments of thick film, bonded-phase, open tubular columns have eroded the single advantage remaining to the packed column - large sample capacities (Jennings 1981a). The increased inertness and superior resolution of siliceous glass capillary columns has attained wide attention (Jennings 1981b).

The columns increased resolving power is of considerable benefit particularly for compounds of sensory importance; notably those containing nitrogen or sulphur, which in the past had suffered alteration or simply failed to pass through packed columns. However, capillary columns did suffer from differences in their upper temperature limits, bleed rates and tailing or abstraction of selected test compounds (Jennings 1981b). Most authorities now agree that both Lewis acid sites and silanol groups at the surface of the glass contribute to performance defects of a column (Jennings 1981b).

These defects may take the form of catalytic effects (evidenced by total or partial component abstraction), or by absorptive interactions (leading to abstraction or tailing), of susceptible solutes. The former usually varies directly, and the latter inversely with column temperature (Grob 1980).

The advent of fused silica open tubular columns (Dandeau and Zerenner 1979) has revolutionized capillary gas chromatography. Nonpolar columns were originally prepared by undercoating with a polar phase such as Carbowax 20 m. While this is effective in deactivating

the glass surface, it causes other undesirable consequences such
as distortion of retention indices (Lipsky *et al.* 1980). New deactiv-
ation treatments (for example Grob *et al.* 1978 and 1979) resulted in
preparation of a range of non-polar fused silica columns, inert and
thermally stable to 300°C without Carbowax 20 m undercoating (Lipsky
et al. 1980).

Fused silica glass, in general, provides better capillary columns
than those made from natural quartz (Lipsky *et al.* 1980). The
chemically pure fused silica glass, when coated externally with a
suitable polymer, provides a degree of flexibility and handling prev-
iously unknown. It is possible to have a fused silica glass system
extending from injection port to the base of the jet in the flame
ionization detector. This avoids unnecessary connections, unswept
"dead volumes", metal or glass lined tubing surfaces, and improves
overall chromatographic performance by at least 10-25% (Lipsky *et al.*
1980).

4.3   Combined Gas Chromatography - Mass Spectrometry

Combined gas chromatography/mass spectrometry (GC/MS) has become
the major instrumental technique of component identification in flavour
research. It makes possible studies of complex mixtures of organic
components which would otherwise be impossible, at least within a
realistic time frame (Flath 1981). For example, Davies and Menary
(1982) identified 64 components in six varieties of hops; Murray *et al.*
(1972) found 81 components in passionfruit and Ismail *et al.* (1981a and b)
identified 73 components of Victoria plums using GC/MS techniques.

In order to be useful in combination with a GC, a mass spectrometer
needs fast scan capabilities (approximately 1 sec scan from 15-300 amu),
with sufficient sensitivity to yield a mass spectrum with 1 to 10 ng

of material when operated in the electron impact (EI) mode (Flath 1981). In this mode a beam of energetic electrons is employed to ionize sample molecules.

The biggest instrument changes in recent years have been in the area of sample ionization modes. The most common complementary technique to EI is chemical ionization (CI), which is now a standard feature of commercial GC-MS units (Flath 1981). CI involves the preliminary ionization of a reagent gas (usually isobutane) followed by ion-molecule reactions in the ion source region. The energy transfer is much lower in such ion molecule reactions than is the case with electron impact ionization, so fragmentations observed in CI vary considerably from those observed in EI (Flath 1981). Butane or isobutane reagent gas yields a rather simple CI mass spectrum of a sample with a pseudomolecular ion MH^+ or $(M-H)^+$. This is especially useful if conventional EI does not yield a molecular ion.

4.4 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) has become the most important analytical technique in the last decade for separating highly polar and thermally unstable compounds in the molecular range 200-2000. Since analyses are generally performed at ambient, the destructive temperatures needed in gas chromatography are avoided and thermal degradation products are not encountered (Kubeczka 1981).

However, many volatile oil constituents cannot be analysed by high performance liquid chromatography with ultraviolet (UV) detected due to the lack of chromophoric groups in compounds such as the monoterpene hydrocarbons and alcohols (Ross 1978). Ross considers that HPLC cannot normally be considered for the total analysis of volatile oils but rather for quality control where acceptability may be determined

by reference to certain compounds with good chromophoric properties.

Jones *et al.* (1979) consider that while the resolution afforded by gas liquid chromatography for the separation of volatile flavour constituents remains unsurpassed, HPLC will considerably speed up any necessary prefractionation required for optimal semipreparative GLC separations of complex mixtures having varied functionality.

Separation of the constituents of *Lindera umbellate* and *L. sericea* have been described by the reverse phase method on a Bondapak C18 column using methanol/water (1:1) with UV (254 nm) and refractive index (RI) monitoring. It is noteworthy that more peaks were detected by UV which would seem to indicate that RI detection will not be particularly useful in analysis of these types of compounds (Komae and Hayashi 1975).

The alternative detection with UV light limits the selection of solvent systems, of which most (e.g. the widely applied methanol) show high absorptions at lower wavelengths. This is a common problem if gradient elution is necessary to resolve highly complex mixtures (Strack *et al.* 1980). Most communications report on HPLC of compounds which are UV detectable above 240 nm.

The HPLC analysis of cinnamon and cassia oils (Ross 1976) and later of eugenol, isoeugenol, methyl salicylate and thymol (Ross 1978) with UV detection at 260 nm was achieved with methanol/water (1:1). Various 1,2-unsaturated enones (carvone, citral and neral) were separated with acetonitrile/heptane (1:99) on 5 μ Partisil by UV detection at 242 nm (Ross 1978). Affording a simple and rapid method for determination of carvone in spearmint and dill oils.

Tyman (1983) considers it necessary for quantitative work to determine response factors (effectively extinction coefficients), as in gas chromatography, since a minor but strongly chromophoric species can otherwise be overestimated. The method of detection in HPLC is

selective for the UV absorbing species and thus the chromatogram may be simpler than the GC trace (Tyman 1983). In order to attain reproducibility sufficient time for re-equilibration between analysis runs is important, the influence of the solvent (if any) used for the injected sample and the quality of the mobile phase are factors that must be given careful consideration (Tyman 1983).

It seems to have been taken for granted that the minimum requirement for UV detection is conjugation (Tyman 1983), but recent work has been carried out with essential oils rich in sesquiterpenes having isolated double bonds and low intensity UV absorption 200-220 nm (Strack *et al.* 1980).

To some extent the availability of HPLC solvents with improved transparency has enhanced the range of UV detection (Tyman 1983). From results on a mixture of eleven sesquiterpenes on Li Chrosorb RP-18 using acetonitrile/water (85:15) all were resolved by the aid of UV detection at 200 and 220 nm (Strack *et al.* 1980). These workers applied the method to the essential oil from *Cistus ladanifer* and obtained results comparable to GC analysis.

Schwanbeck and Kubeczka (1979) demonstrated an excellent separation of terpene hydrocarbons using n-pentane on a silica gel column with UV detection at 220 nm, at which no applied compound escaped detection. However, this procedure makes it necessary to operate at very low temperatures (-15°C).

Kubeczka (1981) considers there is a second serious limitation, other than detection, to the HPLC separation of flavour volatiles. The restricted peak capacity and relatively small range of K^1 values of a liquid chromatographic system do not lend themselves to the effective separation of multicomponent mixtures in one operation. Kubeczka (1981) considers it necessary to carry out a prefractionation procedure

to produce several less complex fractions for further HPLC analysis.

This pre-separation procedure can also be applied to fractionate natural flavours and essential oils into groups of components in order to simplify GC analysis, since even highly effective capillary columns are not usually able to separate natural flavour mixtures fully in a single run (Kubeczka 1981). A method for HPLC fractionation of mixtures of heterocylic compounds such as furans, thiophenes, pyrroles, thiazoles, oxanzoles, pyrazines and imidazoles has been described by Yamaguchi and co-workers (1979).

Kubeczka (1981) describes a method operating the HPLC on a semi-preparative scale with resolution much superior to ordinary column silica gel chromatography. Using a mobile phase consisting of methanol/water (82.5:17.5) with stepwise elution to pure methanol it was possible to separate up to 0.5 mℓ of a terpene mixture on a LiChroprep R18 column. The mobile phase allowed low UV monitoring at 220 nm and the elution order of the investigated compounds was according to decreasing polarity and within the hydrocarbons to increasing molecular weight. Fraction 1 consisted of oxygenated terpenes, fraction 2 monoterpene hydrocarbons and after changing the mobile phase fraction 3 contained sesquiterpene hydrocarbons. Figures 4.4.1 and 4.4.2 display the pre-separation achieved at a flow of 8 mℓ/min and 4 mℓ/min respectively.

4.5   Isolation and Concentration of Flavour Volatiles

4.5.1   Concentration of Flavour Volatiles

Although outstanding advances in analytical methodology have taken place in recent years, and are continuing to occur, a flavour chemist embarking on a detailed study of volatile aroma consituents is still faced with a task of considerable complexity.

1 = oxygenated terpenes

2 = monoterpene hydrocarbons

3 = sesquiterpene hydrocarbons

CONDITIONS

column:	240 x 10 mm i.d. LiChroprep RP 18 (40μm)
mobile phase:	A) methanol : water (82.5 :17.5 ; v/v)
	B) pure methanol
flow rate:	8 ml/min
detector:	UV, 220 nm
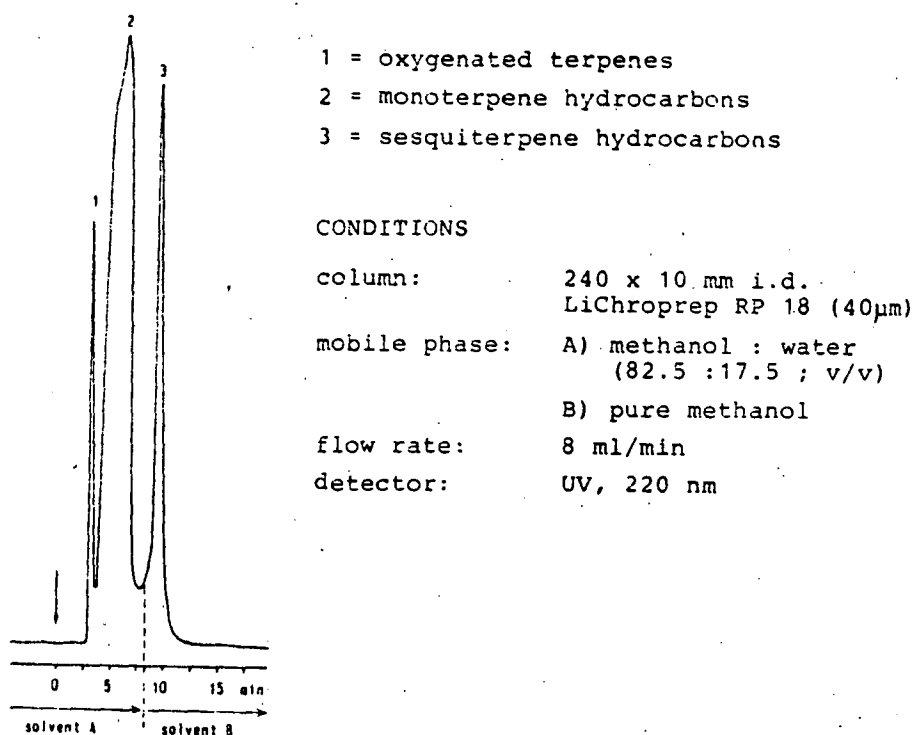
FIGURE 4.4.1   HPLC pre-separation of a terpene mixture (from Kubeczka 1981)
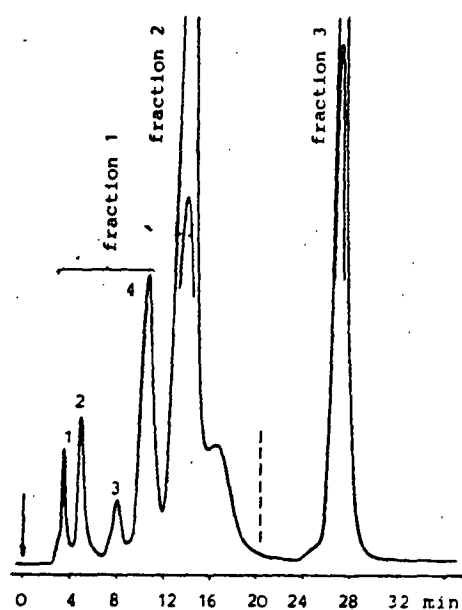
FIGURE 4.4.2   HPLC pre-separation of a terpene mixture at a lower flow rate (4 mℓ/min; other conditions see Figure 4.4.1) (from Kubeczka 1981)

Early methods for flavour isolation generally used steam distillation, followed by solvent extraction of the distillate and then concentration of this extract. This method yields an isolate that preferentially selects flavours with the greatest volatility and solubility in the extracting solvents (Reineccius and Anandaraman 1981). While this method is used only occasionally today, the Nickerson and Likens extractor (Likens and Nickerson 1964), or a modification thereof, is commonly used for flavour isolation: this procedure utilizes simultaneous steam distillation/solvent extraction of the sample. After their isolation aroma volatiles must be obtained in a suitably concentrated form for analysis. The solvent may be removed by a low temperature procedure, involving gas entrainment at reduced pressure (MacLeod and Cave 1975) or by use of a rotary vacuum evaporator; the last traces of solvent being removed under a slow stream of nitrogen (Ayling 1976). It is important to note that some losses of the more volatile flavour components will normally occur during the removal of low-boiling solvents (Cronin 1982).

If steam distillation is not used in the isolation of volatiles only small amounts of water will be present and the volatiles may be readily recovered in a small volume of solvent (Cronin 1982). In many cases aroma volatiles from distillative isolations will be recovered in large volumes as very dilute aqueous solutions. Rather large amounts of organic solvents are then required to extract these, using either separating funnels or a suitable liquid/liquid extractor, such as that described by Williams and Tucknott (1973).

The main criterion which governs the choice of the low-boiling solvent, used to extract flavour volatiles, is the degree of selectivity required. Among the common solvents used in flavour work, diethyl ether (b.p. 35°C) shows the lowest selectivity and is the solvent of choice when optimal recovery of all components is desired (Cronin 1982).

Pentane demonstrates a preferential removal of esters from ethanolic

solutions of esters and alcohols; while trichlorofluromethane (Freon

11) is less selective than pentane, but more selective than ether

(Williams and Tucknott 1973). Aroma distillates often contain high

levels of low-boiling alcohols, especially ethanol, which have little

flavour significance but which may mark important minor components, or

limit the extent to which volatiles may be concentrated. Ethanol may

be largely excluded from these samples by using pentane (b.p. 35°C),

2-methyl butane (b.p. 28°C) or trichlorofluromethane (b.p. 23°C) as

the solvent of choice (Cronin 1982). Other low-boiling alcohols, for

example hexanol, isobutanol or 3-methyl-butanol, may be discriminated

against to varying degrees, depending on the solvent and the extraction

conditions used (Williams and Tucknott 1973).

While solvent extraction can be very useful in sample preparation

it introduces another serious problem: dilution of the sample with

large volumes of solvent. A number of methods have been suggested to

restrict the amount of solvent required, but eventually the extract

still requires concentration by removal of excess solvent (Jennings

1981b). Lower boiling sample components are also lost during this step,

in direct proportion to their partial pressures and concentrations,

relative to those of the solvent. By using a pressurized chamber

(Figure 4.5.1), it is possible to use lower boiling solvents such as

Freon 12 (dichlorodifluromethane, b.p. -30°C STP) or liquid carbon

dioxide (b.p. -78°C) (Jennings 1979, 1981b). A major advantage is

that this extraction method provides a flavour isolate that is free

from solvent, with reduced loss of low-boiling components, and yet can

be directly injected into a gas chromatograph (Reineccius and Anandara-

man 1981).

The solvent power of liquid carbon dioxide is not high compared

with ordinary liquid solvents. For these ordinary non-polar solvents
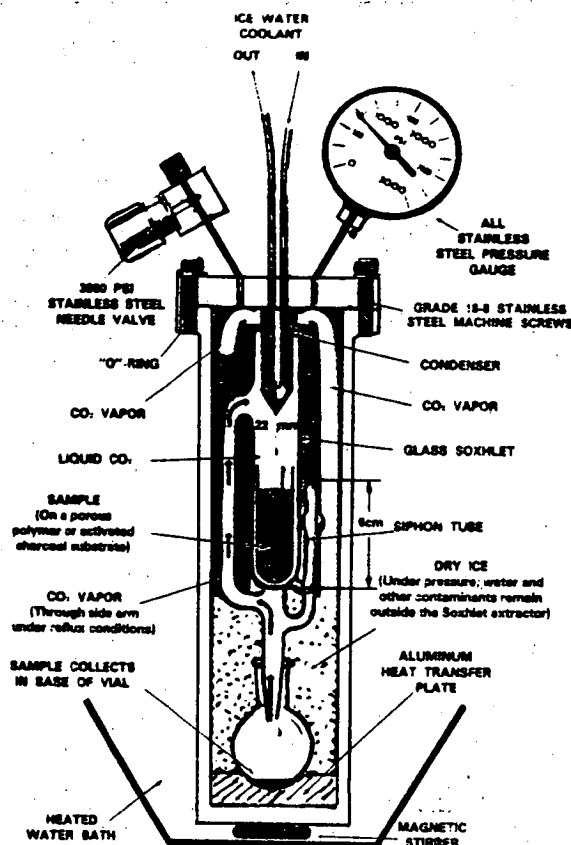
FIGURE 4.5.1   High pressure Soxhlet Extractor
               (from Jennings 1981b)

an increase in temperature usually leads to an increase in solvent

power. This rule is not always valid for CO_2 even if at higher temper-

atures the pressure is also increased to guarantee a liquid phase

(Brogle 1982).   In general, solubility of organic compounds in liquid

CO_2 is determined by polarity and/or molecular weight.   Low molecular

weight oxygenated compounds and hydrocarbons, for example esters, ethers

and terpenes, are soluble whereas high molecular weight compounds,

for example alkaloids, chlorophyll and carbohydrates, are insoluble

(Clarke 1983).

Some discussion has ensued in the literature concerning the

relative merits of using liquid CO_2 for extraction as against using

supercritical CO_2.   Figure 4.5.2 shows a phase diagram for carbon

dioxide; above the triple point (-56.6°C, 416 kPa) and below the

critical point (31.1°C, 7280 kPa) carbon dioxide may exist as a colour-

less mobile liquid: above the critical point it exists as a critical

FIGURE 4.5.2

Carbon dioxide phase diagram
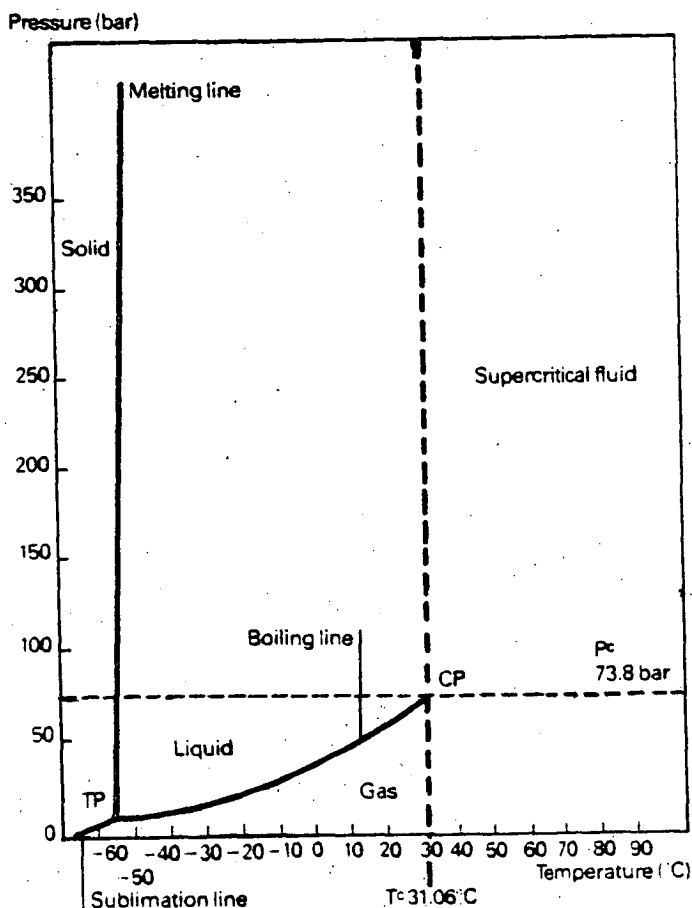
fluid. Liquid CO_2 is non-polar but the polarity and hence the dissolving power of the solvent for polar compounds increases with increasing temperature and pressure in the supercritical region (Clarke 1983). The principal advantage for the use of supercritical CO_2 over liquid CO_2 appears to be a more rapid and efficient but less-selective extraction (Clarke 1983). Supercritical extractions can be made in a wide range of available conditions, thereby permitting the preparation of different extracts from the same starting material (Calame and Steiner 1982).

The selectivity of liquid CO_2 (as a non-polar solvent), its operation at low temperature (sub-ambient) and its relatively low pressure of operation (compared to supercritical CO_2) limits any possibility of chemical change of the constituents being extracted (Clarke 1983). One must be cautious that the purity of the carbon dioxide used to charge the extractor is evaluated as it may contain substantial amounts of low-boiling contaminants (Reineccius and Anandaraman 1981).

4.5.2 *Isolation of Flavour Volatiles*

(a) Trapping Techniques

A large number of procedures have been described for trapping
components eluted from GC columns. Trapping techniques may be very
simple; for example, the collection of components in cooled glass
capillary melting point tubes inserted into the GC column outlet;
these tubes can then be sealed for storage (Cronin 1982). Another
common procedure, which is particularly useful if infrared spectra
are to be recorded, is to bubble eluted components into a small quantity
of chilled carbon tetrachloride contained in a tapered capillary tube
(Cronin 1982).

Considerable interest is centered on the use of simple miniature
systems, most of which use glass or metal tubing containing a suitable
absorbent material, which may be cooled if necessary. These traps may
contain ordinary gas chromatographic packings, such as Chromosorb 105
(Murray 1977) and Porapak Q (Ismail et al. 1980), or other absorbants
such as charcoal (Clark and Cronin 1975b; Sugisawa and Hirose 1981).
An important feature of all these arrangements is the high surface to
volume ratio that facilitates collision of aerosol droplets at the
surface, thereby permitting high trapping efficiency (Cronin 1982).
Short cooled lengths of glass porous layer open tubular (PLOT) capil-
laries containing a layer of alumina or Celite 545 are an alternative
and versatile means for handling small quantities of GC elutes. The
trapped compounds may be subsequently released and their aromas eval-
uated by grinding up the traps in a small quantity of water (Clark and
Cronin 1975a).

(b) Choice of Absorbant

The suitability of various polymers for aroma adsorption has been

examined by a number of workers. Murray (1977) preferred Chromosorb 105 because of its low background and high specific surface area while Chromosorb 102 was rejected due to unacceptably high backgrounds. Tenax GC showed lower absorptive capacity than either Chromosorb 105 or 106 (an acceptable alternative to 105) and demonstrated breakthrough of some components of medium volatility. Williams et al. (1978) showed Tenax GC gave more consistent results than Porapak Q for non-polar compounds with boiling points greater than that of hexyl acetate, but showed greater losses of low-boiling alcohols. Schaefer (1981) in study concerning the suitability of four solid absorbents for head-space sampling determined that, while Porapak Q has a greater retention volume than Tenax GC, the actual choice of absorbent depends on the specific problem involved. It is also necessary to be aware of arte-facts produced by heating the absorbents above their normal operating temperatures. Those produced from Porapak Q and Tenax GC (Lewis and Williams 1980) may interfere with the analysis of aroma components.

(c) Chemical Methods

A wide variety of simple qualitative tests are available which depend on a colour change or precipitation to characterise different types of functional groups. Many of these can be adapted to the analysis of GC eluates and may be of use in the characterisation of food aroma volatiles (Cronin 1982). Using a three-way column effluent splitting device, Cronin and Gilbert (1972) trapped components as very sharp, narrow bands on short lengths of glass PLOT capillaries containing a relatively thick layer of activated alumina. Colour reactions were then developed in situ by the application of appropriate reagents to the trapped bands. For flavour analysis the ability to detect less than one microgram of many components is a most attractive feature of this

technique (Cronin 1982). In the identification of insect sex attractants, Beroza (1975) has also used microchemical tests on active fractions as a preliminary probe for structural information. Boelens *et al.* (1974) used the reaction products of organic sulphur compounds to study their contribution to the overall aroma.

More recently reaction gas chromatography using inline catalysts to cause a range of chemical reactions has become important. Stanley and Murray (1971) reported methods for hydrogenation and hydrogenolysis of submicrogram amounts of flavour materials obtained from GC eluates.

4.6 Headspace Analysis

Headspace concentration is probably the most common technique used for flavour isolation. The volatiles are initially stripped by purging the material to be sampled with an inert gas such as helium or nitrogen. The stripped volatiles are passed through an absorbing column packed with a polymer of choice (Tenax GC, Porapak Q, etc.). When an adequate quantity of volatiles has been concentrated on the trap, they may be desorbed, by using either heat (back flushing with an inert gas while heating to 200-250°C) or solvent extraction (Reineccius and Anandaraman 1981).

Several important flavour components of passionfruit - Edulan I and Edulan II (Murray *et al.* 1972; Whitfield and Stanley 1977); dihydro edulans (Prestwich *et al.* 1976); and 6-(but-2-enylidene) -1,5,5 trimethylcyclohex-1-enes (Whitfield and Sugowdz 1979), have been identified using the headspace concentration methods described by Murray (1977). Murray included the sampling traps within the main flask to allow headspace collection close to the volatile source and so eliminate any risk of contamination. This also provides for isothermal conditions between the liquid and collecting traps, thereby avoiding the risk of

condensation of water in the trap from the saturated gas stream. Volatiles are extracted from aqueous solutions, such as steam dis-tillates and aqueous condensates, by passing the solution through Chromosorb 105 traps connected to a syringe pump: purging with a nitrogen stream effectively removes all water from the traps (Murray 1977).

The volatiles are unloaded from the trap and injected on to a capillary column by flushing them with a stream of nitrogen, from the Chromosorb 105 onto a precolumn of glass-lined steel tubing containing a short plug of 10% OV 101 GC packing (Murray 1977). In an examination of wine volatiles, Williams and Strauss (1977) used a similar system of headspace traps and GC introduction.

In a comparison of various methods for collecting aroma components of plums, Ismail et al. (1980) showed that extracts were more represen-tative if fruit was loosely packed in the collecting vessel; high gas flows were used for purging, and the entrained volatiles were adsorbed onto gram quantities of Porapak Q, kept agitated during collection, and then desorbed with ether. These procedures avoided two difficulties often encountered in this type of collection: firstly, a buildup of moisture in the vessel and deterioration of fruit quality due to low gas flows; secondly, heat regeneration of the trap raises doubts with regard to recovery of high boiling components, degradation and oxidat-ion of heat sensitive components and artefact production (Lewis and Williams 1980). This method has enabled a qualitative and quantitative examination of the aromas above four cultivars of plums indicating that benzaldehyde, ethyl nonanoate, linalool, gammaoctalactone, gamma-decalactone, 2-phenethanol and methyl cinnamate are important components of plum aroma (Ismail et al. 1981a).

If a solvent-free isolate is required, the use of a carbon dioxide

extractor (Jennings 1979) to remove adsorbed volatiles from the various adsorbant materials is very effective (Reineccius and Anandaraman 1981). Alternatively, thermal desorption followed by on column cold trapping can be used.

Automated purge and trap systems, such as the Philips PU4750 Headspace Analyser, provide reasonable sensitivity and reproducibility. However, Jennings (1981b) outlines a system which eliminates the inlet splitter and improves resolution of flavour components with short retention times. The sample accumulates in a first trap, cooled by liquid nitrogen, during analysis of the first sample. The contents of the first trap are then transferred to the second trap, and held while the first re-cools to a point where it again removes entrained volatiles from the carrier gas, and begins accumulation of the next sample: the contents of the second trap are then delivered to the GC for analysis (Jennings 1981b).

4.7 Sensory Evaluation

4.7.1 Olfactory Mechanism

In spite of modern analytical instruments the Flavour and Fragrance industry depends upon the perfumer and his assessment of odour quality as the final arbiter of fragrance value. Instrumentation used in the industry is rapid, sensitive and precise when used correctly. However, it cannot substitute for human judgement; the human nose with its olfactory system is much more sensitive than the finest gas chromatography yet devised (Dorland and Rogers 1977). The human perception of odour is often coloured by subjectivity, and there can be physiological defects in even the most highly trained olfactory system (Dorland and Rogers 1977).

The mechanism of olfaction involves vaporization of the odourant to form a mixture of air and aroma components; this mixture enters the nose and stimulates the olfactory cells of the nasal mucosa to send electrical impulses to a region of the brain, termed the 'olfactory bulb', where the impulses are decoded to give an odour sensation (Nimbalkar 1977). While the actual mode of stimulation of the olfactory cells is as yet unknown there are, at present, five important hypotheses which attempt to explain the olfactory mechanism (Brud 1980):

1. The adsorption/desorption hypothesis: Odourants stay fixed on the olfactory membrane for only a short time, during which some of their molecules penetrate the membrane. The characteristic odour is directly related to the intensity of this reaction and thus, molecular size. Dravnieks *et al.* (1979) take the view that the odourant triggers electrical phenomena (adsorption hypothesis). Steiner, on the other hand, holds that the molecules of odourants are electrically attracted by the membrane, arranging themselves on the receptor cell in accordance with the distribution of electrical charges in the molecule (Sturm 1978).

2. Enzyme hypothesis of Baume and Davis: Olfactory stimuli result from a blocking reaction between odourants and enzymes of the olfactory receptors. It is quite true that numerous metabolic processes involving specific proteins take place in cells, but only in the presence of certain chemical compounds. Additionally, the proteins and enzymes of the olfactory membrane have been shown to behave in this way (Brud 1980).

3. Oscillation hypothesis: Characteristic molecular vibrations are responsible for interaction with receptors. In support of this, Wright (1957) quotes the IR spectra of musk aroma molecules, but

confines himself to the most intensive out-of-plane oscillations which show a certain kinship in these compounds on account of the similar geometry of their molecules. Other work has shown that the entire spectrum of molecular vibrations penetrates the membrane, while the molecules themselves do not.

4. Stereochemical hypothesis: Since contact between the odourant and the olfactory membrane is a pre-requisite for perception, molecular shape is likely to be a factor. Amoore (1970) adopted a classification of odours in seven main groups, founding his hypothesis on the close relationship between the external shape of the molecules and the olfactory impressions to which they gave rise. He postulated hollows on the olfactory membrane into which molecules of aroma chemicals fitted like a key in a lock.

5. Functional group profile hypothesis: The spatial position of the functional groups and their charge determine the dipole moment of a molecule. Fixation of aroma chemicals molecules on the surface of the receptors is achieved, it is argued, by the function of groups, whereas the stimulant effect is brought about by the profile of the molecule and its orientation on the receptor (Beets 1978). Thus, molecules of similar structure should have similar smells (Figure 4.7.1).

All these hypotheses have several points in common: (i) an olfactory impression is caused only by molecules that come into direct contact with the olfactory membrane; (ii) there is a relationship between molecular shape and sensory impression; (iii) different substances may give rise to differing odours, or on the other hand to similar or identical ones (Figure 4.7.2). By contrast, similar chemical compounds may give rise to different olfactory impressions; (iv) the contact between the odour-
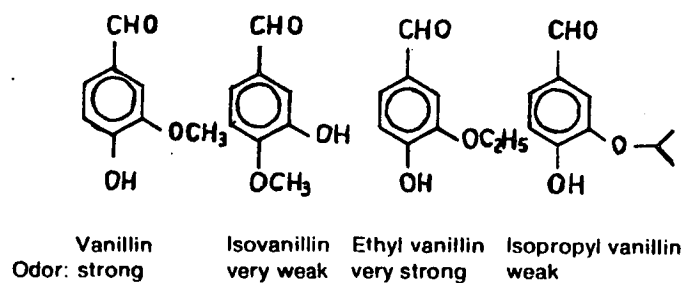
FIGURE 4.7.1   Functional group profile concept
(from Brud 1980)
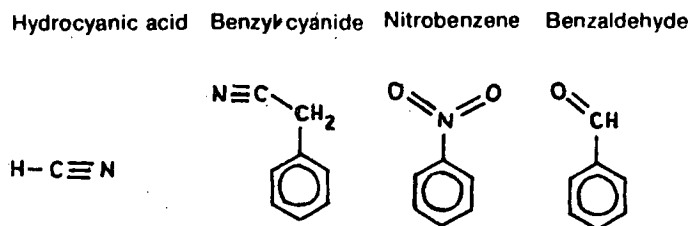
FIGURE 4.7.2   Substances with an odour of bitter almonds
(from Brud 1980)

and molecules and the protein molecules of the olfactory receptors
is of a physical nature (Brud 1980).

The sense of smell is highly subjective since it is considerably
influenced by the environment under which the observer receives an
odour sensation. The sense of smell also depends on the physical and
psychological conditions as well as the frame of mind of an individual.
It is further conditioned by the association of the odourant with
certain recollections (Nimbalkar 1977). A person who is going to
smell products unknown to him for the first time, must realise that
such impressions are irreplaceable for his olfactory memory (Roudnitska
1980).

4.7.2 Sensory Assessment

There are complex interrelationships between flavour and
analytical differences observed between cultivars of vegetables and
fruits. The key to unravelling these is precise sensory "objective"
description (Williams 1978a). Lists of compounds identified in cultiv-
ars are of little value unless they can be related to some aspect of
flavour and quality enabling the information to be used to assist in
both quality control and product improvement (Williams 1978a).

The sensory assessment of components as they are being separated
by gas or liquid chromatographic techniques is a valuable and relatively
simple means of obtaining an indication of the aromas of components or
fractions. One advantage being that it can often be used to trace the
region where a particular flavour character resides before valuable
time is spent in identification (Williams 1978a). Once the organo-
leptically important compounds are known, the influence of the manu-
facturing process on flavour changes can be elucidated in terms of

reaction kinetics and mass transfer conditions. This permits process-ing conditions to be optimized for flavour retention, having due regard for other requirements such as destruction of microorganisms (Casmir and Whitfield 1978). In most flavour laboratories odour evaluation of the gas chromatographic effluent is now fairly common practise; participants being asked to describe the odour of components as they elute, either in their own terms or using predetermined adject-ives, the descriptions in some cases also being given an intensity score. The descriptions are usually written alongside the peak with which they are associated (Williams 1978a).

Simple assessment of the gas chromatographic effluent is unlikely to give very much information if the particular odour note being looked for is due to a combination of compounds; unless these compounds co-elute together they may well be missed (Williams and Lewis 1978). A sensory assessment scheme has been proposed (Parliment and Scarpellino 1977) to overcome this problem. A crude separation is then performed and the various fractions combined with one another; those making a contribution to the aroma note in question are re-separated and the process is continued until individual components are being handled. This procedure has been further refined by Casmir and Whitfield (1978) who separated passionfruit juice and recombined fractions for a taste panel. Results from 'sniffing' the GC effluent of headspace samples were also used to help identify the principle organoleptic compounds. To confirm that the compounds selected were indeed responsible for the flavour profile of passionfruit, the molecular species available as synthetic chemicals were recombined to produce a compounded nature identical drink (Casmir and Whitfield 1978).

There are a number of disadvantages in using simple assessment of GC effluent (Williams 1978a). It does not give information on

interactive effects of compounds which do not co-elute, particularly
if they in themselves have little odour. Assessors quickly get
fatigued and, unless multiple splitting devices are used, only one
person can assess the effluent at a time, which requires multiple
analyses to be performed if reliable information is to be obtained.
The eluting compounds often have to be assessed at the elevated temper-
ature of the carrier gas stream and at a possibly higher concentration
than would be present in the headspace of the original food. Further,
when assessing odours on line, decisions have to be made quickly before
the next peak appears. For this reason, many recognizable odours,
because they cannot instantly be allocated names, often get poorly
interpreted (Williams 1978a).

Several methods, all involving some form of trapping and assess-
ment offline, have been developed to overcome these difficulties. The
simplest uses a gas syringe for collecting the volatiles, which can
then be dispensed to a panel, small portions at a time (Tucknott and
Williams 1974). More elaborate procedures bubble the eluate through
water (Parliment 1976) or trap it on Celite (Clark and Cronin 1975a)
or Lactose (Gramshaw 1976) from which it can be dispensed into the
medium of choice for assessment by a panel of judges (Williams 1978a).
It should be borne in mind that the character of compounds may be con-
siderably affected by the medium in which they are assessed. For
example, von Sydow *et al.* (1970) showed a striking difference in quality
between the odour of aqueous solutions and the equivalent solutions
in 'deodourized' juice. The latter made a clear but uncharacteristic
contribution to the overall odour, probably because of the presence of
substances exerting a very low vapour pressure but with high odour
intensities.

Determination of threshold values and the concept of odour units
is another tool which provides useful information on the relative

importance of compounds (Williams and Lewis 1978). By indicating
the degree by which the concentration of a compound in a food or
beverage exceeds its threshold value in that product, it should be
possible to estimate the contribution it makes to overall flavour
(Williams 1978a; Morrison 1982a and b). The simple application of
such data ignores the interactive effects between compounds (Williams
and Lewis 1978). However, many workers have used this concept, for
example, Williams (1974) in work on ciders, von Sydow (1971a and b)
and Karlsson-Ekstrom et al. (1973) in work on blackcurrants and von
Sydow et al. (1970) in work on bilberry aroma.

The whole concept of odour units is worrying for two reasons.
Firstly, to compare the relative importance of components at concentrat-
ions above threshold the application of odour units assumes a linear
response and an analogous increase in perceived intensity for all
compounds with increase in concentration. Secondly, information obtained
at threshold levels on mixtures cannot necessarily be extrapolated to
the concentrations present in foods and beverages where most components
are present much above this concentration and a different situation
exists (Williams and Lewis 1978).

4.7.3 _Correlation of Analytical and Sensory Data_

While work with individual components and how their properties
are modified in simple mixtures can give insight into the sort of
qualitative and quantitative interactions which may take place, relat-
ing sensory comments to analytical data as a whole is the only true
way to understand what is going on in the food or beverage itself
(Williams 1978b).

In general, the approaches adopted are purely statistical, relying
on such multivariate techniques as multiple regression, discriminant,

canonical and covariance analysis to relate the two sets of data (Williams 1978b). Powers (1981) classifies the aims of multivariate analysis into four categories:

1. to differentiate among products or treatments, evaluate the performance of judges or effect other differentiations;

2. to classify materials, attributes, brands, treatments or panelists responses;

3. to predict sensory quality;

4. to contribute to our knowledge of fundamental sensory knowledge.

In order to achieve these aims Larmond (1979, 1981) considers the choice of sensory method will govern the type of information obtained, and the best method can only be selected for a test by carefully considering the test objective. For example, a flavour profile will yield considerable information but it will not predict consumer acceptability. Sidel et al. (1981) considers scale choice to be a critical factor in achieving maximum precision from sensory tests, since a poor scale can create confusion among subjects or may not lend itself well to statistical analysis. Graphic scaling (use of a diagramatic line) and magnitude estimation are currently the most popular methods (Sidel et al. 1981).

Another important point to realise when relating analytical and sensory data by statistical methods is that any questions asked of the sensory panel should be specific and precise (Williams 1978c). If they are not and people are asked to assess the amount of a poorly-defined or too general an attribute, such as the general term 'flavour', they may well be scoring different things (Lewis and Williams 1978). The sensory profile procedure offers the most promising approach to interpret sensory significance of chemical data in flavour research, but it is

important that terms are defined precisely for the reasons outlined
above (Williams 1978c).

5. ELECTRON MICROSCOPY

5.1 Scanning Electron Microscopy

The use of scanning electron microscopy (SEM) to examine the
surface topography of botanical specimens is a recent but extremely
useful tool in morphological work. Parsons *et al.* (1974) consider
there are two characteristics a good specimen must possess: firstly,
the material must be able to withstand the high vacuum (10^{-5} torr)
needed to operate the electron microscope and secondly, the specimen
must be electrically conducting, to prevent accumulation of surface
charge during examination in the microscope. To overcome these
difficulties a number of preparative techniques have been designed to
preserve material for successful examination.

Parsons *et al.* (1974) examined a number of techniques used to
prepare plant specimens for electron microscopy. These workers found
that fresh material can often be successfully examined without any pre-
treatment other than mounting on specimen stubs before inserting into
the SEM specimen chamber. With fresh samples progressive dessication,
due to high vacuum, caused cellular collapse after 15-20 minutes and
low accelerating voltages (2-3 kV) were required to minimize specimen
charging, which limited the magnification and resolution capabilities
of the microscope. Parsons *et al.* (1974) found these capabilities
could be improved by coating the fresh material with an electrically
conducting layer before scanning; normal accelerating voltages of 10 kV
could then be used.

Freeze drying techniques avoid the artefacts produced at the water/air interface by airdrying because the specimen is rapidly frozen and the ice sublimated away in a vacuum. However, movement of the solid/liquid and solid/vapour phase boundaries can cause distortion in the specimen; such movement can be avoided by using critical point drying preceded by dehydration (Parsons *et al.* 1974). These workers consider the dehydration steps, not fixation, appear to be the most important stage in the preparation of botanical tissues.

The morphology of the glandular hairs of *Cannabis sativa* have been studied using chemical drying, freeze drying and critical point drying (Hammond and Mahlberg 1978 and 1977). These workers consider no single method of preparation of SEM specimens is certain to be free of arte-fact. Chemical alterations and dehydration can cause structural abnormalities, thus, interpretation of real structure can best be achieved through comparison of specimens prepared by a variety of methods.

SEM techniques have also been used to study oil glands in pepper-mint (Clark and Menary 1982), where two types of glands were identified; a ten celled glandular trichome and a three celled glandular hair. In hops (Menary and Doe 1983) the cup shaped Lupulin glands were observed to swell in size, finally forming a structure similar to an ice cream cone due to lifting of the cuticle. The cup shaped layer of secretory cells exuded oily substances into the intra-cellular space below the cuticle, as the volume of secretion increased the cuticle was raised to produce a swollen gland (Menary and Doe 1983).

Oil glands on leaves of *Ribes nigrum* have been examined by scanning electron microscopy previously (Atkinson and Blakeman 1982). These workers reported that the oil glands senesce early in the season, releas-ing their contents onto the surface of the leaf.

5.2 Transmission Electron Microscopy

Hammond and Malberg (1978) extended their work on Cannabis to include ultrastructural studies which involved using transmission electron microscopy (TEM) techniques. The advantage of TEM is that it enables close study of gland structures in relation to their functional secretory activity. The investigations of Hammond and Malberg (1978) revealed the development of the release of secretory materials in Cannabis glands. These workers noted that lipophilic glands of the terpene type are characterized by their dense ribosomal groundplasm and extensive development of smooth endoplasmic reticulum during the secretion phase.

In studies on *Newcastelia visicida*, Dell and McComb (1975) showed that as the glandular head of the trichome developed there was a marked increase in the density of ribosomes. Towards the secretion stage the plastids greatly increased in number and appeared as bodies of various shapes with few internal membranes. This was paralleled by a proliferation of both smooth and rough endoplasmic reticulum which surrounded the plastids. These workers suggest both the endoplasmic reticulum and the modified plastids take part in secretion of terpenes. Similar ontogeny studies of glandular trichomes in *Chrysthemum morifolium* (Vermeer and Peterson 1979) and hops (Menary and Doe 1983) confirmed the likelihood that plastid-entoplasmic reticulum associations function as the site of terpene synthesis.

CHAPTER III

EXPERIMENTAL METHODS

1. PLANT MATERIAL

The material used in this study was obtained from a number of commercial Tasmanian fruit farms; B. Downie, Bream Creek; A.A. Wright, Glen Huon; Elders IXL, Bushy Park; and two research stations; University of Tasmania Horticultural Research Centre, Mt. Nelson and the Huon Horticultural Research Station, Grove.

The material was identified according to Todd's (1962) classification and all cultivars were clearly marked and kept separate. The canes were collected as they were cut, bagged, transported to Hobart the same day and stored at 2°C until the buds could be picked off. All bud material was subsequently stored at -18°C until extraction.

2. HARVESTING

2.1 Manual Harvesting

Harvesting the buds by hand is a laborious, time-consuming task. The buds are readily removed from first year canes by running the hand down the cane. Removal of buds from second and third year wood is much more difficult due to the fact that the buds are produced on tough woody spurs.

2.2 Chemical Harvesting

The difficulties associated with hand picking the bud material prompted an investigation of the suitability of using abcission

chemicals to loosen the blackcurrant buds. A series of three experi-
ments were set up to examine the effectiveness of Ethrel Growth Regulator
300 (a product of Ciba-geigy Australia Ltd) in loosening blackcurrant
buds. Ethrel is 48% active ingredient Ethephon.

Experiment 1: In order to test the effect of Ethrel concentration on
bud attachment a series of five concentration and two control treatments
were applied to ten blackcurrant plants each (cultivar White Bud), stand-
ing in the field. At the time of treatment these plants carried only
first year canes. The treatments applied were:

T_1 control: unsprayed

T_2 control: water and urea (0.5%) spray

T_3 0.01% Ethrel plus urea (0.5%) spray

T_4 0.05% Ethrel plus urea (0.5%) spray

T_5 0.10% Ethrel plus urea (0.5%) spray

T_6 0.20% Ethrel plus urea (0.5%) spray

T_7 0.50% Ethrel plus urea (0.5%) spray

Urea was added to the Ethrel sprays as it has a reported (de
Wilde 1971) biological effect of increasing Ethrel response. The urea
is thought to act by increasing the rate of uptake of Ethephon by tissues
(Poovaiah and Leopold, 1976). Each treatment was replicated three times.
The experimental design was a randomised complete block (3 x 3 x 7).
After treatment, the canes were cut, labelled and packed in plastic bags
for transport to the laboratory; where they were stored at 20°C. A
measure of bud attachment was taken at H_1 = 2, H_2 = 8 and H_3 = 14 day
intervals after treatment. The measure of bud attachment was achieved
by placing the canes in a large polyweave bag, sealing the bag and mech-
anically beating it to dislodge the buds. The buds remaining on the

canes after such treatment were handpicked.   A bud attachment ratio was then defined as:

$$\frac{\text{weight of buds removed by beating}}{\text{total weight of buds removed (beating + handpicked)}} \quad x \quad \frac{100}{1}$$

Experiment 2: The effect of temperature on Ethrel induced bud abcission was examined by a series of storage temperature treatments at one Ethrel concentration.   The Ethrel was applied at a rate of 0.5% (plus 0.5% urea), to canes cut from the field that morning.   These canes were sealed in plastic bags and stored separately for three weeks at four temperatures; 2°C, 10°C, 20°C and 25°C; each having two replicates. Two harvests were taken at 12 and 21 days after treatment. The control treatment consisted of unsprayed canes.   The bud attachment ratio was measured at harvest as previously described.

2.3 Mechanical Harvesting

In order to simplify harvesting operations the design of a simple machine to pick buds from one year old canes was investigated. After considering the following characteristics of the blackcurrant bush (Figure 2.3.1) - canopy shape, distribution of buds on wood of different ages, attachment of buds to the cane (Figure 2.3.2) and its ability to produce new shoots from the base after pruning; it became obvious that an efficient mechanical harvester design would need to incorporate the following principles:

1. Destructive harvesting in a stool bed situation, as first
   year canes possess the greatest number of buds.

2. A directional force, F, would be required to lift the
   bud up from the cane and break the pedicel (Figure 2.3.3).

This was achieved by using a pair of rotating brushes
to produce the directional force (F) required; at the
same time employing infeed and outfeed rollers to hold
the cane firm in the path of these brushes. On this
basis, several prototype models were constructed and
tested.

An estimate of the picking efficiency of the prototype harvester
was made by running batches of single and bunches canes through the
machine. The numbers of buds removed in one, two or four passes
through the machine with various brush configurations were totalled
and percentage efficiency figures calculated.

FIGURE 2.3.1 Canopy shape of the blackcurrant bush

FIGURE 2.3.2 Attachment of bud to cane
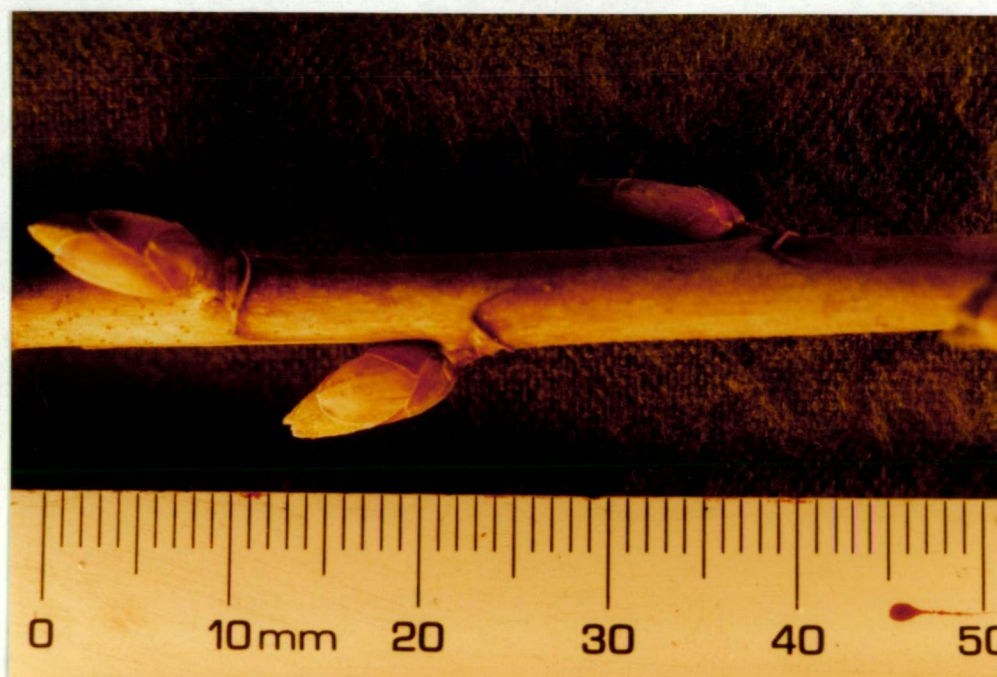
FIGURE 2.3.3
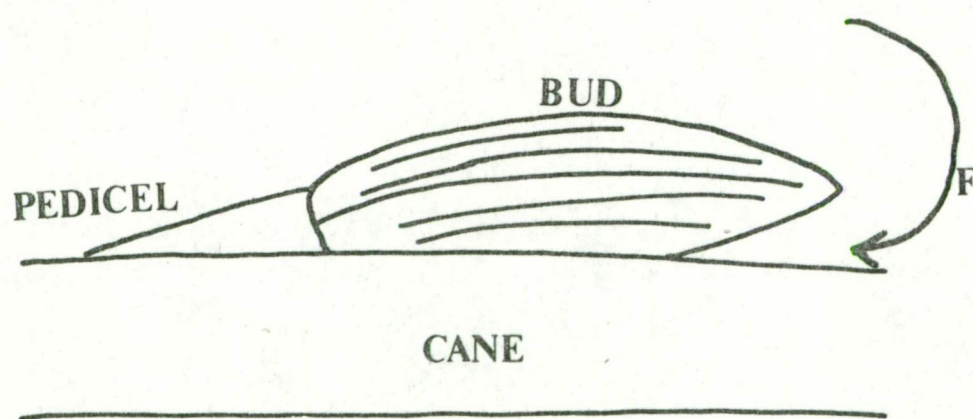
3. EXTRACTION PROCEDURES

3.1 Solvent Extraction

The bud material was macerated with petroleum ether (40-60 BP)
in a 3 ℓ stainless steel blender; small samples were placed in glass
preserving jars, and agitated on a mechanical shaker for three days.
Larger samples were placed in a 200 ℓ stainless steel extraction drum
which was rotated overnight (Figure 3.1.1). In both cases the solvent
was drawn off and transferred to a rotary vacuum evaporator (Bucchi);
it was then removed by evaporation under conditions of reduced pressure
(30°C, 0.7 mmHg). A dark green resinous concrete with a strong black-
currant aroma was obtained. All solvents used were acid washed with
concentrated sulphuric acid, neutralized with 1 M sodium hydroxide,
and redistilled before use to remove rubbery notes detected when the
untreated solvent was evaporated to dryness. To determine if a higher
quality product could be obtained, a series of extraction experiments
were undertaken using a range of solvents other than petroleum ether.
The solvents used were: n-pentane, n-hexane, methanol, methanol/hexane
and methanol/pentane.

An absolute was prepared by redissolving the concrete in redistilled
ethanol, and allowing the mixture to stand at -20°C for several hours;
allowing for settling of undissolved waxes. The ethanolic solution
was filtered using Buchner filtration and Whatman No. 1 filter papers.
A Bucchi rotary vacuum evaporator was then used to reduce the ethanol
volume.

3.2 Liquid Carbon Dioxide

By utilizing a pressurized container fitted with a cold finger

FIGURE 3.1.1   Rotary Extraction Drum
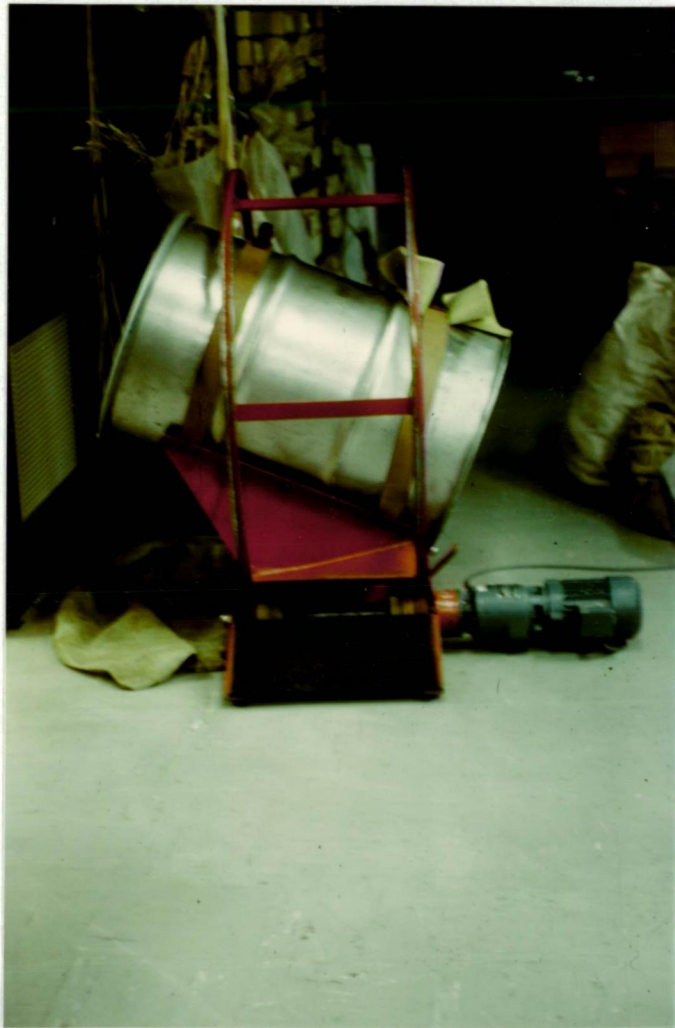
condensor, it is possible to use liquid carbon dioxide as the solvent in a standard glass Soxhlet extractor. Such a device has been described by Jennings (1979) and is commercially available from J & W Scientific. The sample is placed in a Whatman extraction thimble in the neck of the Soxhlet extractor. A predetermined quantity of dry ice is placed in the chamber outside the extractor, the needle valve is fully opened and the cover-plate condenser assembly secured in place. During this step, the rising blanket of carbon dioxide displaces air from the chamber. The needle valve is then closed, the chamber placed in a shallow water bath (35-40°C), and ice water is passed through the cold-finger condensor. At these pressures any impurities in the dry ice (including water) remain in a condensed state outside the Soxhlet, and pure carbon dioxide drips into the extractor. At the end of the extraction period the apparatus chilled to sub-zero temperatures by placing it in a liquid nitrogen bath. The carbon dioxide solidifies, and the needle valve can be opened to discharge the carbon dioxide under conditions where the vapour pressures of the extracted materials are extremely low. When the carbon dioxide has sublimed, the apparatus is opened to yield a solvent-free extract. A later modification provides a method of supplying carbon dioxide in the liquid form direct from the supply bottle; this provides for much easier operation. However, it is necessary to ensure the quality of liquid carbon dioxide by running a test using an empty sample thimble.

3.3 Vacuum Distillate

Approximately 2 g of concrete was placed in a sidearm flask and vacuum distilled (0.7 mmHg) for 45 minutes, while applying gentle heat (30°C) to keep the concrete liquified. The volatile oil was collected in two U-tube traps placed in the vacuum line, both of which were immersed

in liquid nitrogen.

4. SEPARATION TECHNIQUES

4.1 Liquid Solid Chromatography

(a) Silica gel: Blackcurrant bud concrete was dissolved in 10 mℓ of

petroleum ether and centrifuged at 3000 rpm for 5 minutes.   The super-

natant was eluted on a 30 cm silica gel column (60-200 µ mesh) packed

in pentane.   The silica gel was prepared for chromatography by treat-

ment with 0.01N hydrochloric acid (to remove metal impurities) then

washed with deionized water until neutral. To prevent possible acid-

catalysed reactions (Scheffer et al. 1976a), the acid washed silica

gel was treated with dilute ammonia pH 8.2 and then washed again until

neutral.   It was dried at 105°C, then wetted to a water content of 5%

for improved separation of terpenes (Scheffer et al. 1976a).

Fractions were eluted with a series of 20 mℓ volumes of three

diethyl ether/pentane mixtures (5%, 10% and 15%), followed by 30 mℓ each

of 25% and 50% diethyl ether in pentane.   The volume of the first two

fractions collected was 10 mℓ, all succeeding ones were 5 mℓ.   The

solvent was removed under a gentle stream of nitrogen to concentrate

the samples. The fractions were then examined by gas chromatography

and mass spectrometry.   The vacuum distilled oil was separated by the

same procedure.

(b) Florisil: A second liquid-solid chromatography method using

Florisil as a packing was also developed to confirm results obtained

on the silica gel.   The Florisil was activated by ignition at 600°C

for two hours using a furnace oven, then allowed to cool in the oven

overnight.   Before use it was reactivated by heating for two hours at

130°C and then stored in a dessicator.

Two mℓ of blackcurrant vacuum distillate was introduced to a 350 mm x 26 mm LKB column packed with Florisil in petroleum ether. Elution being started at a flow rate of 3 mℓ/min. Fractions were eluted with 1500 mℓ petroleum ether, followed by 600 mℓ diethyl ether and finally 300 mℓ dichloromethane. Fractions were collected as 100 mℓ samples and reduced in volume under a slow stream of nitrogen before injection.

(c) Micro Column: The simple micro column pre-fractionation technique described by Murray and Stanley (1968) was modified to use a variety of different polarity solvents. The silica gel was prepared as described above (Section 4.1a) and then dry packed into 6 mm diameter teflon tubing. The tubing was cut into 16 cm lengths and plugged with silanized glass wool. A 20 µℓ sample of vacuum distillate was loaded onto the top of the column, a further 1 cm of silica gel was added and the column sealed with silanized glass wool. The column was supported inside a glass tube, inverted and developed for approximately three hours in one of the following solvents: methylene chloride/pentane, 40/60 ($^*\epsilon° = 0.24$); methylene chloride ($\epsilon° = 0.32$); diethyl ether ($\epsilon° = 0.39$); and methanol ($\epsilon° = 0.75$). The column was then removed from the solvent, sectioned into 2 cm pieces, each of which was washed with a small amount of developing solvent, filtered through sintered glass funnels (porosity 3) and reduced in volume under a slow stream of nitrogen.

4.2 High Performance Liquid Chromatography (HPLC)

A satisfactory procedure to separate blackcurrant bud oil into oxygenated monoterpenes, monoterpene hydrocarbons and sesquiterpene

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[*] $\epsilon°$ polarity values for developing solvents taken from Snyder and Kirkland (1978).

hydrocarbons was developed using an octadecylsilane-bonded silica
phase (Radial-PAK μ Bondapak C18, 10 μ particle size). The HPLC
system was operated on a semi-preparative scale with a resolution
much superior to the ordinary column silica chromatography. The
mobile phase consisted of methanol and water, which allowed for low
UV monitoring and resulted in a good separation of the three groups.

The HPLC system specifications are as follows:

- Waters Associates dual solvent delivery pump system (model
  6000A)
- a radial compression module (RCM - 100)
- universal injector (model UK6)
- discrete multiwavelength absorbance detector (model 440)
  with an extended wavelength module fitted with a 214 nm
  Absorbance Kit
- an Omniscribe B-5000 series recorder.

The solvent program consisted of stepwise elution with methanol/
water 82.5:17.5 or 75:25 for twenty-five minutes and then pure methanol
for fifteen minutes at a flow rate of 4 mℓ/min. The column used was
a Radial Pak μ Bondapak C18 reverse phase cartridge.

4.3 Gas Liquid Chromatography

(a) Chromatograph

Gas chromatography (GC) analysis of oil samples and chromatography
fractions was conducted using a Pye Unicam Series 104 chromatograph
fitted with an FID detector. Initially this chromatograph was connected
to a Pye Unicam PD88 integrator and a Rikadenki chart recorder. How-
ever, in later work a Sigma 10 (Perkin Elmer) data station was used to

collate the information.

For routine analysis and aroma profile identification a 50 m x 0.5 mm ID OV 101 SCOT capillary column was employed. Operating conditions were as follows: carrier gas was nitrogen at a gas velocity of 153 cm/sec, air flow rate 600 mℓ/min and hydrogen flow rate 60 mℓ/min. At the effluent end of the column, nitrogen was used as a make-up gas at a flow of 60 mℓ/min. The column oven temperature was programmed from 80 to 200°C at 5°C/min.

For determination of KOVAT's retention indices, a 50 m x 0.02 mm ID Fused Silica OV 101 column was used with nitrogen, gas velocity 51 cm/sec, as a carrier. An injection volume of 0.5 µℓ was sampled from all eluted fractions and 0.02 µℓ from all concentrated oil extracts.

A dual detection system utilizing a Hewlett Packard 583A gas chromatograph fitted with an FID and a photometric detector was also employed. The column employed was a SCOT 30 m SP2100, with a helium carrier gas velocity of 51 cm/sec and make-up gas nitrogen. The injector temperature was 230°C with detector temperatures 250°C and 230°C for FID and photometric detectors respectively. The oven temperature was held at 60°C for 5 minutes, then programmed 60-175°C at 5°C/min, then held for 2 minutes before being programmed to 190°C at 5°C/min. This system was employed to examine the three regions determined to be of organoleptic interest for any sulphur containing compounds.

(b) Effluent Traps

For trapping of single components from GC analysis, a splitter (100:1) was attached to the effluent end of the column. A teflon sleeve was slipped over the sniffing port and capillary haematocrit tubes were inserted into the other end of the teflon sleeve to act as traps. These were sealed with teflon caps and stored at -20°C until

analysed by gas chromatography/mass spectrometry.

(c) Headspace Analysis

In order to examine the headspace above samples a Pye Unicam Headspace analyser (model 4750) was connected to the injection end of an OV 101 SCOT glass capillary column (50 m x 0.5 mm ID). The head-space analyser comprises a control unit, head unit and valve unit. The free-standing control unit contains all the instrumentation necessary to determine and maintain the basic parameters of the system - gas flows, head temperature and cooling of the precolumn. The head unit, mounted on the injection port of the Pye Unicam 104 Chromatograph, houses the sample chamber and heater. The valve unit, mounted on the side of the chromatograph, has two needle valves, to set the split ratios, and a three-way valve.

In the standby mode carrier gas flows through the standard carrier line to the column, but not to the head unit. The heaters are off and the precolumn is not being cooled by carbon dioxide. The top section of the head unit is opened and a liquid sample, impregnated on filter paper, is placed in the glass sample chamber. The head unit is reassembled and equilibrated to the desired temperature, while the precolumn is cooled with carbon dioxide. The head unit is flushed, by diverting the carrier gas to the sample chamber, to sweep the headspace vapours onto the pre-column (which is still being cooled). The components of interest in the gas stream are cryogenically trapped, while the carrier gas is vented through a splitter vent. The heater and the carbon dioxide gas are then shut off, the sample chamber sealed with the three-way valve, and the column oven temperature allowed to stabilize. The splitter valve is switched over to the low flow setting and the precolumn flushed with carrier gas to inject the volatiles onto the OV 101 column.

The basic temperature program of 80-220°C at 5°C/min was used and later modified to start the program at various temperatures from 50 to 80°C. The headspace analyser was also connected to the combined GC/MS facility described in Section III 5.2 in order to identify early peaks detected.

5. IDENTIFICATION OF COMPONENTS

5.1 Retention Indices

For determination of KOVAT's retention indices (Kovats 1958 and 1965, Ettre 1964 and 1972), a 50 m x 0.02 mm ID Fused Silica OV 101 column was used. A series of n alkane standards from n-octadecane to n-hexadecane (Mix 1), and a series from n-octadecane to n-dodecane (Mix 2), were mixed in equal proportions. Retention indices were determined isothermally at 120°C (using Mix 1 and Mix 2), 140°C (Mix 1) and 160°C (Mix 1). Retention indices were also determined during temperature programming from 80-220°C at 2°C/min using Mix 1. To ascertain the retention indices the Pye Unicam 104 chromatograph was linked to a Perkin Elmer Sigma 10 data station.

5.2 Gas Chromatography/Mass Spectrometry

The combined gas chromatography/mass spectrometry facility con-sists of a Pye Unicam 204 chromatograph directly coupled, via a glass-lined steel tube (heated at 200°C) to a VG Micromass 70/70F mass spectro-meter. The spectrometer is a high resolution, double focussing model operated at an ionizing energy of 70 eV, a 4 KV accelerating voltage and an ion source temperature of 200°C. The range M/Z 300 to 20 was scanned exponentially downward at 1s/decade, resulting in a full mass

* footnote: The triangle tests were used for comparison of the
strength of the catty and blackcurrant fruit notes using an
objective scale of 1 to 4 (most intense). A subjective assess-
ment as to whether the fruity and catty notes lacked balance
with each other was also made. These results are described in
Section IV 8.1. The author was the sole assessor, but his ability
to discern comparative differences was proved through a series of
triangle tests presented in Appendix 14. The two French industry
products were used as references to provide guides for desirable
quality extracts.

spectrum every two seconds. The data was stored in a VG2035 data
system. Spectra were enhanced by background subtraction, generation
of reconstructed spectra and gas chromatograms (Biller and Biemann
1974) where necessary. Gas chromatograms were represented by Total
Ion Current (TIC) changes with time. Library search facilities were
also available using a seven major peak search capability. A fused
silica OV101 column was used with a hydrogen carrier flow rate of
1.5 mℓ/min.

6. ORGANOLEPTIC ASSESSMENT

6.1 Comparative Analysis

The method of analysis consisted of olfactory examination of two
French industry products, made available from commercial sources, and
comparison of the aroma impression of these standards with products
extracted in the laboratory using triangle tests (Larmond 1977). For
calibration of the operator see Appendix 14. The two standards used were:

(i) CAL cassis concrete, a benzene extract produced by
Camilli, Albert and Laloue, a division of Pfizer,
Grasse, France. This sample was taken up in propylene
glycol and donated by Bush, Boake and Allen Ltd, London.

(ii) Bourgeons de Cassis Absolu B. Tradition 6 39002
obtained direct from the producer J & E Sozio S.A.
Grasse, France as a gift.

In addition to this comparison, a range of samples was prepared
and sent to two companies. These companies undertook an organo-
leptic evaluation of these samples to test commercial acceptance of

Tasmanian Blackcurrant Concrete. The three companies involved were J & E Sozio S.A. Grasse, France; Dracogco (Far East) Ltd, Hong Kong, and Hasegawa Co Ltd, Tokyo, Japan. A list of samples submitted and comparisons requested are as follows:

Samples 800 1980 Bulk concrete

810 1981 Bulk concrete

820 1982 Bulk concrete (a) handpicked whole buds

821 1982 Bulk concrete (b) machine harvested

822 1982 Varietal selection Grahams No. 1 WB

823 1982 Varietal selection Goliath

824 1982 Varietal selection Baldwin

825 1982 Varietal selection Boskoop

826 1982 Varietal selection Lees Prolific

827 1982 Varietal selection Kerry

828 Liquid CO_2 extract

Comparisons requested

1. Any difference in quality over time?

(800, 810, 820)

2. Any effect of machine harvest on quality?

(820, 821)

3. Any varietal preference?

(820, 822, 823, 824, 825, 826, 827)

4. Quality assessment of liquid CO_2 extract

(828 with all others).

6.2 Aromagram

Aromagrams were collated using the glass capillary OV 101 column with a splitter (100:1) attached to the effluent end of the column.

The sniffing port was maintained at about 50°C to prevent condensation of effluent gases. Aroma sensations were written on the chart paper as they were detected at the sniffing port. This type of assessment requires a high degree of concentration due to the number and intensity of aroma sensations present. To maintain an efficient sniffing program no more than three runs attempted before a break in the fresh air was taken. The terminology used to describe aroma sensations was flexible using vocabularies developed by Williams (1975) for cider and Meilgaard et al.(1979, 1982) for beer, as well as free interpretation impressions detected.

7. MICROSCOPY

7.1 Scanning Electron Microscopy (SEM)

Blackcurrant buds were sampled at two intervals; the first, from early May to late July 1980, and the second, early October 1980 to February 1981; using plants (cultivar White Bud), growing at the Horticultural Research Centre. The buds were dissected under a light microscope and each was found to consist of up to eleven bracts. The bracts were fixed in 1% Osmic Tetroxide vapour for two hours, then removed to clean vials and placed in a deep freeze for a minimum of thirty minutes. The bud bracts were then freeze dried overnight in a Dynavac FD16 High vacuum freeze drying unit. The following morning the bracts were removed from the deep freeze and allowed to warm to room temperature for 1-2 hours before the vacuum was released. The bracts were then stored in a vacuum dessicator until mounted on brass stubs using Dotite, a silver conducting paint. The samples were then coated with a 20 nm layer of carbon and then gold before examination in a Joel KXA 50A scanning electron microscope-electron probe micro analyzer (operating with

an accelerating voltage of 15 KV). The electron micrographs were recorded on Polaroid type 107 film. In the second interval fresh bracts were mounted and coated with carbon and gold before examination in the microscope, thus avoiding the freeze drying and fixation procedures.

7.2 Light Microscopy

Blackcurrant bud samples were collected from plants, cultivar White Bud, growing at the Horticultural Research Centre. The buds were separated into their component bracts under a dissecting light microscope.

The bud bracts were fixed overnight in a 4% glutaraldehyde solution containing picric acid, then washed with 0.1 M sodium cacodylate buffer pH 7.2 for thirty minutes. The bracts were then transferred to clean vials, containing cotton wool, for post fixation with one drop of 1% Osmium Tetroxide. After two hours of exposure to Osmium Tetroxide vapour the bracts were transferred to clean vials and washed with 1% Uranyl Acetate solution for thirty minutes. The bracts were then dehydrated in a series of ethanol/water solutions, 60% (30 mins), 70% (1 hr), 80% (1 hr), 90% (2 hr), 95% (1 hr) and 100% (1 hr) ethanol.

The bracts were then immersed in a 50:50:Ethanol/Spurrs medium.

Spurrs medium is a low viscosity embedding medium recommended for electron microscopy since it readily polymerizes at 70°C and has good sectioning qualities (Spurr 1969). The bracts were then cast in the medium and when set sectioned using an LKB ultra microtome. The sections were mounted on glass slides stained with crystal violet and examined under a high power light microscope.

8. GAS EXCHANGE MEASUREMENTS

An infra red gas analyser (IRGA, Grubb Parsons model SB2) was used to measure the change in carbon dioxide concentration of air passed over an attached leaf of a potted blackcurrant plant, cultivar White Bud. An air stream of known carbon dioxide concentration was divided into two components, the first passing directly to the IRGA reference cell while the second was metered at a constant flow through a clear perspex chamber which enclosed part of the leaf (Figure 8.1). Partly depleted of carbon dioxide, the air is then passed to the IRGA sample cell. In order to avoid disturbing the photosynthetic rate it was ensured that the flow rate of fresh air to the chamber was sufficient to avoid depletion greater than 30 $\mu l/l$ (Montieth et al. 1981). Details of this open circuit carbon dioxide monitoring system are contained in Figure 8.2 (reproduced with permission from Clark 1980).

The temperature of the leaf chamber was controlled by adjusting the temperature of water circulating in the chamber's water jacket. This temperature was continuously monitored using a thermocouple placed inside the leaf chamber on the under surface of the leaf. The leaf was placed in position with the petiole in a groove in the lower perspex block, the 'O' ring, petiole and thermocouple were smeared with vase-line as were the adjoining surfaces of the two blocks, this was done to ensure the chamber remained airtight during the experimental period.

In order to ensure control of temperature and humidity in the leaf chamber air supply, humidification was carried out in a water bath maintained at the leaf temperature and the room containing the IRGA system was maintained, as far as was possible, at the temperature of the leaf chamber. To avoid differences in temperature and humidity

FIGURE 8.1   Leaf Chamber

1.   Perspex block;

2.   Perspect water jacket;

3.   Leaf cell (1 dm^2);

4.   Gas inlet (1000 mℓ/mℓ);

5.   Gas outlet;

6.   Water inlet;

7.   Water outlet;

8.   Wing nuts and bolts to tighten chamber;

9.   Neoprene 'O' ring.

5 CM
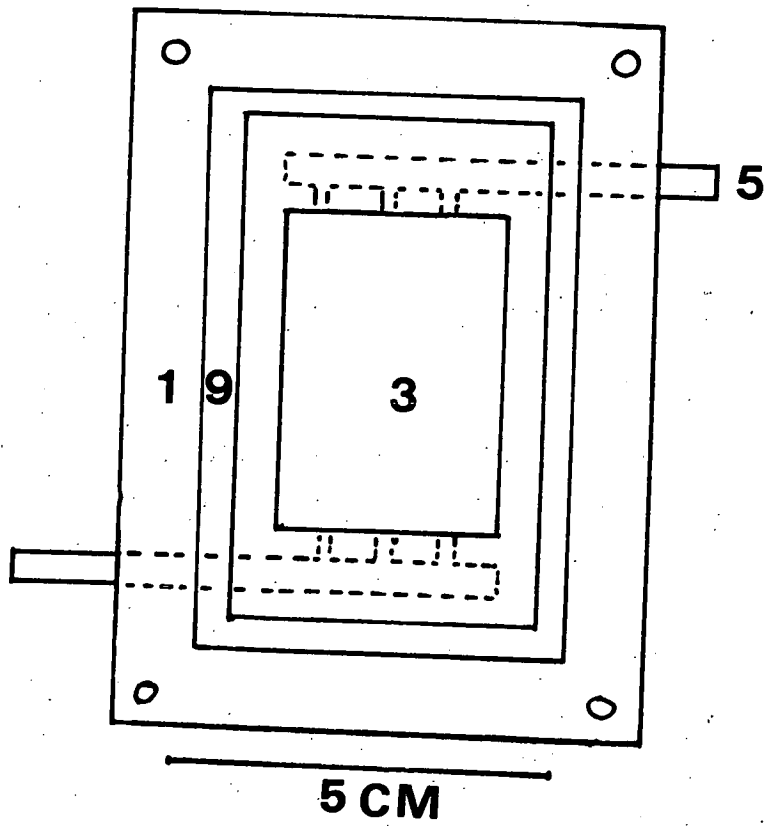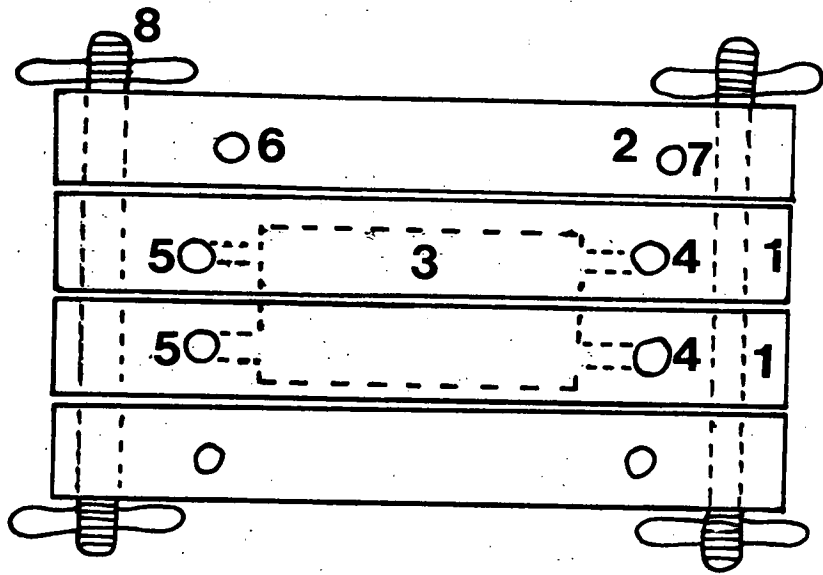
FIGURE 8.2   Diagrammatic representation of the open circuit
             carbon dioxide monitoring system

A.   Gas supply (compressed medical air or 310 ppm CO_2 in N_2).

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

C.   Gas temperature control system and humidification system.

D.   Tubes to remove excess water.

E.   Light cabinet (lined with aluminium foil):

     (i)   Lighting. 4 x 150 W Lugon bulbs,

                       2 x 700 W Philips HPLP lamp.

     (ii)   Light intensity control. Sarlon shade screens.

     (iii) Water bath.

F.   De-humidification system. Test tubes immersed in ice-salt

     mixture contained in vacuum flasks.

G.   Drying tubes containing Drierite.

H.   IRGA, Grubb Parsons SB2.

I.   Flowmeters (1000 mℓ/min^{-1}).

J.   Chart recorder.

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

polythene joints).

1.   Reference line.

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

          correction).

     (b) Chamber supply line.

FIGURE 8.2 Open circuit carbon dioxide monitoring system
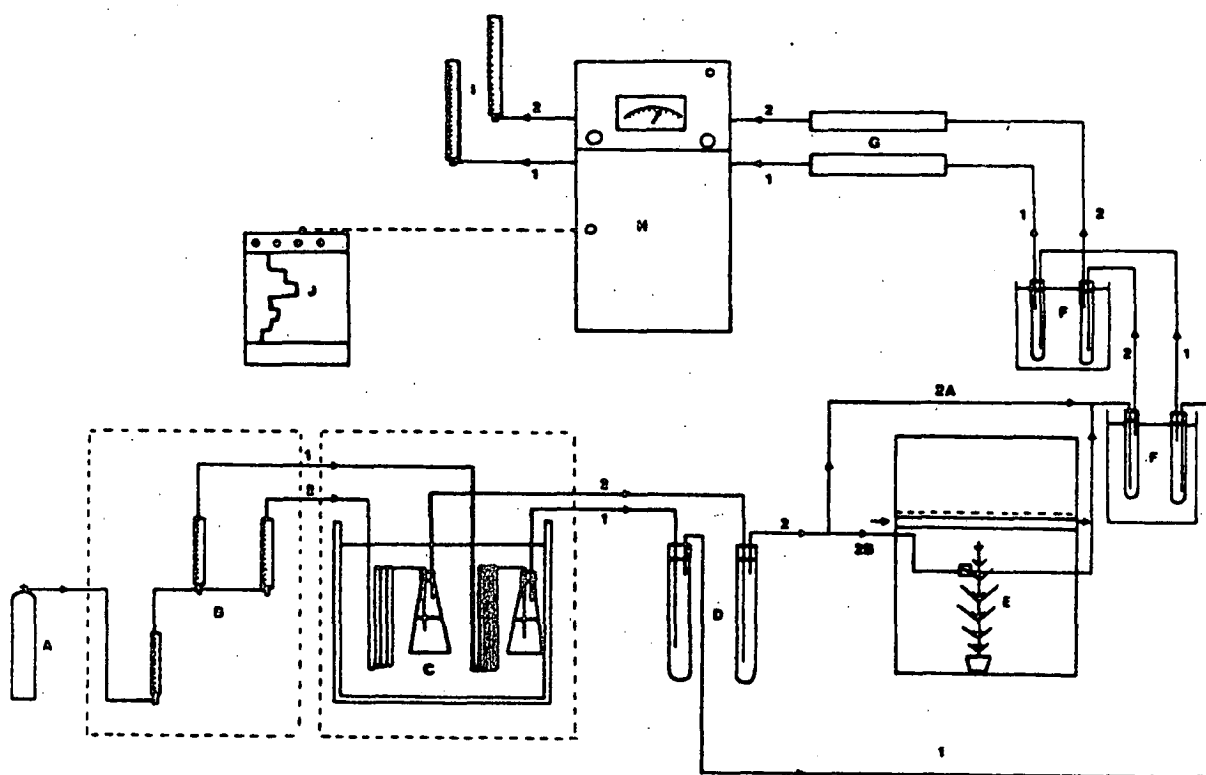
between the leaf chamber and the reference air supply, as well as any effect of the humidification system on carbon dioxide concentration, both reference and leaf chamber air supplies were subjected to the same treatment; with the exception that the reference line did not pass through the leaf chamber.

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

The IRGA was calibrated using gas mixtures of known carbon dioxide concentration (supplied by C.I.G. Hobart). The carbon dioxide concentration was varied in the reference and leaf chamber by-pass line by using mixtures to produce a known concentration differential between the two lines (ΔCO_2). The chart recorder response to changes in carbon dioxide concentration is provided in Appendix VIII 3. From this response it is possible to convert observed chart responses to ppm CO_2 differential between the two lines according to the relation:

$$\Delta CO_2 \text{ (ppm)} = \frac{\text{(chart response)} - 0.2443}{0.4872}$$

At commencement of each day and every two hours the ΔCO_2 between the two reference gases was rechecked. Base line correction of the chart recorder was obtained by passing air, with the same carbon dioxide concentration, through both lines (i.e. $\Delta CO_2 = 0$).

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

$$\text{mg CO}_2 \text{ dm}^{-2}\text{hr}^{-1} = \frac{44\times10^3}{22.4} \times \frac{(1\ 60)}{1} \times \frac{\Delta CO_2}{10^6} \times \frac{100}{10} \quad \frac{\text{mg}}{\ell} \times \frac{\ell}{\text{min}} \times \frac{\text{min}}{\text{hr}} \times \frac{\text{cm}^2}{\text{cm}^2}$$

$$\text{mg CO}_2 \text{ dm}^{-2}\text{hr}^{-1} = 1.1786 \times \Delta CO_2$$

To investigate the response of net carbon dioxide exchange to light intensity, leaves from plants grown in pots (cultivar White Bud) were exposed to varying levels of light intensity, in the leaf chamber. Net carbon dioxide exchange was measured at 24°C from 8 to 1100 $\mu\Sigma\text{m}^{-2}\text{s}^{-1}$. Leaves from these same plants were used to investigate the influence of temperature on net carbon dioxide exchange. Net carbon dioxide was monitored using 21% O_2 310 ppm CO_2 in the light (apparent photosynthesis), and in the dark (dark respiration), while the temperature increased from 10°C to 35°C.

Enhancement of net carbon dioxide using 0% O_2 310 ppm CO_2, over the same temperature range, was to estimate the contribution of photorespiration to the overall net carbon dioxide exchange. All temperature response experiments were conducted at a saturated light intensity of 800 $\mu\Sigma\text{m}^{-2}\text{s}^{-1}$.

9. GLASSHOUSE EXPERIMENTS

9.1 Oil Quality at Bud Burst

Mature field grown blackcurrant plants, cultivar White Bud, were pruned in the winter of 1981 so by May 1982 they consisted of only first year canes approximately 1 m in length. These canes were cut and sealed in polythene bags before being placed in a 5°C cool room, in the dark, for three weeks. After this vernalization treatment the canes were placed in buckets of water in a temperature glasshouse (day temperature 25°C approx. and night temperature 15°C approx.),

under a sixteen hour photoperiod. Samples of opened and unopened buds were then taken at various time intervals up to fifty days after release from the cold room. These were then extracted separately in petroleum ether (40-60 BP) to examine quality differences.

9.2 Carbon 14 Tracing of Oil Synthesis

Mature potted blackcurrant plants (cultivar White Bud), were pruned in the winter of 1981 so that by June 1982 they consisted of first year canes. These plants were first maintained in a bush house but were later moved into the open, at the Horticultural Research Centre, and connected to a drip irrigation system. Bud burst occurred in early September and the plants grew vigorously until the end of the experimental period. Single plants were labelled with $^{14}CO_2$ by first encasing the plants in large clear polythene bags; 0.25 mℓ of radioactively labelled sodium $[C^{14}]$ carbonate (supplied by Amersham International Ltd, Buckinghamshire England) with a specific activity of 57.5 mCi/mmol was then measured into a vial, excess hydrochloric acid 1N (about 5 mℓ) was placed in a second vial. The two vials were then mixed inside the sealed bag using rubber gloves attached to the bag for that purpose. The polythene bag was left in place for one hour to allow the plant time to absorb the labelled carbon dioxide. Samples of stems and leaves were taken for determination of soluble carbohydrate (sugars etc.) and available polysaccharides (starch) according to the method published by Priestly (1965 and 1973).

Leaf and stem samples were dried before extraction at 70°C in a forced air Unitherm drier (Birmingham and Blackburn Construction Co., Birmingham, England). The samples were then extracted in batches of three using 50 mℓ 75% methanol refluxed on a water bath for six hours. This extracted principally sugars and sorbitol as radioactive

components.   The stems and leaves were filtered from solution,

redried and then extracted with 50 mℓ of 5% trichloroacetic acid and

35% methanol under reflux for two hours in order to remove available

polysaccharides.   The stem and leaf matter was filtered out and

again redried.   5 mℓ aliquots of each extract were taken and placed

in glass scintillation vials to which 2 mℓ of Dimilume (a Packard

scintillation liquid) had been added. The samples were then counted

in a Packard PRIAS model PL/PLD scintillation counter using a channels

ratio method of quench correction.

Bud samples were also taken at the same sampling intervals. These

were ground in 3 mℓ of n-hexaneand 0.5 µℓ of tridecane standard using

a mortar and pestle.   A 0.5 mℓ aliquot of this solution was added

to 5 mℓ of Dimilume and counted as described above.

10. FIELD EXPERIMENTS

10.1 The Effect of Plant Density on Yield Factors

The layout used for this experiment is a specific systematic

fan design first described by Nelder (1962) and later by Bleasdale

(1967b).   Within the fan design (Figure 10.1.1), individual plants

are planted in rows which radiate from a single point, with the distance

between plants along a radius approximately equal to the distance be-

tween arcs at that point; i.e. a square plant arrangement. In this

type of design a large range of plant densities, in this case from

11.1 to 1.0 plants/m^2, can be grown in a small area, overcoming the

need for large numbers of guard plants required by randomised complete

block designs.   The calculations required to construct this design

are contained in Appendix VIII 1.

The plant material used in this experiment was a White Bud selection, which had been used as a stool bed in previous years on the Coniston property, Bushy Park, Tasmania. The mature plants were ploughed out of the ground, then pruned heavily to produce a crown for replanting (Figure 10.1.2). The heavy pruning was required to reduce the transfer of blackcurrant borer moth larvae to the new planting site at Sunbury, Bushy Park.

The soil at the experimental site is classified as being part of the lower terraces in the Derwent series, ranging from 18 to 36 m above the present river level (Dimmock 1961). These soils show strongly differenciated profiles and consist of a grey, sandy loam surface over a bleached sandy subsurface; beneath which lies a prismatic or columnar structured clay subsoil, at about 25 cm. The top of the clay horizon is dark stained with organic matter and becomes sandy with depth overlying waterworn gravels (Dimmock 1961).

The experiment was planted in the first week of August 1980 using a specially marked string and protractor to pinpoint the intersection of radii and arcs (Figure 10.1.3). Each intercept was marked with a white wooden peg, and a crown was planted at that point, using a spade (Figure 10.1.4). Each crown was completely covered with soil to reduce the chance of survival of any borer moth larvae.

Fertilizer was applied at the rate of 800 kg/ha of 8-4-10 during August and 200 kg/ha ammonium nitrate spread over three applications in early January of each year. Copper oxychloride was applied at the green tip stage and in late November, at a concentration of 200 g/100 ℓ and a water application rate of 1,150 ℓ/ha to control Septoria leaf spot.

Weed control was achieved by a herbicide program which varied over the three years. In August 1980 Casuron G was applied at a rate

FIGURE 10.1.1

Systematic designs for spacing experiments

A fan design with a 'square' plant arrangement.
The plant positions are represented by dots.
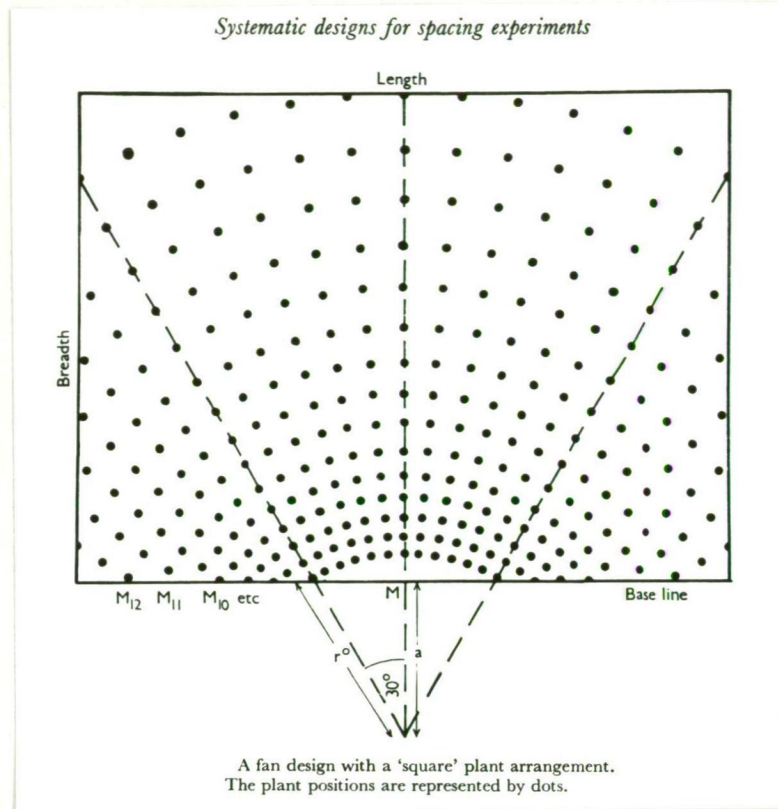
FIGURE 10.1.2

Blackcurrant crown

FIGURE 10.1.3 Laying out fan design with protractor and marker string

FIGURE 10.1.4 Planting blackcurrant crowns at marker points

of 75 kg/ha and gave control of broad-leafed weeds, but not docks, chickweed, clover and a range of grasses. It was subsequently necessary to use Tryquat twice, at a concentration of 2.5 ℓ/ha and a water application rate of 1000 ℓ/ha, and hand hoeing to keep the experimental plots free of weeds. As a result in August 1981 a mixture of Mecopropamine 50 (5 ℓ/ha) and Simatox 80 (1.4 kg/ha) were used in a high volume of water (1000 ℓ/ha) to give good control of the remaining problem weeds. A Tryquat (2.5 ℓ/ha) spray was used to dessicate weeds left from the previous year. In the following August Caroguard 50 F.W. (5 ℓ/ha) was applied mixed with Tryquat (2.5 ℓ/ha) at the same water application rate used above. This last spray gave excellent control of all weeds including docks.

The plots were connected to a fixed overhead irrigation system. Irrigation was used to supplement rainfall such that total available water was 35 mm per week. Any supplementary irrigation required was applied in two applications per week, throughout the growing season.

The plots were harvested in June each year. Each arc was considered to be a plant density treatment. Each bush was pruned at the base of the stems, the canes bundled up and placed in plastic bags with all other plants from the same treatment. After being transported to the laboratory the canes were stored in a 5°C cool room in the dark. For each plant the number of canes, the length of every cane, the total fresh weight of canes with buds still attached, and the yield of handpicked buds was recorded. In 1983 the basal girth of each cane was also measured.

10.2 The Effect of Harvest Date and Plant Density on Oil Yield and Composition

This experiment used the same plots laid out for the plant density trial, outlined in Section III 10.1. Specimens of fifteen

*footnote: Decolourisation with charcoal was to obtain standardisation
of samples presented for GC analysis. No checks were
carried out by the author as to compounds removed by
absorption, but the technique has proved very satisfactory
for peppermint (Clark 1980). Estimate of quantity of oil
was given by total peak area.

buds per density were randomly sampled from seven density treatments over the three blocks; first at weekly intervals from 5 November 1982 to 23 December 1982, then two weekly intervals till 18 March 1983 then three weekly intervals till harvest on 14 June 1983.

Each sample was weighed, then ground in a mortar and pestle with 3 ml of n-hexane. 0.5 µl of a tridecane standard was added at this time along with approximately one gram of sodium sulphate (anhydrous), which assisted the grinding process and removed any water present. The n-hexane extract was transferred to a small vial and decolourized with activated charcoal pellets. The extract was then concentrated under a slow stream of nitrogen and 0.5 µl was injected onto a SCOT OV101 50 m column using the apparatus described in Section III 4.3.

10.3 The Effect of Bud Burst on Oil Quality and Yield

Five kilograms of buds were picked at weekly intervals from 12 August 1983 to 2 September 1983 in the commercial fruit plantation at Sunbury, Bushy Park. These were each subsampled and three 100 g samples set aside. Each sample was itself subsample for a 10 g weight of buds. The total number of buds per 10 g and the percentage of opened buds were recorded. Each 100 g sample was extracted with petroleum ether 40-60 BP, according to the method described in Section III 3.1, and the yield of concrete obtained. The quality of the oil was examined organoleptically by comparison to standard samples as described in Section III 6.1. The composition of the oil was determined by gas chromatography as outlined in Section III 4.3.

10.4 Light Interception, Utilization and Relationship to Planting Density

This experiment utilized the replicated plots at Sunbury, Bushy Park, previously described in Section III 10.1. Fixed sampling sites

were marked at eight selected densities and replicated over the three blocks. The actual sites were randomly selected along each density arc. The sites were 0.5 m² quadrats marked by four pegs constructed from 20 cm of PVC pipe (25 mm diameter). A 0.5 m² quadrat with adjustable legs up to 1.2 m in height was constructed; the tips of the legs just fitting inside the marker pegs.

At weekly intervals from 22 October 1982 until 20 January 1983, and then at twice weekly intervals until leaf fall, measurements of leaf area and light transmission were taken. For the leaf area measurements the quadrat described above was fitted into place at each site, and using infra red film a photographic record was taken. The film used was Kodak High Speed Infra red film 2481; the camera was a Pentax Spotmatic F with 50 mm f4 macro lens, fitted with a red 25A filter. As the crop grew in height it was necessary to use a small step ladder to take the photographs. The film was developed in Kodak D19 high contrast developer for 8 minutes and fixed with Kodak rapid fixer for 5 minutes. The photographs were then printed on ILFORD Ilfospeed 5.1 M grade 5 paper. The leaves appeared white against a grey background. The leaves were carefully cut out and by differential weighing, a measure of leaf area made.

Solarimeter measurements were made using two sets of three tube solarimeters (Delta-T Devices, Cambridge England). Each set was connected through a junction box to a millivolt integrator (Type MV1, Delta-T Devices). A single solarimeter tube and integrator were permanently located in an east-west orientation between blocks 2 and 3. In taking measurements the three tubes were clipped onto a wooden cross-piece to keep them evenly spaced; this crosspiece then slipped into the site marker pegs. The solarimeter tubes were always orientated in an east-west direction. Ten minute readings (I) taken at each site

were compared to complementary readings (I_o) taken at the same time
with the single solarimeter tube.   All readings were taken between
10.00 a.m. and 2.00 p.m. in an effort to keep some control over sun
angles.

The total count given by the integrator is divided by 1.5 to
express the total irradiance in kJ/m^2.

10.5 Varietal Differences in Oil Quality

A number of different cultivars have been collected from 1980 to
1983 at three sites in southern Tasmania. A bud sample of about 100 g
was taken from each variety for extraction and examination of quality.
One site at Marion Bay became unavailable after the first year due to
a change of ownership. The other two sites were at Grove Horticultural
Research Station where Tasmanian Department of Agriculture Officers
were undertaking a variety trial, and at the University's Horticultural
Research Centre where a variety collection is maintained.

The bud samples were extracted as described in Section III 3.1,
analysed by gas chromatography (Section III 4.3) and submitted to
organoleptic assessment (Section III 6).

CHAPTER IV
RESULTS AND INTERPRETATION

1. HARVESTING METHODS

1.1 Manual Harvesting

During the winter of 1980, buds were hand picked from canes grown on a fruit farm at Glen Huon, Southern Tasmania. Table 1.1.1 details the yield of buds picked by each worker on successive eight hour days.

TABLE 1.1.1 Yield of buds (g/day) by hand picking

Day	Worker A	B	C	D
1	1009	1109	1255	1191
2	1157	1306		1066
3	–	1008		1323
Mean	1083	1141	1255	1193

Overall Mean = 1168 g/8 hr day = 0.15 kg/hr

During 1981 and 1982 cane material was not available to pick buds on a large scale. In August 1983 buds were again hand picked but instead of harvesting the canes first, the buds were picked directly from first year canes in the field at Sunbury, Bushy Park, Southern Tasmania. Table 1.1.2 lists the yields harvested from the bushes planted in rows 3 m apart, with 30 cm between plants.

1.2 Mechanical Harvesting

The construction of a mechanical harvester to pick blackcurrant buds was an evolutionary process beginning with the consideration of the forces

TABLE 1.1.2 Yield of buds hand picked in the field

Date	Man hours worked	% buds open	Bud Weight (kg)	Yield (kg/hr)
12/8	8	0	5.2	0.65
19/8	9	7.3	5.8(5.38)	0.64(0.60)
26/8	8½	42.1	10.3(5.96)	1.21(0.70)
30/8	38	48.4	49.9(20.71)	1.31(0.55)
2/9	9	94.2	16.6(0.96)	1.84(0.11)

The figures given in brackets are adjusted figures based on the percentage of buds open at harvest time.

required to remove the bud, as outlined in Section III 2.3. The first prototype consisted of a pair of steel drums covered with pieces of stipuled rubber (Figure 1.2.1) similar to that used on the backs of cricket gloves (supplied by Thomson Rubber Products, Woongoolba Queensland). The rollers were mounted on a T shaped frame (Figure 1.2.2), with the two picking rollers rotating at the same speed and in the same direction. Originally the rollers were mounted a little way apart, but were later moved closer so the protruberances just touched (Figure 1.2.3).

Progress of the stem through the picking rollers had to be slowed to allow sufficient dwelling time between the rollers for picking. A pair of feeding rollers, which were constructed from steel shafts (37 mm diameter) covered with high vacuum rubber hose (supplied by Dynavac, Australia), were used for this purpose (Figure 1.2.4).

This design proved partially successful in removing buds (40% efficient), although several disadvantages were apparent. Firstly, the rubber material used was very soft and deteriorated quickly. Contamination of the buds with the rubber also meant that the quality of the flavour product produced by solvent extraction was poor, containing many rubbery off notes.

FIGURE 1.2.1 Three types of picking surface use in prototype harvesters
(a) stipuled rubber; (b) rubber strips; (c) nylon brushes
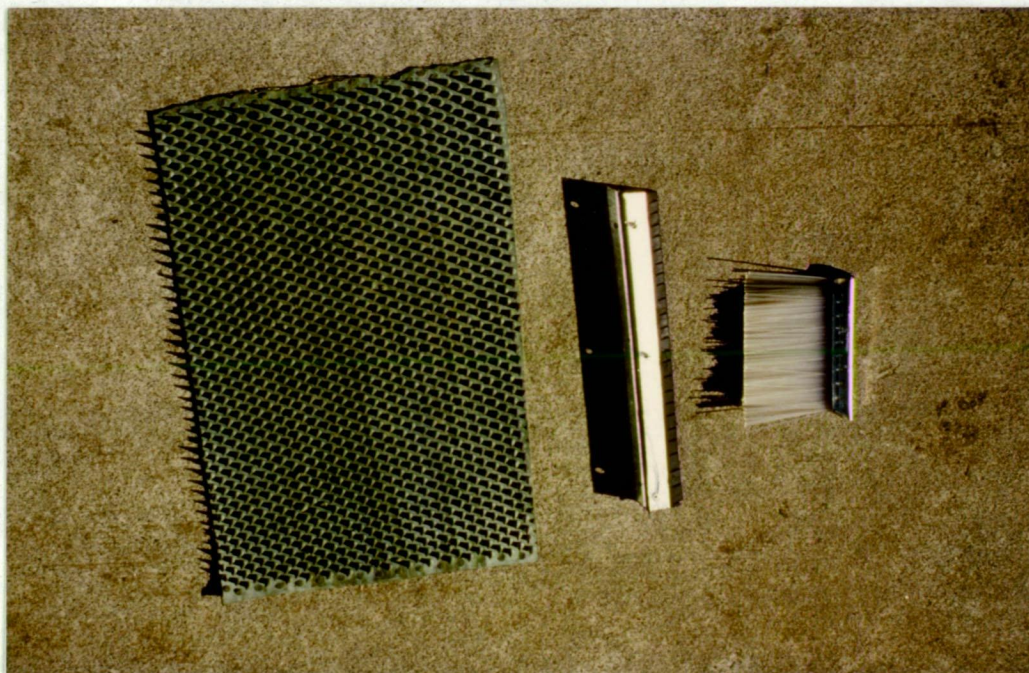
FIGURE 1.2.2 Prototype harvester I
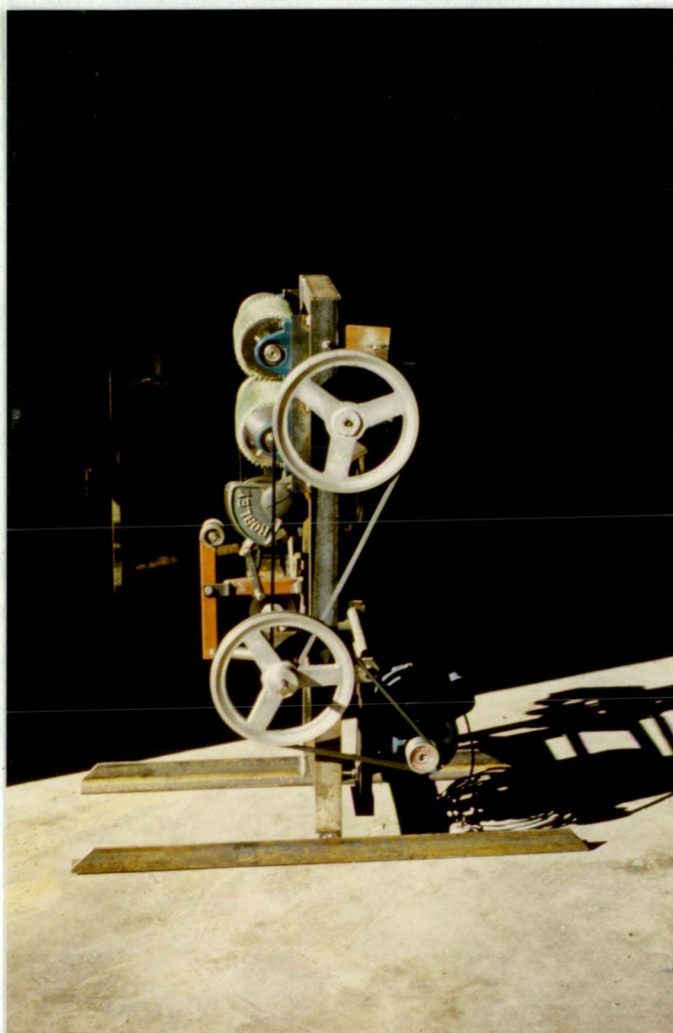
FIGURE 1.2.3   Picking rollers Prototype I
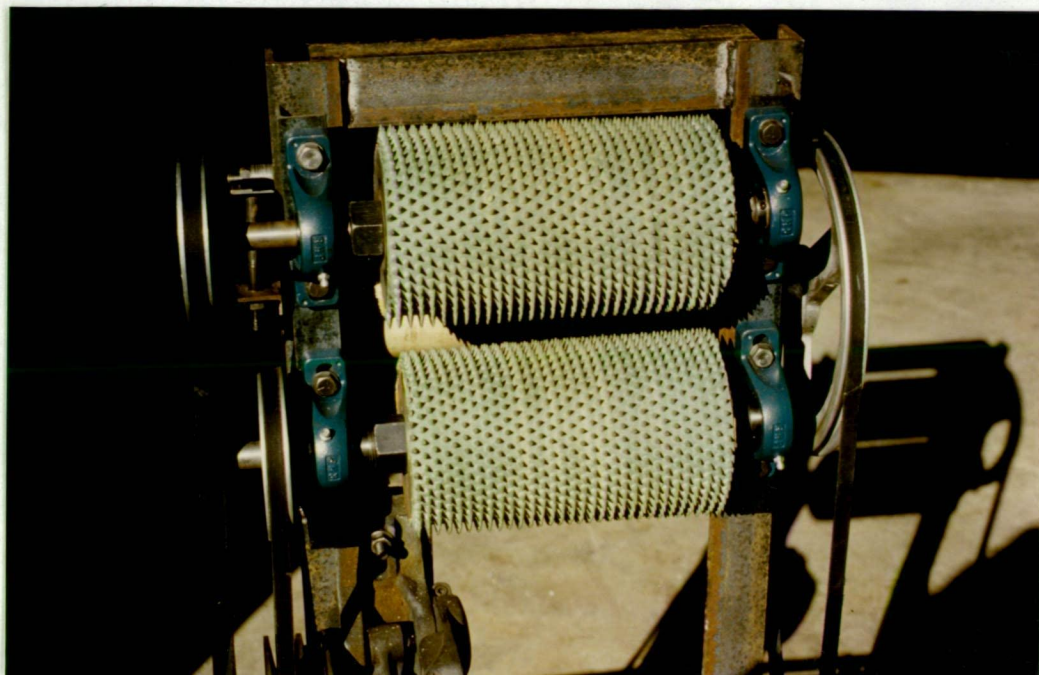
FIGURE 1.2.4 Infeed rollers Prototype I
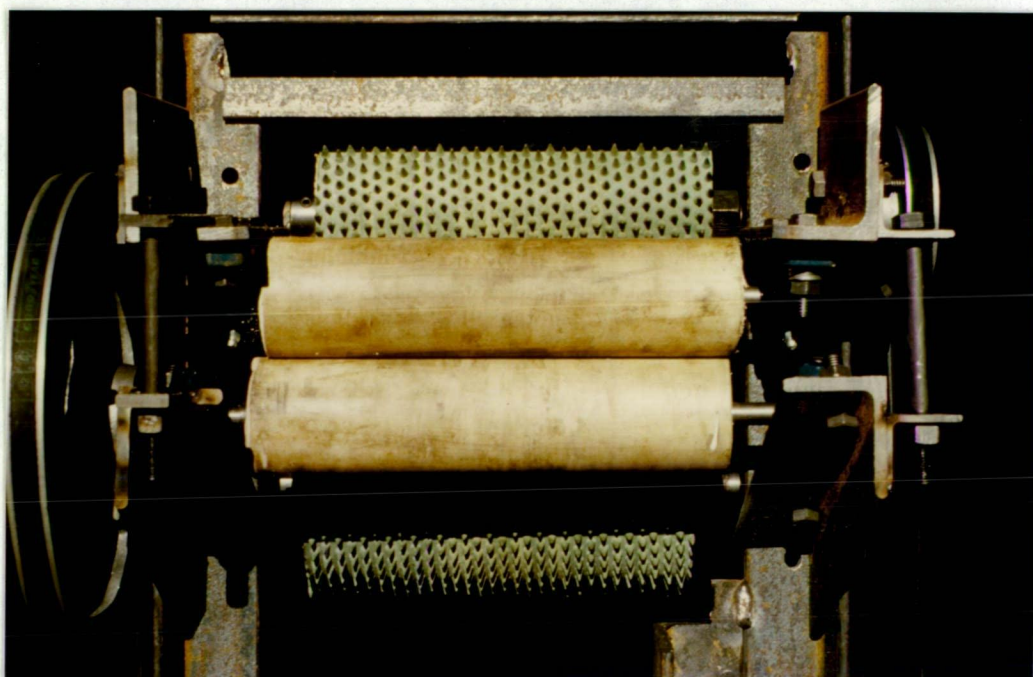
Secondly, having only a single pair of infeed rollers meant that buds from the posterior end of the cane were not picked at all. When the cane left the infeed rollers there was no resistance to forward movement and it was carried through at the same speed as the picking rollers. It was found that a brushing action would remove the buds. This brushing action was investigated by replacing the rubber material on the drums with rubber strips (Figure 1.2.1). These were made from canvas impregnated black-filled polyurethane and mounted on the rollers with tinplate supports. The rubber protruded from the rigid supports by 2 cm and was cut into a fringe every 2 cm. This principle worked adequately and in the final design rollers were covered with nylon brushes (Figure 1.2.1).

This design (Figure 1.2.5) consisted of a single pair of picking rollers, constructed from twelve stiff nylon brushes which were mounted on a steel roller to give an overall outside diameter of 15 cm (Figure 1.2.6). These rollers were set so that they just mesh with each other and rotate in the same direction. The canes being fed to the machine by infeed rollers (Figure 1.2.7). These feeding rollers are driven at a slower speed (originally 1:8 and later 1:14) than the picking rollers and are mounted so that they just intermesh. As the canes pass through the picking rollers they engage a set of outfeed rollers (Figure 1.2.8), of identical design to the infeed rollers, which are driven in tandem with the latter by a chain drive. The design drawings are attached as Appendix VIII 2.

The basic principle incorporated in the design is that the infeed and outfeed rollers restrict the rate of passage of the canes through the picking rollers, enabling the nylon brushes to provide sufficient force to remove the buds, by lifting the bud to break the pedicel. With a single pair of picking rollers, it is important to orientate the canes and feed them in base first, as the picking rollers rotate anticlockwise.

FIGURE 1.2.5   Prototype Harvester II

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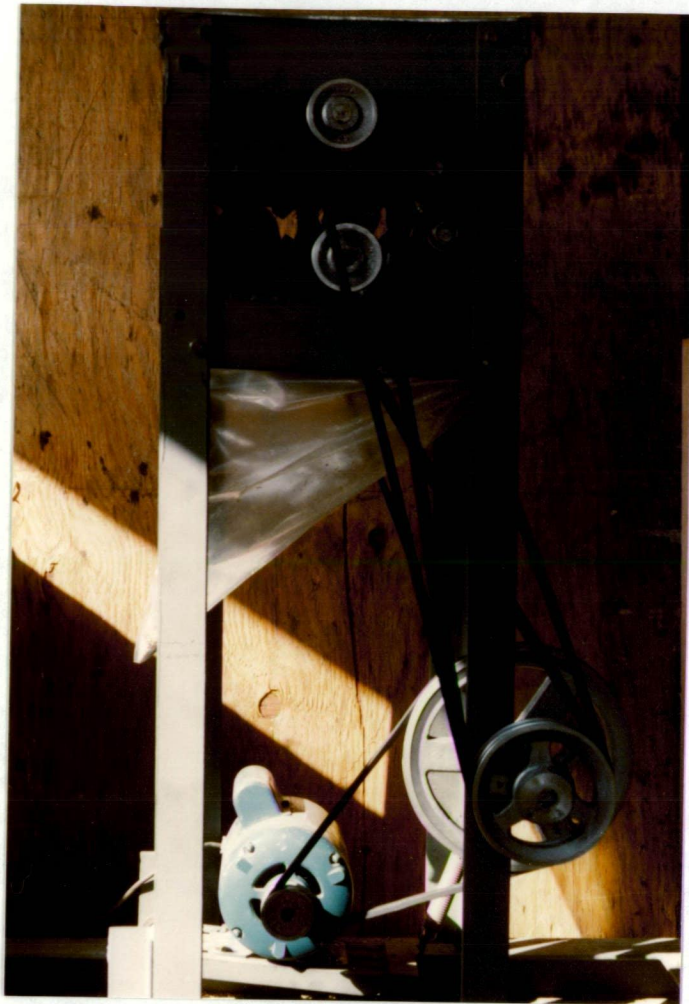
FIGURE 1.2.6   Side view of nylon brush picking rollers
               (note intermeshing)
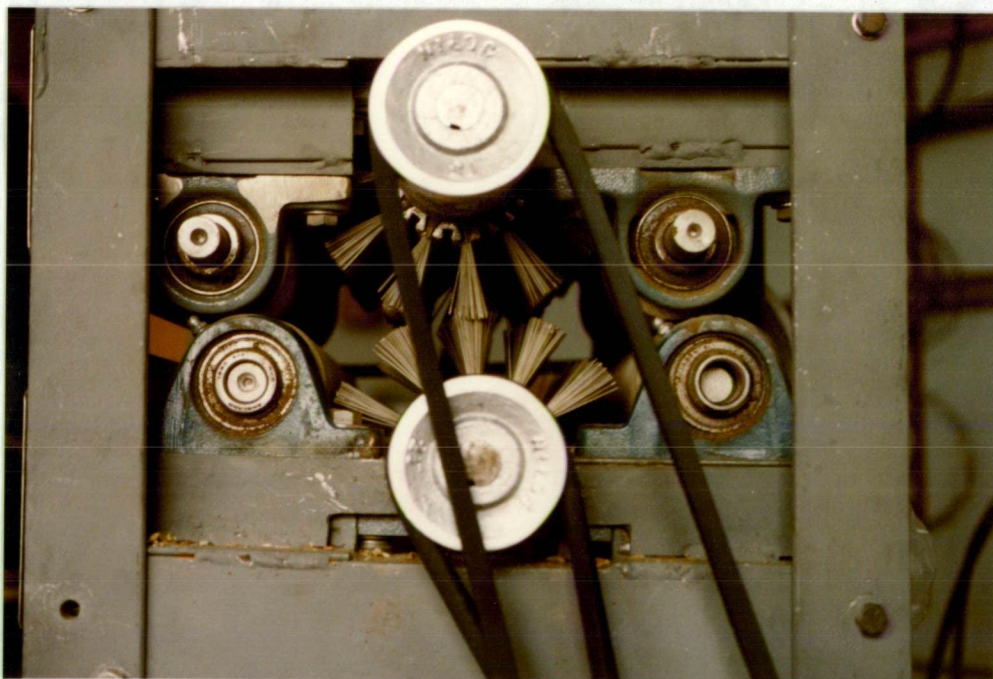
FIGURE 1.2.7 Canes entering infeed rollers Prototype II

FIGURE 1.2.8 Outfeed roller design Prototype II

In this configuration the canes cannot be easily fed into the picking rollers tip first due to the supple nature of the tip.

If the picking rollers are contra-rotating the canes can be fed base or tip first; however, the picking action in this configuration is very harsh and causes considerable damage to the bud material picked (Figure 1.2.9). While this method is very efficient in removing buds, as shown by Table 1.2.1, the damage done to the bud is unacceptable as many glands are ruptured. Feeding the canes tip-first to clockwise rotating rollers is not possible, once again due to the supple nature of the tip; in this case the canes will simply not feed through the rollers. Table 1.2.1 details the efficiency with which buds are removed by various roller configurations and Figure 1.2.9 shows the type of bud material picked as compared to that produced by hand harvesting.

The efficiency of the final prototype has been measured on single canes and bunches of canes with dormant buds and buds just breaking dormancy (Table 1.2.2). Both types of buds are readily removed on a single pass through the machine, with approximately a further 15% removed on a second pass. Figure 1.2.10 shows canes with dormant buds before, and Figure 1.2.11 canes after picking by the harvester.

In the present configuration, the final prototype when fed with canes by two labourers picked 3.5 kg/4 hrs (0.88 kg/hr).

1.3 Chemical Harvesting

1.3.1 *The Effect of Ethrel Concentration*

The effect of ethrel concentration on bud attachment was measured as described previously in Section III 2.3. The percentage of buds removed after ethrel treatment, are recorded in Table 1.3.1. The data was analysed by analysis of variance using a GENSTAT computer package

FIGURE 1.2.9

1. Machine harvested   (rollers anticlockwise, canes tip first)

2. Machine harvested   (rollers contra-rotating, canes tip first)

3. Machine harvested   (rollers anticlockwise, canes base first)

4. Handpicked buds   White Bud

5. Handpicked buds   Lees Prolific

TABLE 1.2.1 Efficiency of bud removal by a mechanical harvester

Roller Configuration	Number of Passes through rollers	Replicate	% Buds removed	Mean % buds removed
anticlockwise	2 base first	1	78.1	
		2	86.7	85.2
		3	85.0	
		4	90.9	
anticlockwise	4 tip first	1	52.9	
		2	46.2	
		3	84.6	60.2
		4	43.8	
		5	73.3	
contra-rotating	4 tip first	1	93.3	
		2	75.0	
		3	100.0	90.0
		4	87.5	
		5	92.3	
		6	91.7	
clockwise	canes will not feed tip first			

TABLE 1.2.2 Efficiency of bud removal

Treatment	Replicate	% dormant buds removed		% breaking buds removed	
		Pass 1	Pass 2	Pass 1	Pass 2
single canes	1	72.7	81.8	62.5	75.0
	2	72.7	81.8	56.3	81.3
	3	68.8	75.0	45.5	81.8
	4	76.2	85.7	88.9	94.4
	5	47.4	63.2	46.7	66.6
	6	52.9	82.4	60.0	60.0
	7	52.6	68.4	47.4	73.7
	8	47.1	82.4	37.7	50.0
	mean	67.9	77.6	55.6	72.9
bunches of canes	1	66.1	85.6	72.2	91.8
	2	67.3	84.2	78.1	91.7
	3	79.3	86.2	71.4	86.7
	mean	70.9	85.3	73.9	90.1

FIGURE 1.2.10 First year canes
before entering harvester

FIGURE 1.2.11 First year canes
after buds have been picked by
harvester

TABLE 1.3.1 Percentage of buds removed by beating after ethrel treatment

Treatment	Harvest Date	Replicate			Mean
		I	II	III	
T_1	H_1	54.4	49.6	44.7	49.5
	H_2	64.5	62.6	65.0	64.0
	H_3	67.3	60.9	65.8	64.7
T_2	H_1	38.2	44.8	40.1	41.0
	H_2	57.9	59.1	55.5	57.5
	H_3	73.8	55.5	71.6	67.0
T_3	H_1	47.5	50.4	60.2	52.7
	H_2	61.4	59.8	50.9	57.4
	H_3	60.5	55.4	49.5	55.1
T_4	H_1	46.5	68.4	49.5	54.8
	H_2	60.5	50.0	71.5	60.7
	H_3	62.6	65.2	72.7	66.8
T_5	H_1	16.7	39.3	47.9	34.6
	H_2	77.4	49.0	66.6	64.3
	H_3	72.4	75.4	76.3	74.7
T_6	H_1	52.6	48.0	54.0	51.5
	H_2	73.0	74.3	76.3	74.5
	H_3	73.5	69.1	73.5	72.0
T_7	H_1	79.6	78.3	67.8	70.1
	H_2	59.5	72.6	87.7	74.7
	H_3	71.3	73.6	92.7	82.7

(Mark 4.03 Rothamsted Experimental Station England, 1980). This analysis showed (Table 1.3.2 and Figure 1.3.1) both harvest date after ethrel application and concentration of ethrel used were highly significant at $P = 0.05$ with a small interaction effect. To investigate these treatment effects further, Duncan's multiple range test (Duncan, 1955) was applied at $P = 0.05$ and $df = 40$. Table 1.3.3 lists the marked means and their association as a result of the test. Treatment, T_7, with the highest concentration of ethrel (0.50%) was observed to be significantly different from the controls and other treatment means at the three harvest dates.

Harvest one, two days after application of ethrel, was partly confounded by the fact that the area control (T_2) and treatment T_5 were not associated with the others. As their means are less than those in the observed grouping, it is clear that these treatments are not effective in removing buds after two days. At the second harvest treatment, T_6 (0.20%), as well as T_7 (0.50%), was significantly different from both controls. By the third harvest treatment T_5 (0.10%) was grouped with T_6 and these, as well as T_7, were significantly different from the control group. This harvest was also confounded by the fact that treatment T_3 was not associated with the control group, but as it had a lower mean it was not sufficiently effective in removing buds. The ranking of harvest date means against ethrel concentration shows an association of T_1 means at harvest II and III. This is the interaction effect identified by the analysis of variance, and shows that storage of canes at 20°C for eight days or longer will enable removal of 64% of bud material by beating with only urea and not ethrel treatment.

During mechanical removal of buds (refer Section III 2.2) it appeared that the number of prunings used affected efficiency. This has not been substantiated as block effects have been shown not to be

TABLE 1.3.2 Analysis of variance table for ethrel concentration
and harvest date

Source of Variation	df	SS	MS	F
Blocks	2	127.16	63.58	1.018 n.s.
Treatment				
Harvest	2	3890.42	1945.21	31.090***
Ethrel conc.	6	2946.07	491.01	7.848***
Harvest x Ethrel conc.	12	1626.14	135.51	2.166*
Residual	40	2502.69	62.57	
Total	62	11092.49		

TABLE 1.3.3 Table of ranked means and their association

(a) Ethrel concentration

	Harvest Date		
Ethrel Concentration	H_1 (2 days)	H_2 (8 days)	H_3 (14 days)
T_1 = unsprayed control	T_5 34.63	T_3 57.37	T_3 55.13
T_2 = 0.5% urea control	T_2 41.03	T_2 57.50	T_1 64.67
T_3 = 0.01% Ethrel + urea	T_1 49.53	T_4 60.67	T_4 66.83
T_4 = 0.05% " "	T_6 51.53	T_1 64.03	T_2 66.97
T_5 = 0.10% " "	T_3 52.07	T_5 64.33	T_6 72.03
T_6 = 0.20% " "	T_4 54.80	T_6 74.53	T_5 74.70
T_7 = 0.50% " "	T_7 70.13	T_7 74.83	T_7 82.73

(b) Harvest Date

Harvest date	Ethrel Concentration						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
H_1	49.53	41.03	52.70	54.80	34.63	51.53	70.13
H_2	64.03	57.50	57.37	60.67	64.33	74.53	74.83
H_3	64.67	66.97	55.13	66.83	74.70	72.03	82.73

FIGURE 1.3.1 Legend

T1 Control treatment unsprayed

T2 Control treatment 0.5% urea spray

T3 Ethrel treatment 0.01% plus urea (0.5%)

T4 Ethrel treatment 0.05% plus urea (0.5%)

T5 Ethrel treatment 0.1% plus urea (0.5%)

T6 Ethrel treatment 0.2% plus urea (0.5%)

T7 Ethrel treatment 0.5% plus urea (0.5%)

FIGURE 1.3.2 Legend

C1 Unsprayed control, first harvest (12 days)

E1 Ethrel treatment (0.5%), first harvest (12 days)

C2 Unsprayed control, second harvest (21 days)

E2 Ethrel treatment (0.5%), second harvest (21 days)

FIGURE 1.3.1 The effect of Ethrel concentration on bud removal
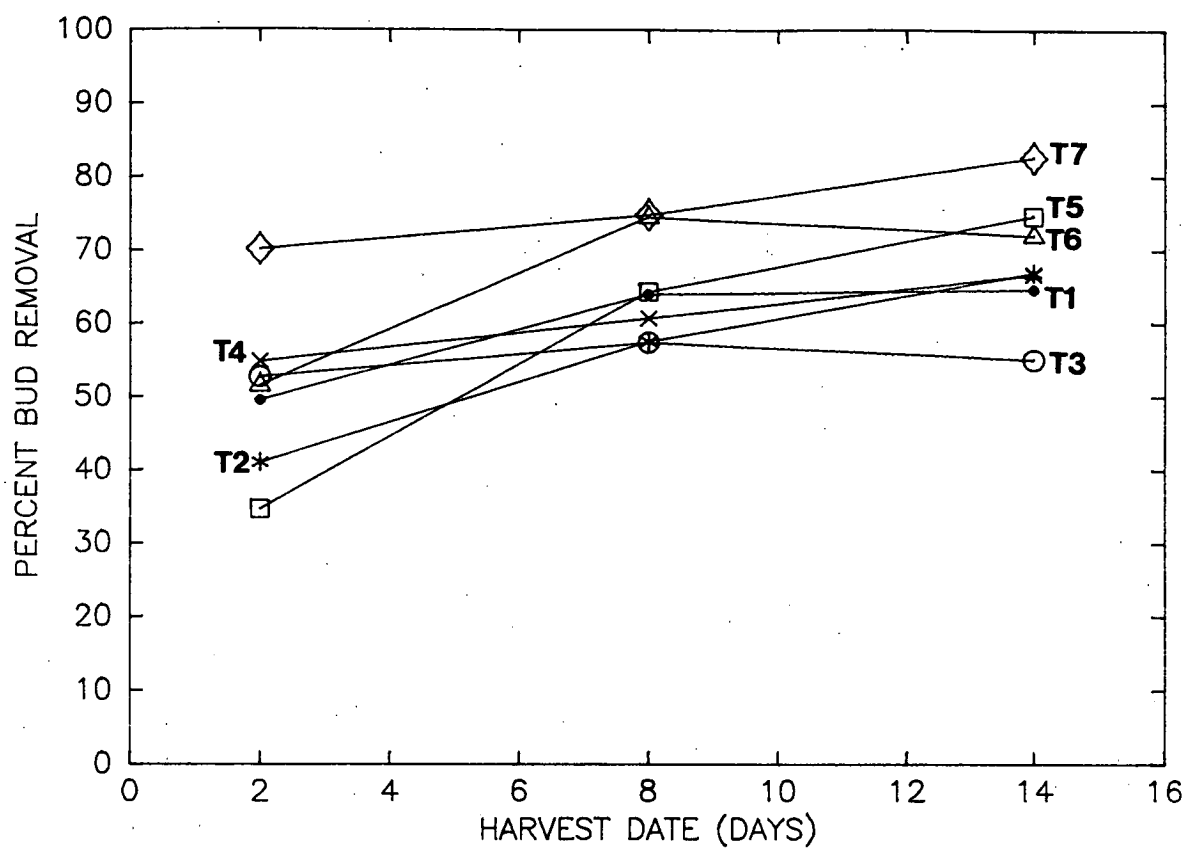
FIGURE 1.3.2 The effect of temperature on bud removal
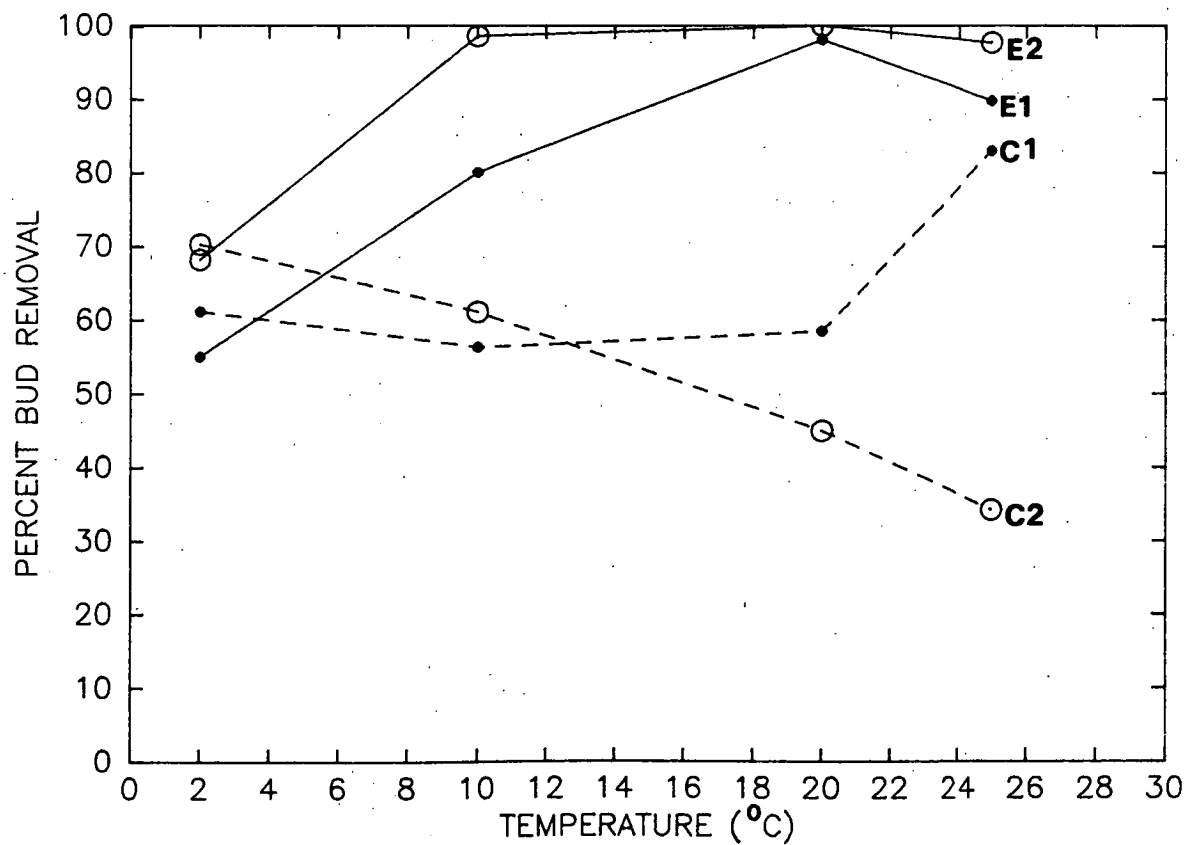
significant. The presence of leaf and grass material amongst the canes gave rise to some problems in measurement of bud yields. All bud material was sieved to remove such material and distribute any introduced errors normally over all treatments.

1.3.2 The Effect of Temperature and Harvest Date on Ethrel Concentration

The effect of temperature on ethrel mediated bud abcission was evaluated as previously described in Section III 2.2. The percentages of buds mechanically removed are recorded in Table 1.3.4. Analysis of variance was applied to the data using a GENSTAT computer package (Mark 4.03 Rothamsted Experimental Station, England, 1980). This analysis showed (Table 1.3.5) that while the application of ethrel had a highly significant effect on bud removal, storage temperature and harvest date alone had no significant effect. There are significant interaction effects between ethrel application, harvest date and storage temperature. Both harvest date and storage temperature increase the effectiveness of the applied ethrel; these effects are better demonstrated in graphical form (Figure 1.3.2) and by examination of means using Duncan's multiple range test (Table 1.3.6).

Storage temperatures of 10°C and above increase the effectiveness of ethrel application (Figure 1.3.2). A temperature of 10°C is required for 21 days or 20°C for a shorter period of 12 days to ensure the most efficient removal of buds. Thus at lower temperatures longer storage times were necessary to remove buds. The importance of harvest date is demonstrated by the 10°C treatment where 80.1% of buds are removed at the first harvest, as compared to 98.5% at the second.

Application of Duncan's multiple range test (Table 1.3.6) shows five pairs of the eight treatments removed different percentages of buds at the two harvest dates. This demonstrates the importance of harvest

TABLE 1.3.4 Percentage buds removed by ethrel treatment at different temperatures

Treat-ment		Storage Temp.	Harvest I (12 days)				Harvest II (21 days)			
			Buds hand picked (g)	Buds mechanic-ally removed (g)	% mechanic-ally removed	% Mean	Buds hand picked (g)	Buds mechanic-ally removed (g)	% mechanic-ally removed	% Mean
Control	CI	2°C	5.17	11.20	68.4	61.2	2.36	9.83	80.6	70.3
	CII		8.70	14.14	61.9		7.25	10.87	60.0	
Ethrel	EI		14.55	15.12	51.0	55.0	6.15	15.40	71.5	68.2
(0.5%)	EII		13.75	19.78	59.0		10.03	18.49	64.8	
	CI	10°C	7.82	9.74	55.5	56.4	5.19	9.57	64.8	
	CII		9.63	12.85	57.2		5.33	7.25	57.6	
	EI		4.20	13.07	75.7		0.59	19.83	97.1	98.6
	EII		3.37	20.39	85.8	80.1	0	21.39	100.0	
	CI	20°C	8.64	6.00	41.0		4.91	3.77	43.4	45.0
	CII		4.19	13.30	76.0	58.5	8.78	7.66	46.6	
	EI		0.60	15.15	96.2		0	8.03	100.0	100.0
	EII		0	13.23	100.0	98.1	0	6.38	100.0	
	CI	25°C	4.47	13.46	75.1		9.60	2.20	18.6	34.2
	CCII		9.78	5.05	90.8	83.0	7.40	7.30	49.7	
	EI		0.72	12.83	94.7		0	14.80	100.0	97.7
	EII		2.03	11.39	84.9	89.8	0.40	8.10	95.3	

date in increasing ethrel effectiveness at all storage temperatures,
except 20°C.

TABLE 1.3.5 Analysis of variance table for temperature and ethrel
            concentration

Source of Variation	df	SS	MS	F
Blocks	1	98.0	98.0	0.940 n.s.
Treatments				
Ethrel conc. (EC)	1	5745.9	5745.9	55.140***
Storage temperature (ST)	3	689.3	229.8	2.205 n.s.
Harvest Date (H)	1	16.8	16.8	0.161 n.s.
EC x ST	3	3185.4	1061.8	10.189**
EC x H	1	1081.1	1081.1	10.375**
ST x H	3	1312.9	437.6	4.200*
EC x ST x H	3	757.9	252.6	2.424 n.s.
Residual	15	1563.1	104.2	
Total	31	14450.4		

TABLE 1.3.6 Table of means and their association (from Duncan's
            multiple range test)

		Control (EC_0)		Applied Ethrel (EC_1)	
		Harvest 1	Harvest 2	Harvest 1	Harvest 2
Temperature	2	65.2	70.3	55.0	68.2
	10	56.4	61.2	80.1	98.5
	20	58.50	45.0	98.1	100.0
	25	82.90	34.1	89.80	97.6

2. EXTRACTION PROCEDURES

2.1 Solvent Extraction

A series of extractions were undertaken using a range of solvents
to determine if a product of higher quality could be obtained. The
French perfume industry has been using benzene (Thomas 1979) to extract
blackcurrant buds but has recently changed to hexane - due to new health
regulations. The concretes produced from the solvents used were com-
pared organoleptically with two standard samples of French origin (refer
to Section III 6.1) to determine the better quality product. Table
2.1.1 contains a quantitative comparison of the different concretes
produced from the same source of bud material. This comparison is based
on percent gas chromatographic trace peak areas without correction for
FID response factors for the individual components involved.

The hexane and petroleum ether extracted have a reasonably similar
composition while those first extracted with methanol and then re-
extracted with pentane, hexane or petroleum ether show considerable dif-
ferences. The samples extracted with methanol first are enriched in
sesquiterpenes compared to those extracted with petroleum ether or
hexane.

A comparison based on organleptic qualities showed a preference
for the petroleum ether concrete over the hexane concrete due to the
overall richness of the former. The petroleum ether sample has had a
stronger 'catty note' than the hexane sample, but neither possessed the
spicy, pepper notes of the French benzene extracted samples. None of
the methanol extracted samples were acceptable due to a lack of catti-
ness and an overall flat impression.

Liquid cardon dioxide extracts of blackcurrant buds have been
obtained in two ways. Firstly, by extraction using the apparatus

TABLE 2.1.1 Percentage peak area data for various solvent extracts

Name	n hexane	pet ether	MeOH/pentane	MeOH/hexane	MeOH/pet ether
Alpha thujene	1.94	1.04	0.35	1.89	7.96
Alpha pinene	1.02	0.89	0.23	tr	tr
Sabinene/beta-pinene	6.33	4.06	6.48	3.20	2.20
Myrcene	2.22	0.99	0.64	0.81	0.56
Alpha phellandrene	0.56	0.45	1.91	0.38	tr
Delta-3-carene	15.62	3.69	4.02	1.07	2.28
Alpha terpinene	-	2.02	-	0.64	1.42
P-cymene	-	2.55	-	0.99	-
Beta phellandrene/limonene	5.24	2.88	1.78	1.75	1.26
Cis beta ocimene	tr	tr	0.57	1.52	tr
Trans beta ocimene	3.03	0.29	0.69	1.98	2.38
Gamma terpinene	7.21	10.59	1.72	0.80	2.62
Cymenene	1.60	tr	1.90	1.19	tr
Linalool	1.11	tr	1.52	1.19	tr
Alpha terpinolene	5.58	8.83	1.46	1.50	2.14
Non-an-2-one	3.77	1.29	0.56	0.85	0.74
Unknown MW152 (17)	tr	0.84	tr		tr
Terpinen-4-ol	4.20	1.69	1.42	2.50	2.34
Alpha terpineol	2.69	6.14	0.73	3.95	1.18
Trans piperitol	1.90	4.09	0.54	2.02	2.28
Carvone	0.97	1.75	0.49	1.30	1.18
bornyl acetate	1.46	2.69	1.92	1.14	1.62
4 terpinyl acetate	0.00	3.23	0.24	1.10	0.36
Beta terpinyl acetate	0.00	1.71	0.28	0.24	0.54
Beta elemene	0.04	0.05	0.07	tr	0.22
Beta caryophyllene	8.67	12.76	25.28	10.10	9.22
Humulene	3.94	5.69	11.22	4.87	4.50
Alloaromadrene	0.20	tr	tr	0.35	tr
Germacrene D	0.59	1.22	9.57	1.87	1.94
Gamma elemene	0.30	1.17	3.88	1.05	0.70
Gamma cadinene	0.52	1.06	0.52	0.21	0.20
Beta cadinene	0.16	0.28	tr	1.26	1.06
Caryophyllene epoxide	7.26	1.54	6.96	20.63	8.26
Humulene epoxide	4.47	0.31	2.54	6.85	3.34
Unknown (45)	0.45	0.35	1.01	2.24	0.82
Unknown (46)	1.17	0.24	1.10	3.19	1.22

TABLE 2.1.2 Percentage peak area data for various extracts

Name	Pet ether	CUB CO$_2$ (waxy)	TU CO$_2$ (wax free)	Vacuum distillate
Alpha thujene	2.90	3.93	0.37	0.38
Alpha pinene	1.45	0.29	2.24	0.33
Sabinene/ Beta pinene	31.16	14.32	8.77	16.15
Myrcene	1.84	0.30	2.55	2.81
Alpha phellandrene	0.65	0.33	0.63	0.69
Delta-3-carene	17.43	8.19	6.80	31.96
Alpha terpinene				
P-cymene				
Beta phellandrene	0.78	0.11	1.87	3.25
Limonene	2.66	1.02	0.76	3.25
Cis beta ocimene	0.42	0.12	0.61	1.26
Trans beta ocimene	1.49	1.98	1.27	6.75
Gamma terpinene	1.07	1.13	9.78	0.82
Cymenene				
Linalool				
Alpha terpinolene	7.88	3.14	8.16	11.63
Non-an-2-one	0.43	1.39	0.20	1.11
Unk MW152 (17)	0.32	0.15	0.82	0.35
Terpinen-4-ol	0.10	0.15	3.06	0.51
Alpha terpineol	1.70	2.67	0.92	3.78
Trans piperitol	0.53	0.27	0.17	0.53
Carvone	0.30	0.32	0.10	0.05
Unknown 168 (34)	0.14	0.47	0.61	tr
Unknown 182 (35)	0.32	0.50	0.44	0.39
bornyl acetate	0.23	0.28	0.82	0.15
4-terpinyl acetate	0.56	1.24	2.38	0.17
Beta terpinyl acetate	0.55	0.51	0.34	1.86
Beta elemene	0.10	0.16	0.31	0.84
Beta caryophyllene	10.33	13.95	16.99	12.39
Unknown 204 (39)	0.10	0.08	0.37	0.06
Humulene	3.45	6.27	8.05	3.79
Alloaromadrene	0.08	0.38	1.70	0.25
Germacrene D	3.11	1.90	9.38	2.61
Gamma elemene	0.38	1.13	9.79	0.83
Gamma cadinene	0.46	1.66	0.75	tr
Caryophyllene epoxide	1.75	5.40	3.74	-
Humulene epoxide	1.07	6.41	1.36	-
Unknown (45)	0.57	3.50	0.85	-
Unknown (46)	0.30	1.67	0.17	-

described in Section III 3.2 and secondly, in a semi-commercial pilot plant at Carlton United Breweries (Melbourne, Australia). The composition of extracts obtained is compared in Table 2.1.2 where petroleum ether extracts from the same bud material are used as controls. The major compositional differences involve a preferential enrichment of sesquiterpenes and a concomitant decrease in amounts of monoterpene hydrocarbons extracted by liquid carbon dioxide.

The yields of oil produced by the two liquid carbon dioxide extraction methods (Table 2.1.3), were the same but they were dissimilar products. The former is a green, waxy aromatic material, while the latter extract is more like an absolute but of higher quality (Section IV 5.1). It is lemon yellow in colour and has a viscous nature like the distillate. The vacuum distillate, produced from the petroleum ether extracted concrete, contains only the volatile portion of this material. Its composition was similar to the solvent extracted concrete but enriched in terpenes as would be expected (Table 2.1.2).

TABLE 2.1.3 Oil yields of various extraction solvents

Extracting Solvent	Percentage Yield Replicate			
	1	2	3	Mean
Petroleum ether	4.1	5.2	3.9	4.4
CUB CO_2	1.92	-	-	1.92
TU CO_2	2.62	1.49	1.80	1.97
Vacuum distillate*	8.03	5.70	4.02	5.92

* pet ether concrete is the source of the distillate

3. SEPARATION TECHNIQUES

3.1 Liquid Solid Chromatography

(a) Silica Gel:

Silica gel was found to be effective in separating hydrocarbons from oxygenated compounds (Table 3.1.1). It should be noted that none of the fractions obtained by silica gel chromatography possessed a catty aroma, suggesting that, despite the precautions taken (Section III 4.1a), the compound responsible for this aroma was labile and decomposed during treatment. Fractions were selected on the basis of aroma and gas chromatographic evidence, for analysis by GC/MS (Section IV 4.2).

(b) Florisil:

Florisil was found to be effective in allowing separation of hydrocarbons and oxygenated compounds. However, the technique is unable to allow the distinctive catty note of the blackcurrant to pass through the chromatography column unaltered. The catty note, present in the vacuum distillate before chromatography, cannot be detected in any single fraction afterwards (Table 3.1.2). This infers that the compound was either altered on the column by some form of chemical decomposition, or the characteristic note is the result of two or more compounds which have separated into different fractions.

(c) Microcolumn:

The microcolumn technique of Murray and Stanley (1968) was used with a series of different polarity solvents as described in Section

TABLE 3.1.1 Chromatography separation achieved using silica gel packing

Fraction	Elution Volume (ml)	Chromatographic Run			
		3	6	9	11
1	10				
2	20				
3	25				
4	30				
5	35				
6	40				* MHC
7	45				** M/SHCC
8	50			* MHC	*** "
9	55	*** MHC		* "	*** "
10	60	** "		** M/SHC	* "
11	65	*		** "	* "
12	70	* M/SHC	* MHC	* SHC	* SHC
13	75	* "	* "	* "	* "
14	80	** SHC	*** M/SHC		*
15	85		** "		* M/SOXY
16	90	* SHC	** M/SHC	* M/SOXY	* "
17	95	* "	* "	* "	* "
18	100		*		** "
19	105			* M/SOXY	*** M/SOXY
20	110		*	* SOXY	* "
21	115				* SOXY
22	120		* M/SOXY		
23	125		* "		
24	130		*		

MHC - monoterpene hydrocarbons
SHC - sesquiterpene hydrocarbons
MOXY - monoterpene oxygenated compounds
SOXY - sesquiterpene oxygenated compounds

M/S - mono/sequi terpene
HC - hydrocarbons
OXY - oxygenated compounds

TABLE 3.1.2 Chromatography separation achieved by using florisil packing

Fraction	Components		Aroma Impression
	Level	Type	
1	*		naphthalene
2	****	hydrocarbons	hydrocarbon
3	*		ether, sweet
4			
5			
6	*		ether, musty
7	***	monoterpene hydro-carbons	sweet, woody, dull
8	***		light, musk
9			
10	***	sesquiterpene hydro-carbons	ether
11	*		
12	*	monoterpene oxygenated compounds	strong musty
13	*	" "	
14			
15			
16			
17	**	sesquiterpene and monoterpene oxygenated compounds	oregano, sweet
18	*		" "
19	*	"	
20			musty
21	*	sesquiterpene oxygen-ated compounds	green, musty
22			v. unpleasant, musty
23			
24			dank, musty

TABLE 3.1.3 Microcolumn chromatography separations

Solvent	Methanol	Diethyl ether	Methylene chloride	Methylenechloride/ pentane (40:60)
Polarity ϵ°	0.75	0.39	0.32	0.24
Fraction 1				
2	**		**	*
3		***	** MHC	
4				
5		*		
6				*
7		*	** MHC	*
8		***	** MHC	*
9	***	**		

MHC - monoterpene hydrocarbons

III 4.1c.   The solvents used and the separation achieved with each, as determined by gas chromatography (Section III 4.3), are listed in Table 3.1.3.

The fractions for the methylene chloride run were analysed by GC/MS and found to contain only monoterpene hydrocarbons (Section IV 4.2). This separation technique was not perservered with since examination of the gas chromatographic traces showed none of the other solvents gave a satisfactory separation.   In addition, the catty note was not detected in any of the fractions.

3.2 High Performance Liquid Chromatography

In order to develop a satisfactory high performance liquid chromatography (HPLC) method, the vacuum distillate was subjected to examination

by a scanning ultra-violet spectrophotometer from 190 - 450 nm. This analysis (Figure 3.2.1) showed that a wavelength of 216 nm was optimal for detection of the terpene constituents by ultra-violet light. A A variety of solvents were then examined (Figure 3.2.1) to determine their ultra-violet absorption characteristics. From this examination a methanol/water mixture was used as the solvent of choice.

Preliminary gradient elution programming from methanol/water (50/50) to pure methanol resulted in selection of a program starting with methanol/water (70/30) for 20 minutes then pure methanol for 15 minutes at 4 mℓ/min using a Rad Pak µ Bondapak C18 reverse phase column. Further refinement of the program was carried out by running samples of the distillate under three selected program conditions (Figures 3.2.2 - 3.2.4). These three programs demonstrated that increasing solvent polarity (i.e. decreasing the proportion of methanol) improves the separation of the oxygenated compounds that are of interest. From this work a methanol solvent mixture of 70/30 was chosen as the starting point for chromatographic runs involving collection of individual peak samples. The fractions collected are delineated on Figure 3.2.4; all fractions were reduced in solvent volume and those with interesting aromas examined by combined gas chromatography/mass spectrometry.

3.3 Gas Liquid Chromatography

(a) Routine Analysis

For routine gas chromatography (GC) analysis a SCOT OV 101 50 m column was used to separate blackcurrant bud oil. A typical GC trace is presented as Figure 3.3.1. To obtain higher resolution a Fused Silica OV 101 50 m column was employed (Figure 3.3.2). The carrier flows and operating conditions for both columns are contained in Section III 4.3a. The OV 101 chromatographic phase provides excellent resolution of the

FIGURE 3.2.1 Absorbance characteristics of HPLC solvents

1. Blackcurrant vacuum-distillate
2. Methanol
3. Methanol/water (75/25)
4. Acetonitrite

sesquiterpene hydrocarbons

monoterpene hydrocarbons

oxygenated compounds
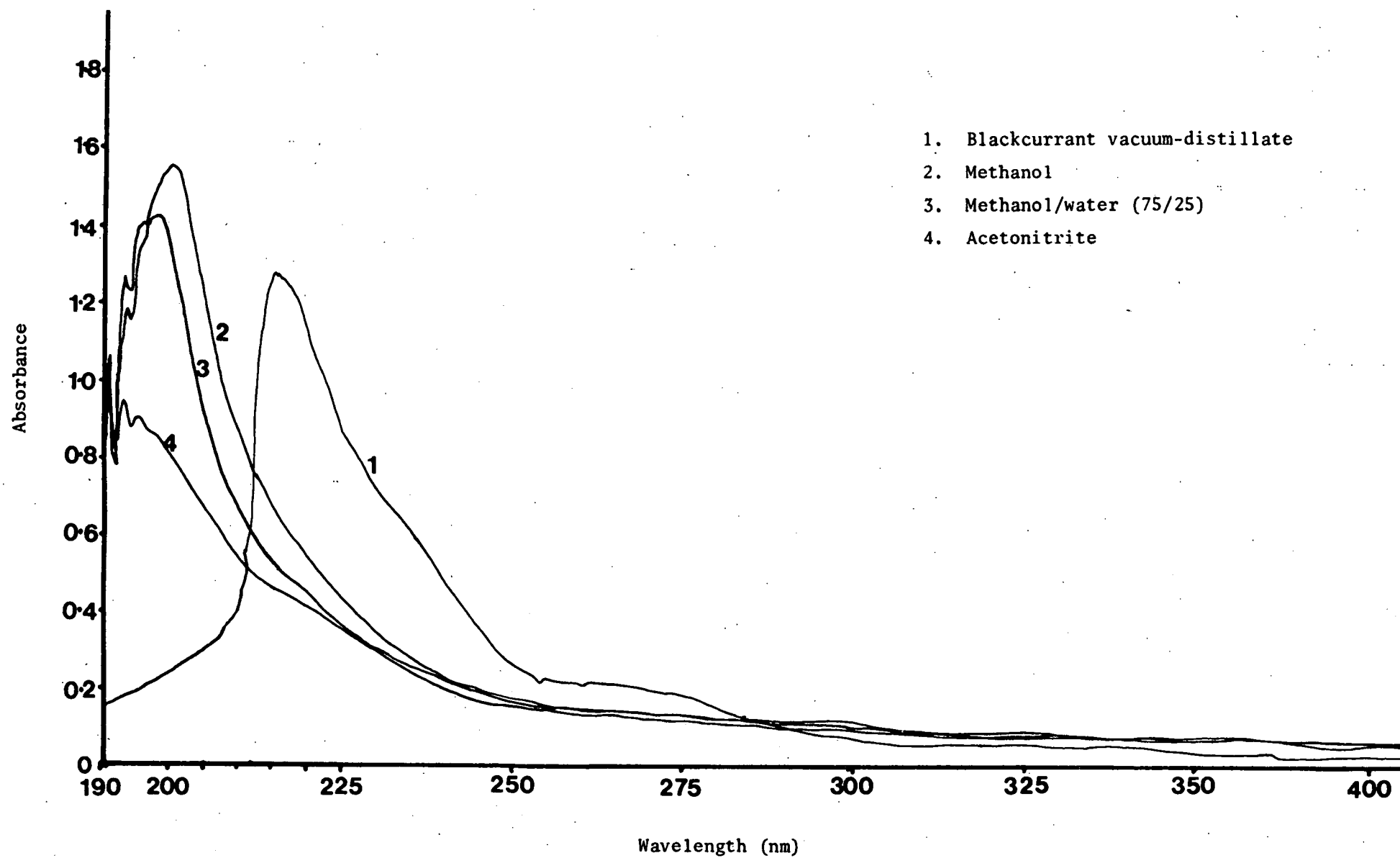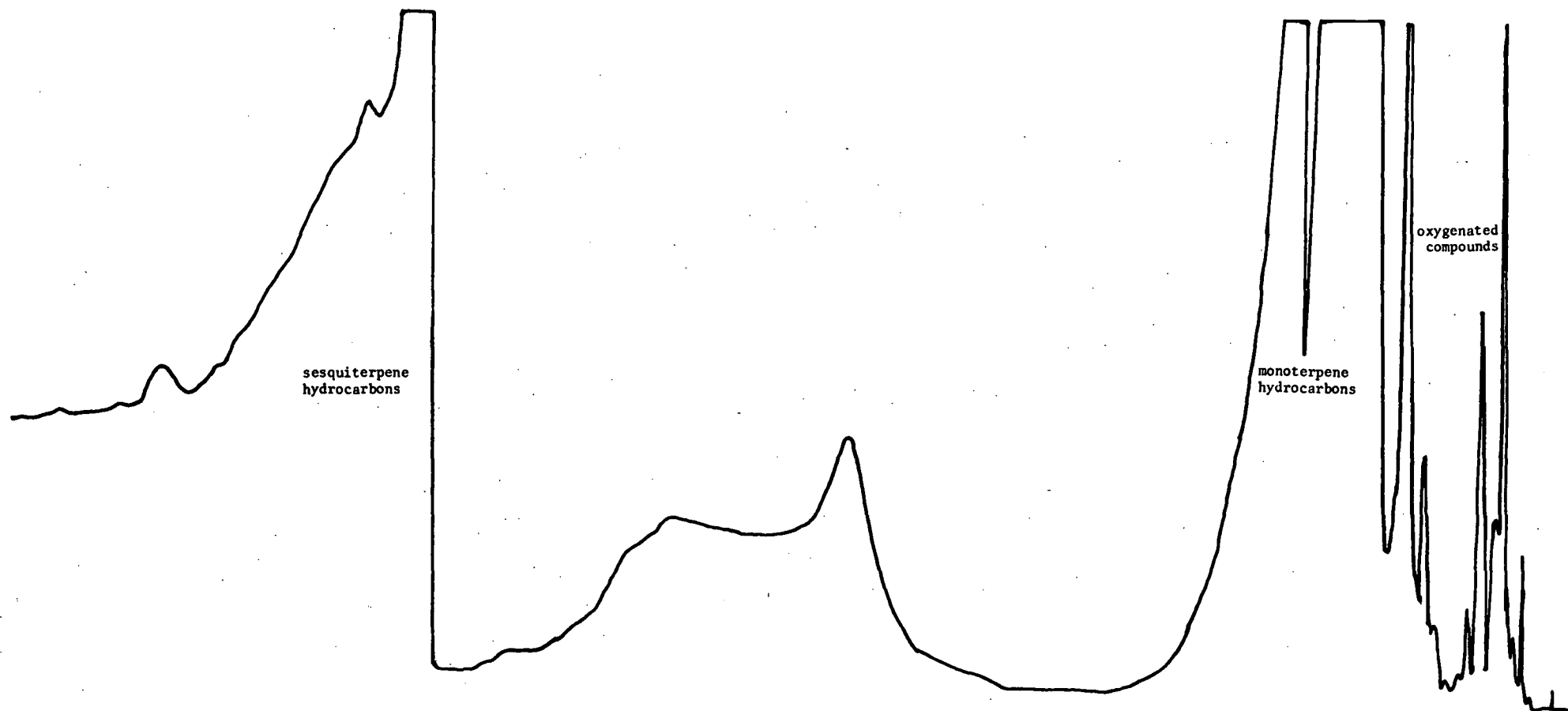
FIGURE 3.2.3 HPLC separation methanol/water (75/25) 20 min then pure methanol

Chart speed 1 cm/min

sesquiterpene
hydrocarbons

monoterpene
hydrocarbons

oxygenated
compounds
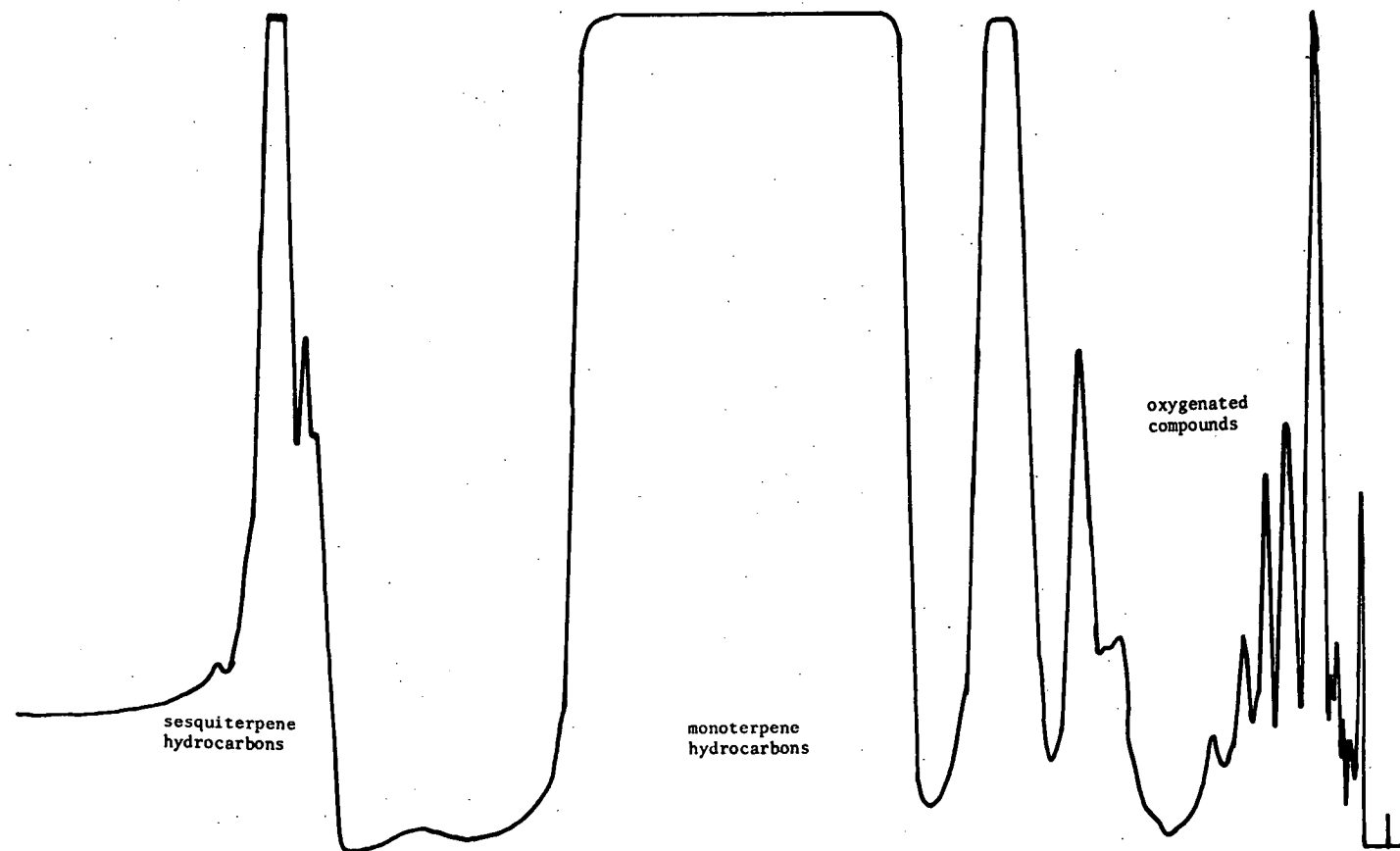
FIGURE 3.2.4 HPLC separation methanol/water (70/30) 20 min then pure methanol

Chart speed 1 cm/min
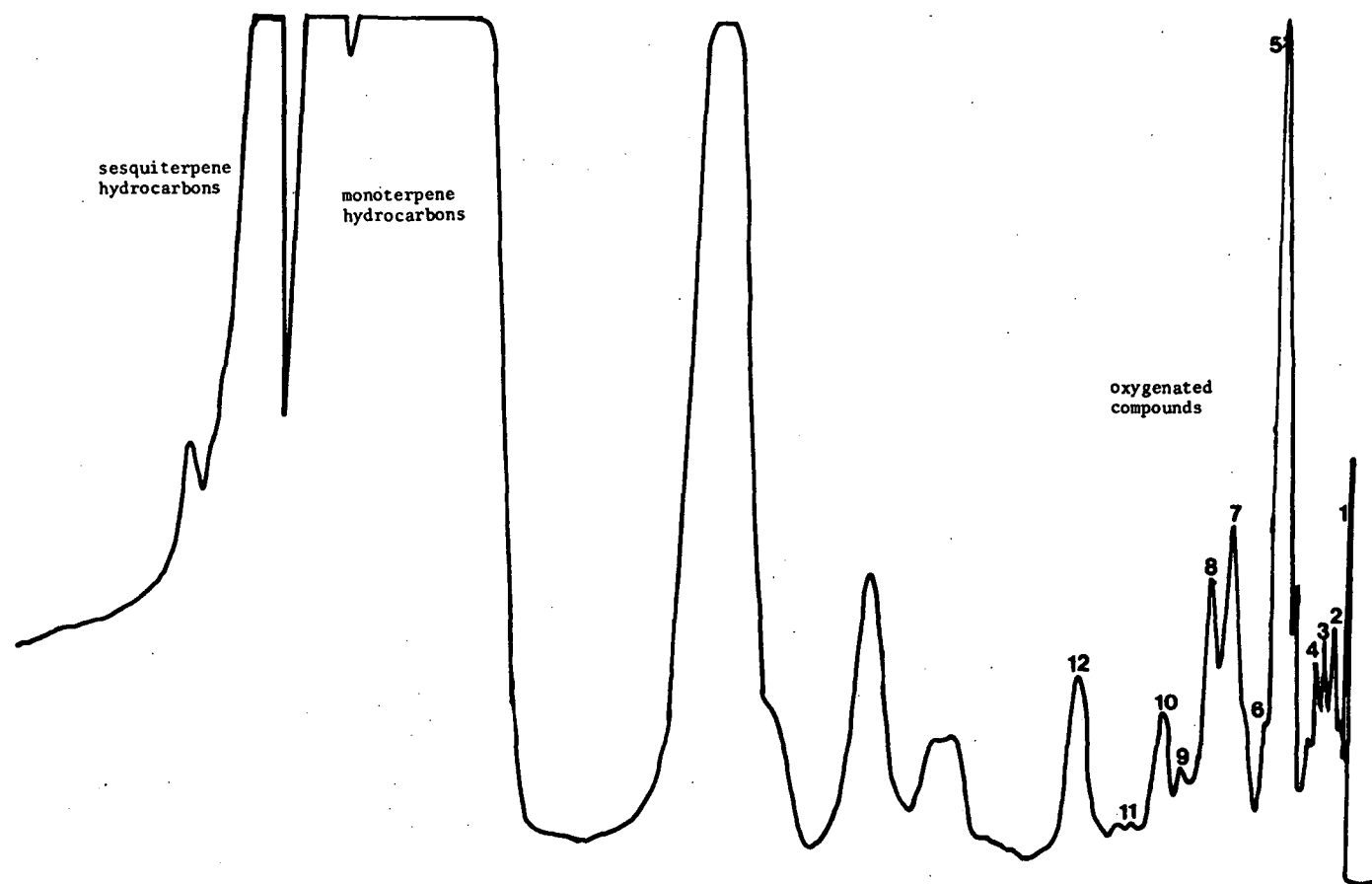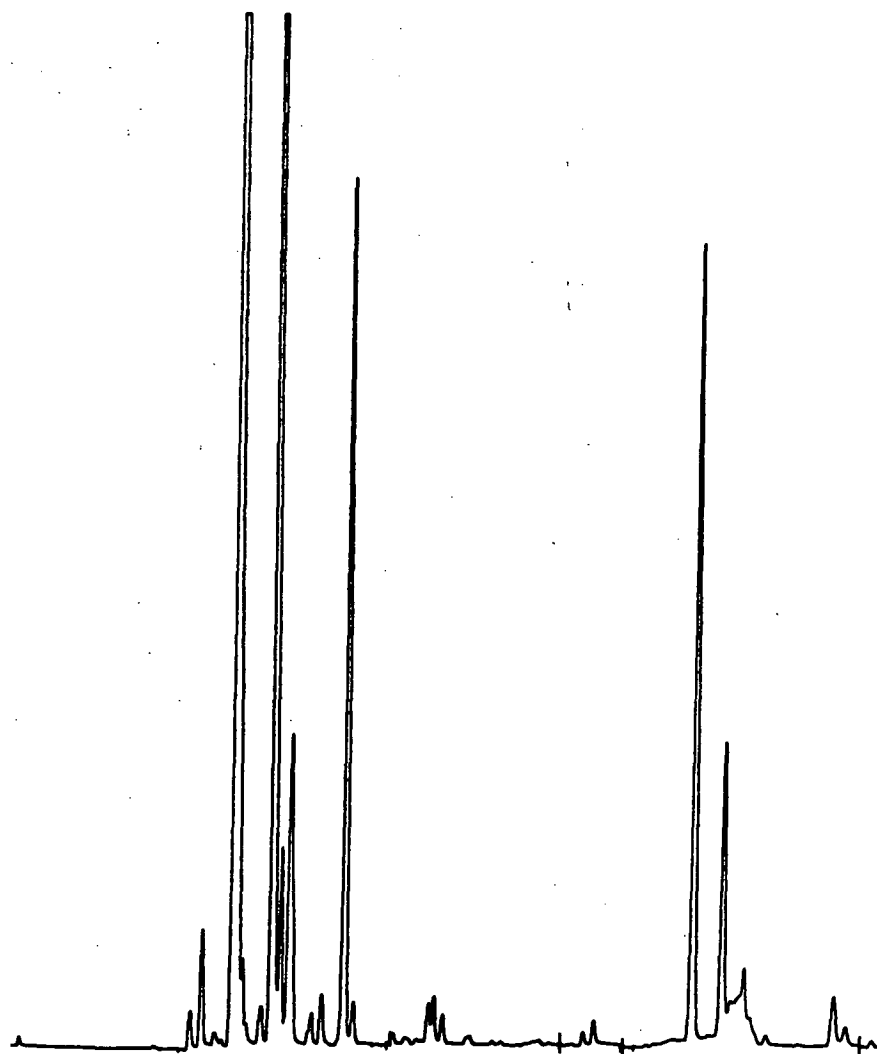
FIGURE 3.3.1   GC trace of blackcurrant bud oil
               80-220 at 4°C/min.   SCOT 50m OV 101
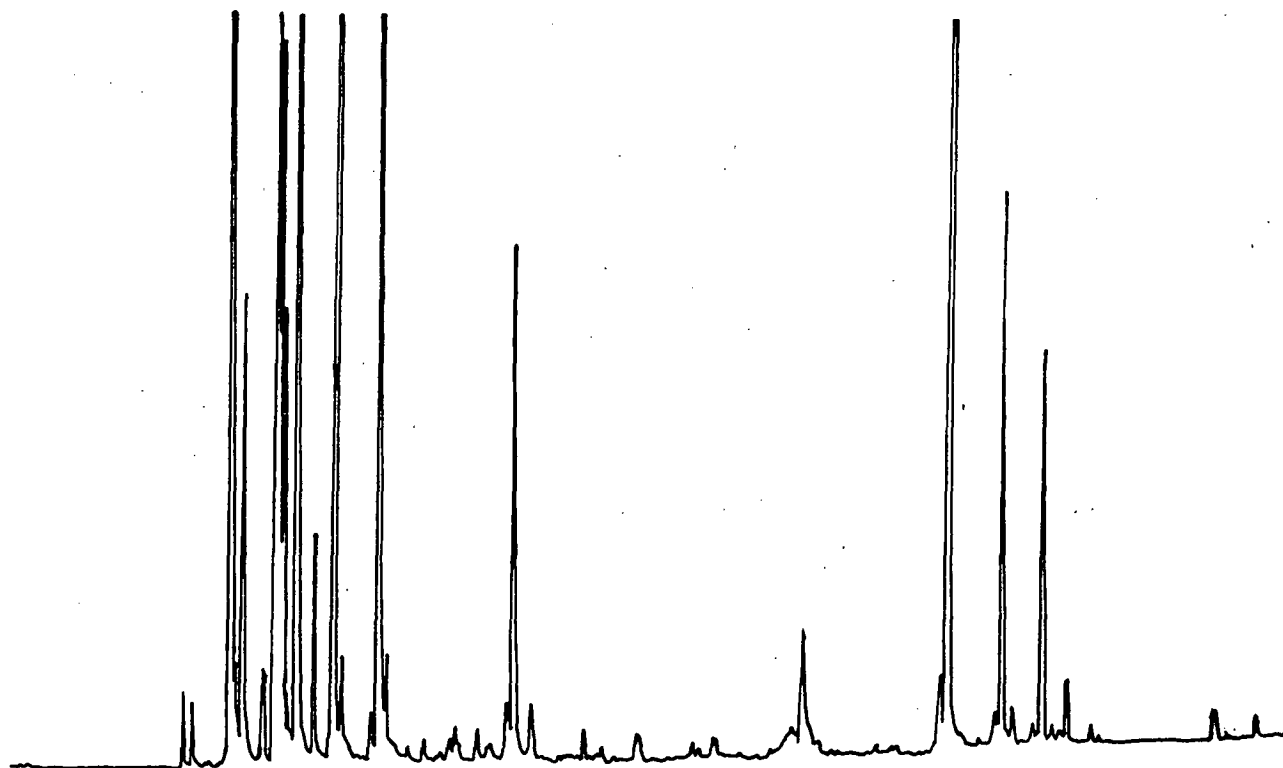
FIGURE 3.3.2   GC trace of blackcurrant bud oil
               80-220 at 2°C/min. FUSED SILICA 50m OV 101

components of blackcurrant bud oil. Gas chromatographic analysis of the regions of organoleptic interest did not detect any sulphur containing compounds.

(b) Effluent Traps

The effluent traps were used to trap peaks in the regions determined to be of organoleptic interest (Section IV 5.2), in an attempt to identify the components responsible for particular aroma sensations. This technique was found only to confirm the identity of components, which had already been detected by other fractionation methods.

(c) Headspace Analysis

A Pye Unicam Headspace analyser (Section III 3.3c) was used to examine the aroma of blackcurrant concretes. A good separation of monoterpenes and early components was achieved (Figure 3.3.3). Components were identified by connecting the headspace analyser to the GC/MS facility described in Section III 5.2. The early components were never seen in routine GC analysis of blackcurrant concretes but their presence and identity was confirmed by routine GC/MS analysis of a liquid carbon dioxide extract of blackcurrant buds.

FIGURE 3.3.3 GC separation of blackcurrant concrete headspace

pk	Component
1	n-hexane (solvent)
2	isobutanol
3	butanol
4	pentan-2-ol
5	2 methyl butan-1-ol
6	tricyclene
7	α thujene
8	α pinene
9	beta-thujene
10	beta-pinene/sabinene
11	myrcene
12	alpha-phellandrene
13	delta-3-carene
14	alpha-terpinene
15	p-cymene
16	limonene/Beta-phellandrene
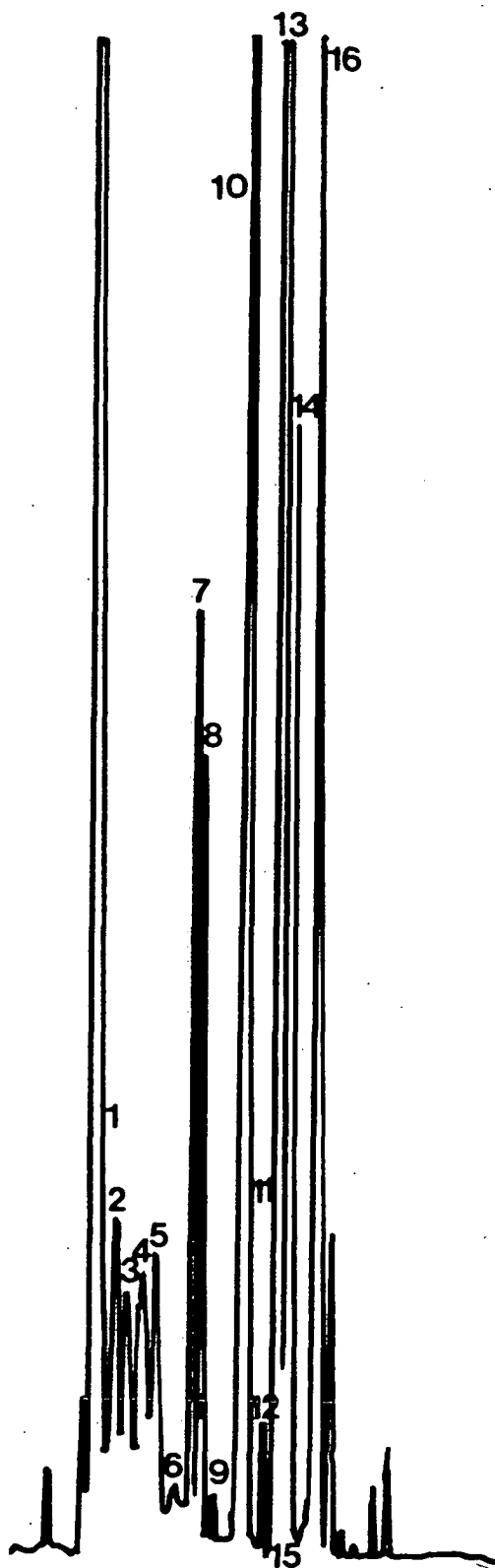
FIGURE 3.3.3 GC trace of blackcurrant concrete headspace
OV 101 SCOT 50 m column 80-220 at 5°C/min

4. IDENTIFICATION OF COMPONENTS

4.1 Retention Indices

KOVATS Retention indices were determined using a fused silica OV 101 column (Section III 5.1) in three isothermal temperature runs (120°C, 140°C, and 160°C). In addition, retention indices were determined during linear temperature programming from 80-200°C at 2°C/minute. Identification of components using retention indices was achieved by comparison with published work (Jennings and Shibamoto 1980; Andersen et al. 1969, 1977) and determination of indices using pure standards (verified by GC/MS) in the laboratory. The retention data obtained is collated in Table 4.2.1.

4.2 Gas Chromatography/Mass Spectrometry

The combined GC/MS facility described in Section III 5.2 was used to examine blackcurrant oils in a variety of forms - as concretes, vacuum distillates, column chromatography fractions, HPLC fractions and liquid carbon dioxide extracts. The information obtained from these extracts is contained in Table 4.2.1 and Figure 4.2.1. Identification of component peaks was made by comparison with published work (Heller and Milne 1978, Hirose 1967, Mashonas and Lund 1970, Stenhagen et al. 1974) using VG data systems library search capabilities. A total of one hundred and twenty three components have been detected in the blackcurrant oil, of which sixty six have been positively identified and are named in Table 4.2.1. Good quality mass spectra have been obtained for a further fifty seven components which are not named due to limitations of the library data system. These mass spectra are to be found in Appendix VIII 4. High resolution GC/MS has enabled formulae and structural

FIGURE 4.2.1 Some components identified in blackcurrant
bud oil
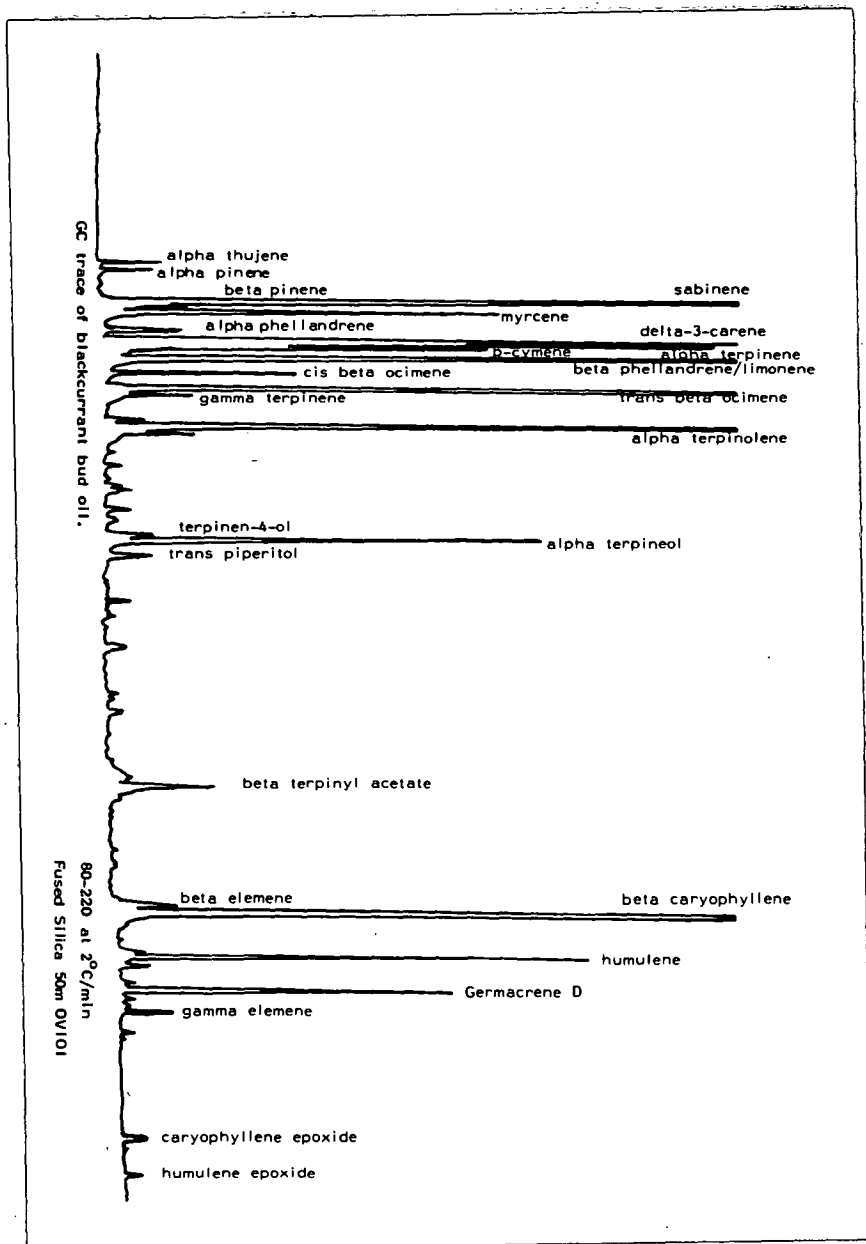
TABLE 4.2.1   Identification of components in blackcurrant bud oil

Peak Number	Name	% Peak Area	Component identified[**]	Chromatogram peak number (Figure)	I_{120}	I_{140}	I_{160}	$I_{Programmed}$	Source for Identification[**]
1	acetic acid	-	BF						4
2	isobutanol	-	F						4
3	n-butanol	-	F						4
4	pentan-2-ol	-	F						4
5	2 methyl butan-1-ol	-	N						4
6	unknown alcohol (1)	-	U						4
7	unknown alcohol (2)	-	U						4
8	unknown alcohol (3)	-	U						4
9	unknown alcohol (4)	-	U						4
10	xylene isomers	-	F						7,11
11	tricyclene	0.01	N						3
12	alpha thujene	0.38	F	1	928	929		928	1
13	alpha pinene	0.33	BF	2	940	941		937	1
14	unknown MW119 (5)	tr	U						10
15	unknown MW120 (6)	tr	U						10
16	benzene	tr	F						11
17	benzaldehyde	tr	F						11
18	propyl benzene	tr	N						10
19	iso propyl benzene	tr	N						10
20	beta thujene	0.04	N						5
21	1-oct-en-3-ol	0.03	BF						11
22	unknown MW136 (7)	tr	U						6
23	1-ethyl 2 methyl benzene	0.03	N						6
24	sabinene	15.44	BF	3	957	970		971	1
25	beta-pinene	0.71	BF	4	972	981		977	1
26	1,2,3 trimethyl benzene	tr	N						10
27	myrcene	2.81	BF	5	990	993		984	1
28	unknown MW136 (8)	tr	U						6
29	unknown MW120 (9)	tr	U						9,10
30	1 methyl 2 ethyl benzene	tr	F						9,10
31	alpha phellandrene	0.69	BF	6	1001	1001		1000	1
32	unknown MW136 (10)	tr	U						5
33	unknown MW136 (11)	tr	U						5
34	delta-3-carene	12.65	BF	7	1008	1012		1010	1
35	alpha terpinene	3.90	BF	8	1011	1014		1014	1
36	p-cymene	2.64	BF	9	1013	1019		1017	1
37	beta-phellandrene	3.25	BF	10	1028	1030		1023	1
38	limonene	3.25	BF	10	1029	1036		1026	1
39	cis beta ocimene	1.26	BF	11	1033	1040		1029	1
40	trans beta ocimene	6.75	BF	12	1036	1057		1041	1
41	3,5,5 trimethyl n-hexanol	tr	N						11
42	2-ethyl-hexanol	tr	F						5,12
43	gamma terpinene	0.82	BF	13	1053	1061		1054	1
44	cymenene	0.02	N		1059	1090		1060	1
45	unknown MW154 (12)	0.02	U						5
46	linalool	0.28	BF					1078	1
47	alpha terpinolene	11.63	BF	14	1008	1097		1083	1
48	non-an-2-one	1.11	N					1089	1
49	unknown MW154 (13)	tr	U						5
50	unknown MW154 (14)	tr	U						11

TABLE 4.2.1 (continued)

Peak Number	Name	% Peak Area	Component identified[**]	Chromatogram peak number (Figure)	I_{120}	I_{140}	I_{160}	$I_{Programmed}$	Source for Identification[**]
51	unknown (15)	tr	U						11
52	unknown (16)	tr	U						11
53	unknown MW152 (17)	0.35	U		1132	1137			5,7,11
54	unknown MW152 (18)	tr	U						4
55	unknown MW152 (19)	tr	U						4
56	unknown MW152 (20)	tr	U						4
57	unknown MW154 (21)	tr	U						4
58	unknown MW182 (22)	tr	U						7
59	cis-p-menth-2-ene 1,8 diol	tr	N						4
60	menthone	0.24	N						7
61	unknown MW182 (23)	tr	U						7
62	naphthalene	0.25	N						7
63	terpinen-4-ol	0.51	BF	15	1166	1172		1165	1
64	alpha terpineol	3.78	BF	16	1172	1178		1168	1
65	p cymen-8-ol	0.53	F						4
66	trans piperitol	0.11	F	17	1180	1186		1179	1
67	sabinene hydrate	0.06	N						4
68	unknown MW152 (24)	tr	U						4
69	unknown MW152 (25)	tr	U						4
70	unknown MW154 (26)	tr	U						4
71	unknown MW150 (27)	tr	U						7
72	unknown MW182 (28)	tr	U						7
73	unknown MW182 (29)	tr	U						7
74	carvone	0.21	F						11
75	unknown MW182 (30)	tr	U						7
76	unknown MW180 (31)	tr	B						7
77	unknown MW180+182 (32)	tr	U						7
78	unknown MW180 (33)	tr	U						7
79	unknown MW168 (34)	tr	U						5
80	unknown MW182 (35)	0.12	U					1237	2,12
81	citronellyl formate	0.17	U					1241	7
82	bornyl acetate	0.17	B		1271				6,7
83	2-undecanone	tr	N						7
84	4-terpinyl acetate	0.78	F		1331	1332			6,7
85	beta terpinyl-acetate	1.87	N	18		1336		1336	7
86	citronellyl acetate	0.01	BF						7
87	geranyl acetate	0.03	F					1353	7
88	methyl undecanoate	0.02	N						7,11
89	alpha copaene	0.08	B						2
90	unknown MW204 (36)	0.05	U						4
91	unknown MW204 (37)	0.10	U						4
92	beta elemene	0.84	B	19		1414	1428	1412	1
93	beta caryo-phyllene	12.39	BF		1413	1427	1439	1421	1
94	unknown MW204 (38)	0.06	U			1431			7
95	unknown MW204 (39)	0.30	U		1427	1441	1455		7
96	humulene	3.79	BF	21	1440	1457	1468	1454	1
97	alloaromadrene	0.25	N		1445	1460	1482	1461	4,5
98	unknown MW204 (40)	0.02	U						5
99	unknown MW204 (41)	0.13	U						7

TABLE 4.2.1 (continued)

Peak Number	Name	% Peak Area	Component identified**	Chromatogram peak number (Figure)	I_{120}	I_{140}	I_{160}	$I_{Programmed}$	Source for Identification**
100	Germacrene D	2.61	N	22	1464	1478	1491	1479	2,4,5
101	gamma elemene	0.83	F	23		1505		1493	1
102	unknown MW204 (42)	0.10	U						6
103	gamma cadinene	0.10	N						4,5
104	beta cadinene	0.08	N						4,5
105	unknown MW204 (43)	tr	U						5
106	beta elemene alcohol?	0.20	N	24		1513	1517	1516	7
107	gamma elemene alcohol?	0.07	N	25			1522		4,7
108	caryophyllene epoxide	0.25	N	26			1574	1574	1,4
109	unknown MW204 (44)	tr	U						8
110	humulene epoxide	0.19	N	27			1580		1,4
111	unknown (45)	-	U						8
112	unknown (46)	-	U						8
113	unknown (47)	-	U						8
114	unknown MW204 (48)	-	U						7
115	unknown MW220 (49)	-	U						4
116	unknown MW220 (50)	-	U						4
117	unknown MW220 (51)	-	U						4
118	unknown MW220 (52)	-	U						4
119	unknown MW220 (53)	-	U						4
120	unknown MW220 (54)	-	U						4
121	unknown MW250 (55)	-	U						8
122	unknown MW250 (56)	-	U						8
123	unknown MW286 (57)	-	U						4

KEY TO TABLE 4.2.1

**
source for identification
1 blackcurrant concrete
2 vacuum distillate
3 concrete headspace
4 liquid carbon dioxide extract
5 liquid carbon dioxide
6 silica gel chromatography Fraction 12
7 silica gel chromatography Fraction 19
8 silica gel chromatography Fraction 24
9 Florisil chromatography Fraction 8
10 Florisil chromatography Fraction 13
11 Florisil chromatography Fraction 17
12 HPLC Fraction 1

**
component
B previously identified in buds
F previously identified in fruit
N newly identified
U unknown

* column 5 refers to peaks in Figures 4.2.1 and 5.2.1

TABLE 4.2.2 Suggested formulae for some unknown components

Unknown	Molecular Weight	Suggested Formulae	Structural Inference
1	86		
2	86		primary alcohol
3	86		
4	86		
5	119		
6	120		
7	136		unusual monoterpene
8	136		" "
9	120		
10	136	$C_{10}H_{16}$	monoterpene hydrocarbon
11	136	$C_{10}H_{16}$	monoterpene hydrocarbon
12	154	$C_{10}H_{18}O$	
13	154	$C_{10}H_{18}O$	
14	154	$C_{10}H_{18}O$	
15			
16			
17	152	$C_{10}H_{16}O$	carbonyl group
18			
19			
20			
21	182	$C_{12}H_{22}O$	
22	182	"	
23	150	$C_{10}H_{14}O$	
24	182	$C_{12}H_{22}O$	
25	182	"	related to menthone
26	182	"	
27			
28			
29			
30			
31	180	$C_{12}H_{20}O$	
32	180 + 182 mix	$C_{12}H_{20}O + C_{12}H_{22}O$	
33	180	$C_{12}H_{20}O$	
34	168		
35	182	$C_{12}H_{22}O$	some similarity to paracumic aldehyde
36	204	$C_{15}H_{24}$	very similar spectra to clovene
37	204	"	
38	204	"	
39	204	"	
40	204	"	
41	204	"	
42	204	"	
43	204	"	
44	204	"	
45			
46			
47			
48	204		
49	220		
50	220		
51	220		
52	220		
53	220		
54	220		
55	250		
56	250		
57	286		

information to be derived for some unknown components; this is included in Table 4.2.2. Of the sixty six components which have been positively identified, some twenty three are compounds not previously identified in blackcurrant fruit or bud oils; as reported in the literature reviewed in Section II 2.3.

5. ORGANOLEPTIC ASSESSMENT

5.1 Comparative Analysis

Comparative analysis results obtained from two manufacturers (1,2) are contained in Table 5.1.1 with the assessment carried out in this laboratory (3) before the samples were submitted for analysis. From these comparisons it is clear that there is a quality difference due to ageing. Concretes of recent origin (820) are preferred to those older products (800). This is probably due to oxidation and loss of top notes on standing (despite storage at below 4°C in airtight containers). All assessors considered the machine harvested product inferior to hand-picked buds, and one (2) even considered it unacceptable.

The varieties, 820 (White Bud selection, Bushy Park), and 822 (Grahams White Bud no. 1 selection), 823 (Goliath) and 824 (Baldwin) are those most preferred. However, manufacturer (2) considers 823 unacceptable despite the other assessors preference for this product. Generally speaking the better quality samples smell more musky and cat-like; whereas the poorer samples are reminiscent of monoterpene hydrocarbon resin aromas. This does not necessarily infer they have a higher percentage of monoterpenes but rather that they lack the characteristic musky, cat-like odour. It is important to note that two selections of White Bud, the main local variety, are considered of high

TABLE 5.1.1

Samples		Comparisons											
		A			B			C			D		
Ref. No.	Identity	1	2	3	1	2	3	1	2	3	1	2	3
800	1980 Bulk concrete	2	3	3									
810	1981 " "	3	2	2									
820	1982 " " (i) hand-picked	1	1	1	1	1	1	2	1	1	3	2	2
821	1982 " " (ii) machine harvested				2	4^U	2						
822	1982 variety - Grahams no. 1 White Bud							5	2^U	3			
823	" " - Goliath							1	6^U	2	2		
824	" " - Baldwin							4^U	3^U	4			
825	" " - Boskoop							7^U	5^U	6			
826	" " - Lees Prolific							3^U		7			
827	" " - Kerry							6^U	4	5			
828	Liquid CO_2 extract										1**	1**	1**

Comparisons requested:

(A) Any difference in quality due to ageing? (800, 810, 820)
(B) Any effect of machine harvest on quality? (820, 821)
(C) Any varietal preference? (820, 822-827)
(D) Assessment of liquid CO_2 extract against other preferred products.

Notes: U - considered of unacceptable quality.
   ** - quality considered very good, cannot be compared to the others being of much higher quality.
        Any product scoring less than 3 must be considered of poor quality and not marketable.

        Scale is a preference ranking of samples included
        in the four comparisons.

quality in themselves, as well as being preferred to Baldwin; the principal English variety.

The liquid carbon dioxide extract produced in the laboratory was considered superior to the solvent extracted products in all respects. This is probably due to the fact that it is solvent-free, and demonstrates the potential of liquid carbon dioxide extracts to retain the natural aroma of the material.

5.2 Aromagram

The outcome of attempts to relate odours to compound eluting from the gas chromatography column are presented in Table 5.2.1 and Figure 5.2.1. The study has indicated that while the aromagram is complex, five regions have been identified as important in the overall blackcurrant aroma impression. The first region that has been identified retains a steely spicy note very reminiscent of the French CAL Cassis absolute (Figure 5.2.2). The second region contains the characteristic 'catty' note with an after impression of blackcurrant fruit. The catty odour is extremely intense, completely overriding the previously dominant pine/resin aromas. The other three regions give impressions of blackcurrant fruit alone.

6. ELECTRON MICROSCOPY

6.1 Scanning Electron Microscopy

Blackcurrant buds were sampled from early May to late July 1980 (Plates A-C), and then from the beginning of October 1980 through until the end of January 1981 (Plates 1-18). The purpose of this study was to determine the optimal time for gland filling and to see any correlation

FIGURE 5.2.1 Aromagram Tasmanian blackcurrant bud oil
50m SCOT OV 101. 80-220 at 5°C/min.

clear resin
dry wood
musty
musty
pine resin
dry woody
clear hollow
pine-like
pine
sweet pine
sweet pine
wet wood
dull woody
bittersweet
lemon
musty sweet pine
sweet floral
earthy
flower blossom
steely spicy
sweet camphorous
blackcurrant fruit
2° cats urine
blackcurrant
musty pine
citronell
lemon woody
compost
damp soil
earthy
lemon
woody
rich sweet
jam-like
sharpwood
sweet
blackcurrant fruit
flatulent
fungi
sweet
damp
musky
sickly sweet
sweet fruity
sharp, acidic, citrus
blackcurrant, fruity
woody antiseptic
citrus
lemon
floral
1*
3*
4*
9*

FIGURE 5.2.2 Aroma of CAL cassis absolute
50m SCOT OV 101. 80-220 5°C/min.

catty
blackcurrant fruity
green herby
steely spicy
sickly sweet
bakehouse
spicy
burnt
paint-like
grassy-hay
raspberry fruity
soapy
rubbery
strawberry jam
mouldy
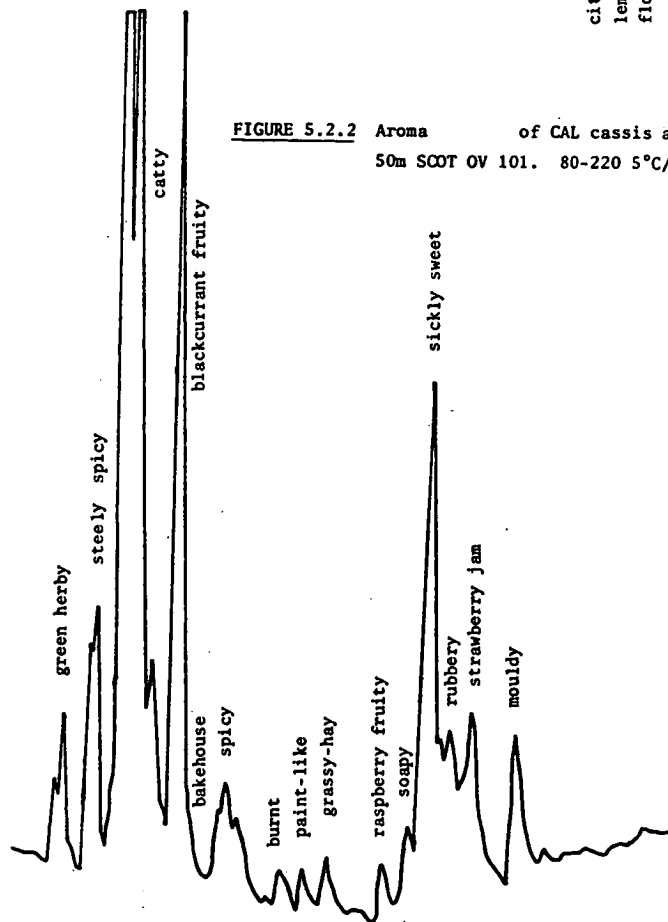
TABLE 5.2.1 Aroma sensations detected with blackcurrant vacuum distillate

Aroma	Peak	Identity
1** steely spicy		
clear hollow	12	alpha thujene
pine	13	alpha pinene
sweet		
camphorous		
dull woody		
pine like	24	sabinene
clear-resin	25	beta pinene
2** blackcurrant fruit		
** cat's urine		
unpleasant sulphur		
sweet pine	31	alpha phellandrene
wet wood		
sweet pine	34	delta-3-carene
dry wood		
eucalyptus		
musty		
musty		
bitter sweet		
lemon	37	beta phellandrene
citrus	38	limonene
pine resin	40	trans-beta-ocimene
sweet/pine	43	gamma terpinene
musty		
dry woody	47	alpha terpinolene
sweet floral		
3** blackcurrant fruit		
musty pine		
citrus		
taint		
lemon		
woody		
sugary		
compost	62	terpinen-4-ol
damp soil	63	alpha terpineol
earthy		
lemon		
woody		
flatulent		
rich sweet		
jam like		
woody		
sharp wood		
sweet		
4** blackcurrant fruit		
flatulent		
fungi		
sweet		
damp		
musky	92	beta caryophyllene
flower blossom		
citrus/lemon/sharp		
wood shavings		
sickly sweet	95	humulene
floral		
sweet fruity	99	germacrene- D
damp wood		
sharp wood		
sharp, acidic, citrus		
jam burnt		
sweet antiseptic		
5** blackcurrant fruit		
woody antiseptic		

*footnote: Peak numbers refer to general numbers in Table 4.2.1.
Five important regions discriminated on the basis that
these aromas are considered to be the important ones
determining quality of the extract.

with oil accumulation studies (Section IV 9.2).

Plates A-C demonstrate little change in gland morphology during the winter or dormant period, indicating that there is no marked gland filling at this time although there may be compositional changes occurring in the oil (Section IV 9.2). Plates 1-18 demonstrate an increase in size of the glands from late October (Plate 1) until the middle of January (Plate 14). Once the glands have reached full size there is no apparent change through to February (Plates 15-18), and indeed during the winter period (Plates A-C). This morphological study suggests the maximum time of filling was late November-December, when the glands swell considerably in size. Compositional changes observed during the growing period are discussed in Section IV 9.2.

A further study was undertaken from late August 1983 to mid September 1983, during the period of budburst (Plates 19-35). In late August, the glands on the bracts were at the swollen stage reached in the middle of January (Plate 14), whether the bracts are from closed buds (Plates 20 and 22) or buds that are just opening (Plates 19-23). The glands on the leaf initials were swollen in the closed bud (Plates 24 and 26), but appeared to have lost some of their oil content once the leaves began to open (Plates 23 and 28). The bracts, which were gradually shed as the bud opened retained their swollen glands until the end (Plates 26, 30 and 31). By the time the bud was fully opened, with three or four leaves, the oil glands on the first emerging leaf were swollen again (Plates 32 and 33). At this time the glands on the youngest emerging leaves were not yet full (Plates 34 and 35); yet the leaf petioles had full glands (Plate 29).

It is interesting to note that there is no detectable difference between the quality of the micrographs produced using the two preparation procedures. Those produced (Plates A-C), using the long process of fixation in Osmium tetroxide and freeze drying before coating, show no

SECTION IV 6 Scanning Electron Micrographs
                Oil gland morphology during the winter.

PLATE A: Oil glands from inner bract (5)
          x160   sampled 3/5/80

PLATE B: Oil glands from inner bract (5)
          x160   sampled 15/6/80

PLATE C: Oil glands from inner bract (5)
          x200   sampled 22/7/80
          Note no change in gland morphology over the
          winter period.
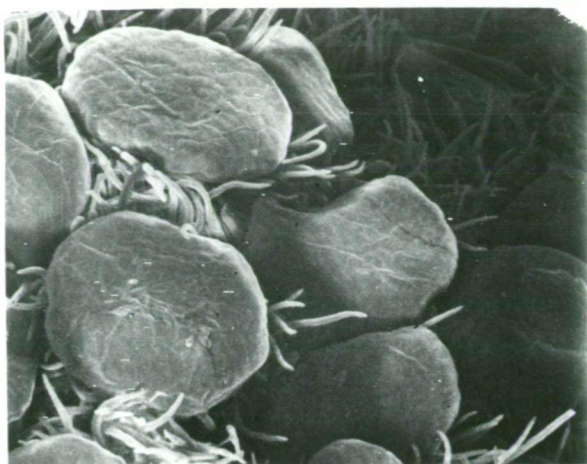
A

B

C

SECTION IV 6 Scanning Electron Micrographs
Oil gland morphology during the growing season

PLATE 1: Micrograph of an oil gland on an outside bract (1)
x1500   sampled 9/10/80

PLATE 2: Micrograph of an oil gland on an outside bract (1)
x1000   sampled 9/10/80
Clearly shown are the individual secretory cells which
secrete the oil into the cuticular space.

PLATE 3: Micrograph of an oil gland on an outside bract (1)
x400   sampled 22/10/80
The dish shape of an empty gland is evident in
Plates 3 and 4.

PLATE 4: Micrograph of an oil gland on an outside bract (1)
x400   sampled 4/11/80

PLATE 5: Oil gland on an outside bract (1)
x320   sampled 18/11/80
Filling of the gland is just beginning to occur

PLATE 6: Oil glands on an outside bract (1)
x250   sampled 18/11/80
The gland filling observed in the previous plate
is not yet a general occurance.

1

2

3

4

5

6

PLATE 7: Oil glands on an outer bract (3)
          x300   sampled 3/12/80
          Note filling has become commonplace.

PLATE 8: Oil glands on an inner bract (5)
          x300   sampled 3/12/80
          Showing filling has become commonplace.

PLATE 9: Oil gland on an outside bract (1)
          x 200   sampled 17/12/80
          The glands are swelling, even on the outside bracts
          which are the last to begin filling

PLATE 10: Micrograph of oil glands on an inner bract (6)
           x300   sampled 17/12/80

PLATE 11: Micrograph of oil glands on an outer bract (4)
           x300   sampled 5/1/81

PLATE 12: Micrograph of oil glands on an inner bract (7)
           x400   sampled 5/1/81

7

8

9

10

11

12

PLATE 13:   Oil glands on an outer bract (3)
            x200   sampled 19/1/81
            Showing maturity in size.

PLATE 14:   Oil glands on an inner bract (8)
            x250   sampled 19/1/81
            Showing maturity.

PLATE 15:   Oil gland on an outer bract (2)
            x400   sampled 2/2/81

PLATE 16:   Oil glands on an outer bract (4)
            x300   sampled 2/2/81

PLATE 17:   Micrograph of oil glands on an inner bract (7)
            x300   sampled 2/2/81

PLATE 18:   Micrograph of oil glands on an inner bract (11)
            x 320   sampled 2/2/81
            Plates 15-18 demonstrate the homogenicity of gland
            filling throughout the bud.
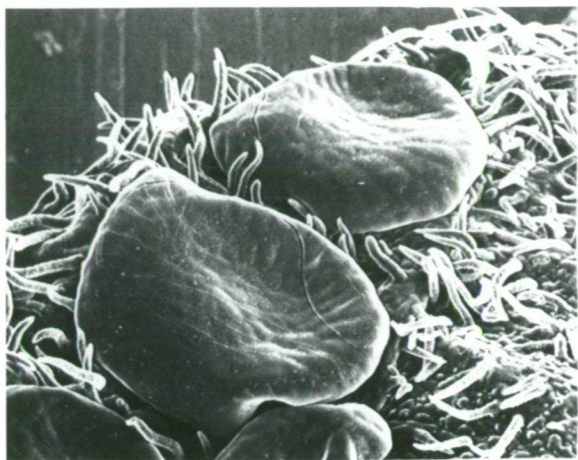
13

14

15

16

17

18

<u>SECTION IV 6</u> Scanning Electron Micrographs
Oil gland morphology at bud burst

<u>PLATE 19:</u> Oil glands on an inner bract (5) of an opened bud
x163   sampled 25/8/83

<u>PLATE 20:</u> Oil glands on an inner bract of a closed bud
x163   sampled 25/8/83

<u>PLATE 21:</u> Oil glands on an inner bract (8) of an opened bud
x156   sampled 25/8/83

<u>PLATE 22:</u> Oil glands on an inner bract (8) of a closed bud
x163   sampled 25/8/83

<u>PLATE 23:</u> Oil glands on the first leaf of an opened bud
x312   sampled 25/8/83
Note the unfilled nature of the glands compared to
Plate 24.

<u>PLATE 24:</u> Oil glands on the first leaf initial of a closed bud
showing full glands
x341   sampled 25/8/83

19

20
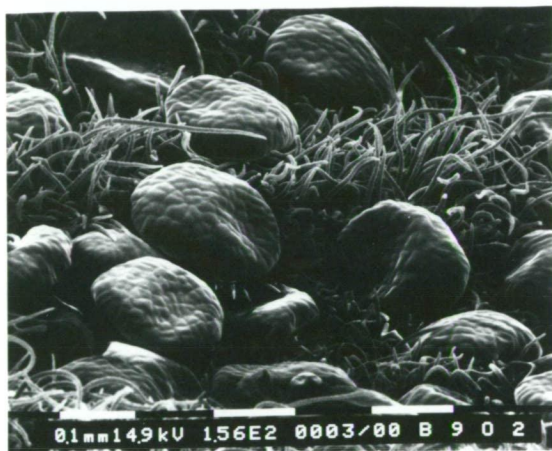
21

22
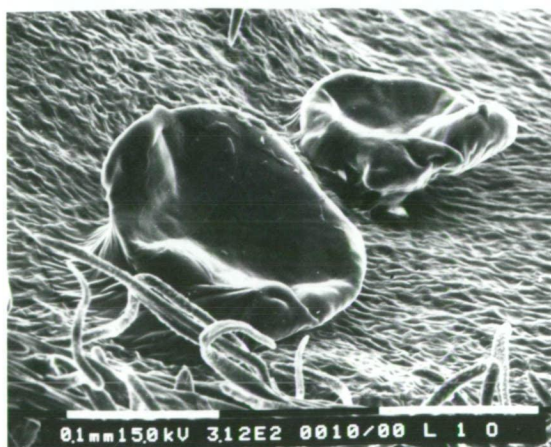
23

24

SECTION IV. 6   Scanning Electron Micrographs
                 Oil gland morphology at bud burst

PLATE 25:   Full glands on the second opened leaf
             x212   sampled 31/8/83

PLATE 26:   Full glands on the second leaf initial of
             a closed bud
             x163   sampled 25/8/83

PLATE 27:   Full glands on an inner bract (4) of an open bud
             x163   sampled 31/8/83

PLATE 28:   Unfilled glands on the second emerged leaf of an
             open bud
             x326   sampled 25/8/83

PLATE 29:   A single full oil gland on the third leaf petiole
             in an open bud
             x388   sampled 14/9/83
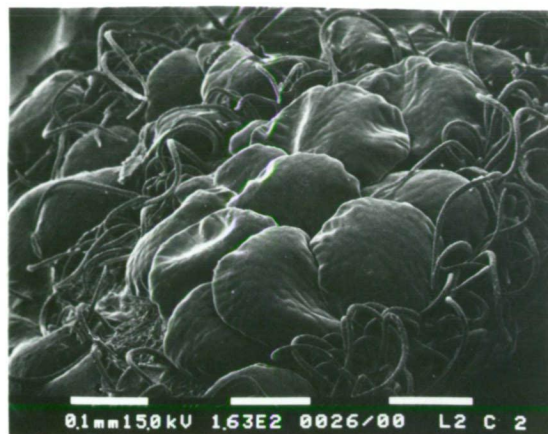
0.1mm 15.0kV 2.12E2 0033/00 10L2J04

25

0.1mm 15.0kV 1.63E2 0026/00 L2 C 2

26

0.1mm 15.0kV 1.63E2 0031/00 B4 J04

27

0.1mm 15.0kV 3.26E2 0006/00 L 2 0

28

0.1mm 15.0kV 3.88E2 0065/00 LP3 06

29

PLATE 30:   Full oil glands on an inner bract (5) of an opened bud
            x221   sampled 31/8/83

PLATE 31:   Full oil glands on an inner bract (6) of an opened bud
            x221   sampled 14/9/83

PLATE 32:   Full oil glands on the first leaf of an opened bud
            x203   sampled 31/8/83

PLATE 33:   Full oil glands on the first leaf of an opened bud
            x312   sampled 14/9/83

PLATE 34:   Oil glands on the third emerging leaf of an open bud
            x356   sampled 31/8/83
            Note these are not yet full.

PLATE 35:   Oil glands on the third emerging leaf of an open bud
            x326   sampled 14/9/83
            Note these are not yet full.

0.1mm 15.0kV 2.21E2 0032/00 B5 05

30

0.1mm 15.0kV 3.41E2 0059/00 B6 06

31

0.1mm 15.0kV 2.03E2 0035/00 8L1 05

32

0.1mm 15.0kV 3.12E2 0060/00 L1 06

33

0.1mm 15.0kV 3.56E2 0063/00 L3 06

34

0.1mm 15.0kV 3.26E2 0034/00 10L3 05

35

advantage over those fresh samples which were coated just prior to examination (Plates 1-35). The fresh samples provide excellent quality micrographs even from leaf samples (Plates 32-35) which have a reasonably high water content.

6.2   Light Microscopy

Light micrographs were prepared by the procedure outlined in Section III 7.2. The plates presented (Figures 6.11 and 6.12) show clearly the structure of the oil glands as discussed in Section II 1.4. The layer of secretory cells is very evident, as is the cuticular space into which the terpene secretions are deposited. The multi-cellular stalk is evident (Figure 6.12), but is not always as well developed. Figure 6.13 taken under low power (x3) shows clearly the position of the yellow oil glands on the bract surface.

FIGURE 6.2.1   Light Micrographs
              Cross-section showing oil gland structure (x32)
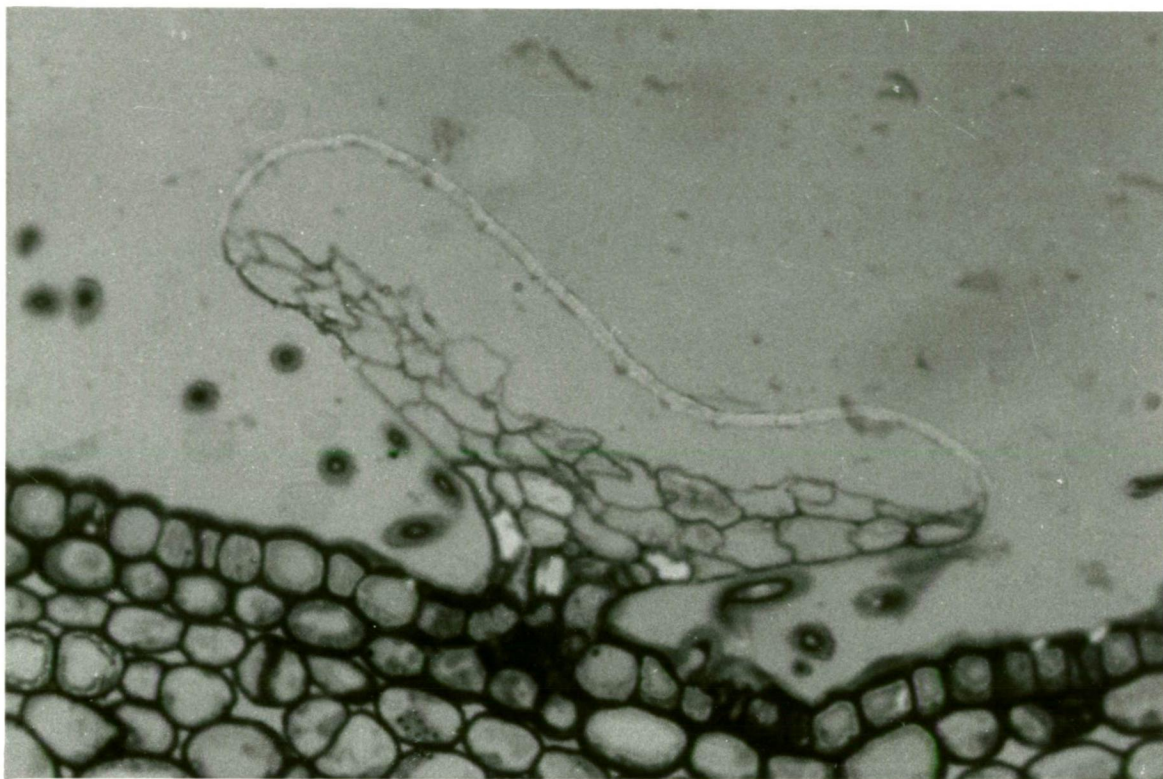
FIGURE 6.2.2   Cross-section showing oil gland structure.
              Note well developed multicellular stalk and the
              layer of secretory cells (x32)
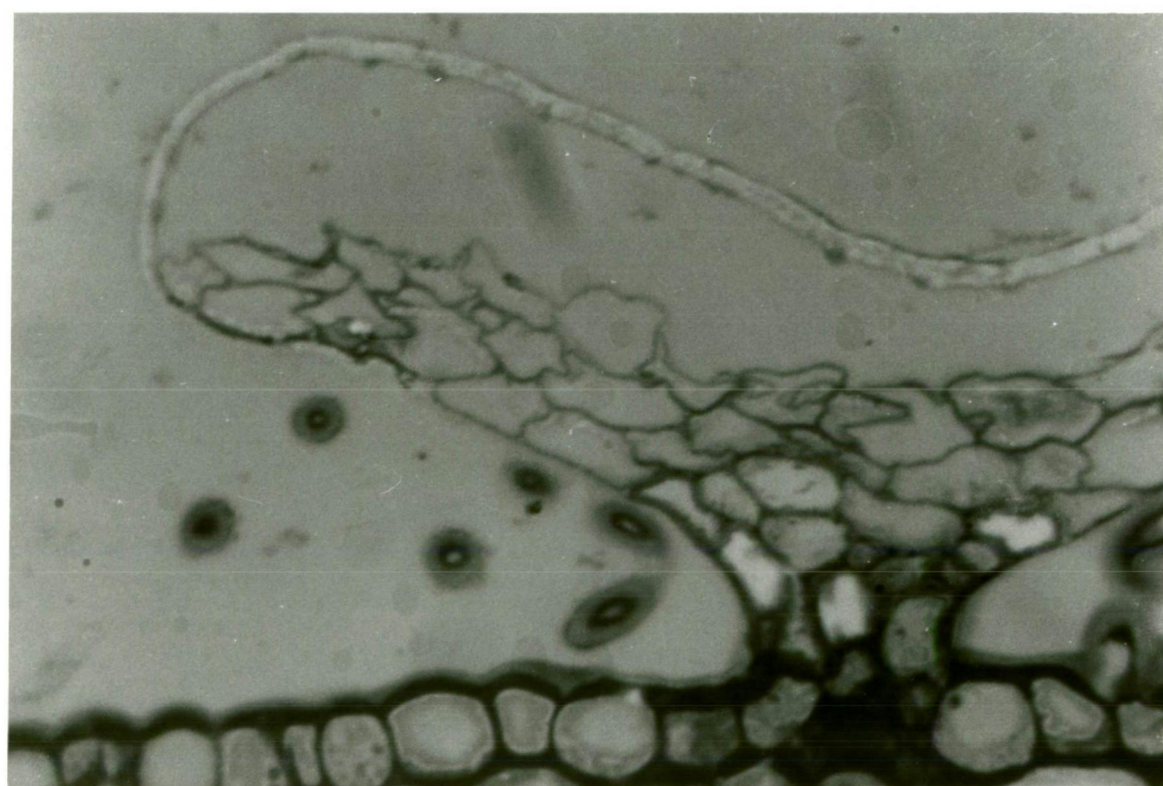
FIGURE 6.2.3 Low power magnification showing distribution of oil
glands on bracts (x3)

7. GAS EXCHANGE MEASUREMENTS

The infa red gas analyser system used in this experiment is fully described in Section III 8. The effect of light intensity on net carbon dioxide exchange is shown in Figure 7.1.1 (data in Appendix 3). Increasing the light intensity from 8 to 400 $\mu\Sigma$ $m^{-2}s^{-1}$ resulted in an increased rate of net carbon dioxide fixation. Light saturation occurred between 400 and 500 $\mu\Sigma$ $m^{-2}s^{-1}$. At a light intensity above saturation (800 $\mu\Sigma$ $m^{-2}s^{-1}$), the effect of temperature on net carbon dioxide fixation was investigated (Figure 7.1.2; data in Appendix 3). Net carbon dioxide fixation in 21%O_2 ('apparent' photosynthesis) reached a maximum at 26°C, and then decreased with increasing temperature. Efflux of carbon dioxide in 21%O_2 in the dark (dark respiration), increased with increasing temperature. The enchancement of net carbon dioxide fixation in 0%O_2 as compared with 21%O_2 increases to a maximum at 24°C and then decreases. This measurement was an estimate of the contribution of photo respiration to the overall net carbon dioxide exchange, and represented an efflux of carbon dioxide from the leaf (Figure 7.1.2 curve 4). By eliminating the contribution of both dark respiration (this assumes that dark respiration continues in the light), and photo respiration from the overall net CO_2 exchange, it was possible to obtain an estimate of 'true' photosynthesis (Figure 7.1.2 curve 5). 'True' photosynthesis reached a maximum at 30°C and decreased when the temperature was increased to 35°C.

8. GLASSHOUSE EXPERIMENTS

8.1 Oil Quality at Bud Burst

An examination of oil quality at bud burst was carried out by

KEY FOR FIGURE 7.1.2

1. 'Apparent' photosynthesis (21% O_2, 310 ppm CO_2, 800 $\mu\Sigma m^{-2}s^{-1}$)

2. Dark respiration (21% O_2, 310 ppm CO_2, in the dark)

3. Enhancement of net CO_2 exchange (2% O_2, 310 ppm CO_2, 800$\mu\Sigma m^{-2}s^{-1}$)

4. Photorespiration (1-3)

5. 'True' photosynthesis (3-2)

FIGURE 7.1.1

EFFECT OF LIGHT INTENSITY ON APPARENT PHOTOSYNTHETIC ACTIVITY
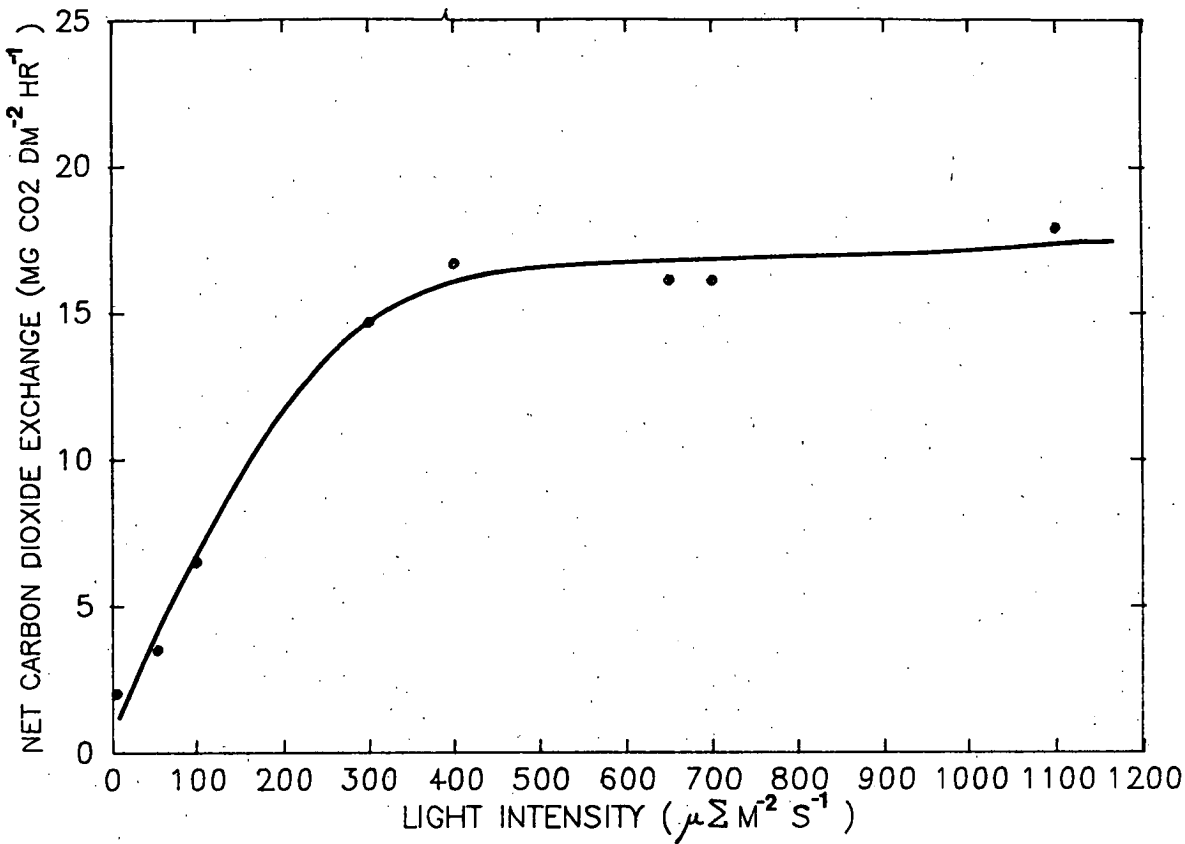
FIGURE 7.1.2

EFFECT OF TEMPERATURE ON APPARENT PHOTOSYNTHETIC ACTIVITY
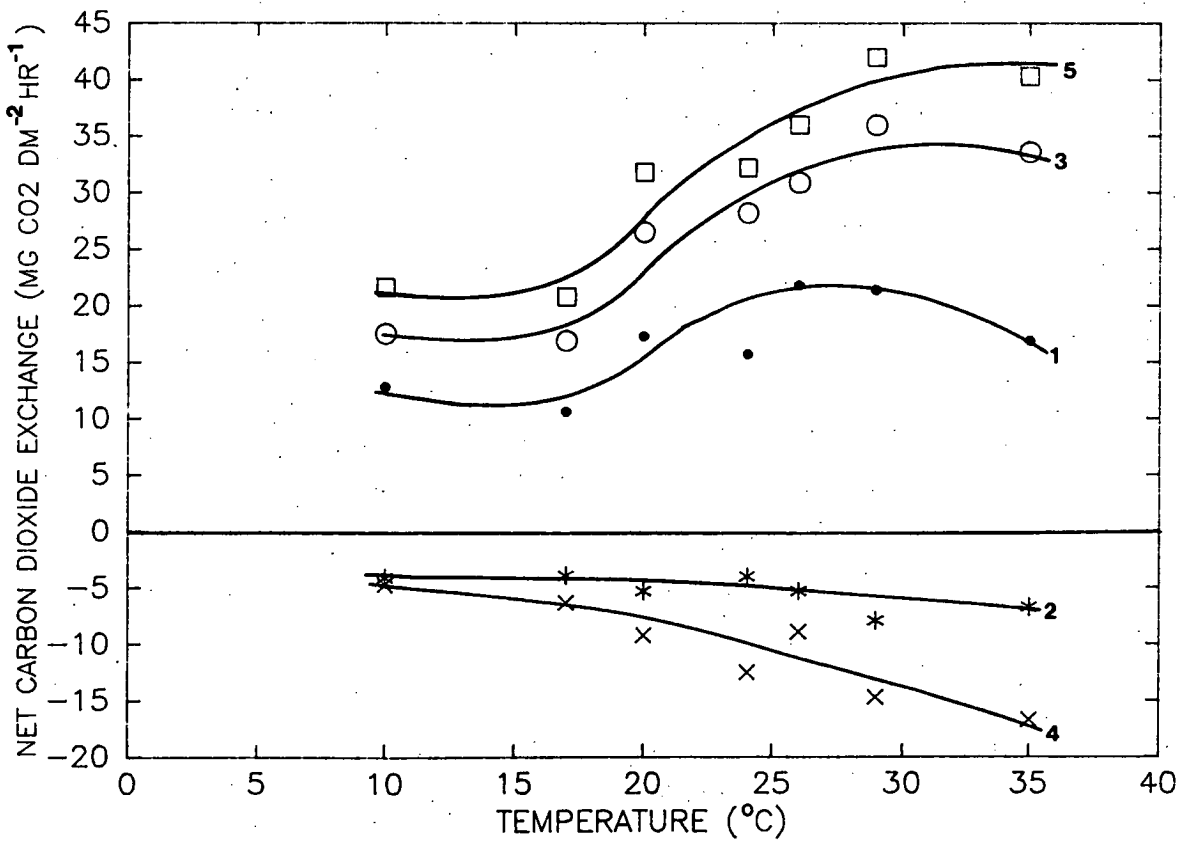
forcing two sets of cuttings to break bud under a 16 hr photoperiod, after three weeks below 5°C. Samples of buds were then taken (Table 8.1.1), at different times after release from the low temperature treatment. The strength of the catty note was then assessed by organoleptic comparison of these samples against standard samples of Tasmanian blackcurrant concrete.* This technique employed two samples of concrete from the preferred cultivar White Bud, extracted from dormant buds. Each sample was then compared with these two controls using the triangle test described by Larmond (1977).

The strength of the catty note was demonstrated to increase as the buds broke from dormancy. The fruity, fresh top notes also decline as the cattiness increases, thus at the time of full bud burst, the product is past its desirable peak where a balance of cattiness and fresh fruity top notes is evident. This balance occurs when 65-70% of the buds are open (Table 8.1.1), probably because the intensity of oil from opened buds is being mollified by that contained in the closed buds.

8.2 Carbon ^{14} Tracing of Oil Synthesis

An attempt was made to identify times of oil synthesis by monitoring the accumulation of a C^{14} label in the bud tissue. The label was applied by utilising labelled carbon dioxide. Table 8.2.1 contains the accumulated data for five separate weeks, of counts of applied label per gram fresh bud weight and the level of oil present in the bud. The amount of label detected in the bud extract varies considerably, without any apparent pattern. In addition, the level of oil present (measured in $\mu\ell/g$ buds) declines from the date of label application, in nearly all cases. This is difficult to relate to data showing oil gland filling was occurring during the January period (section IV 6.1).

footnote: See Section III 6.1 for a full description of aroma assessment procedure.

TABLE 8.1.1 Oil quality at bud burst

Harvest Date[†]		Percentage bud open	Sample	Strength of catty note	Preference rating
May 19th	20	8.4	mixed buds		
	20	"	partly open buds	*	
	28	30.8	open buds	**	
	28	"	closed buds	*	
	28	"	partly open buds	**	
	36	65	open buds	**	
	50	100	"	***	
Jan 1st	17	34.5	open buds	*	3
	17	"	closed buds	weak	
	17	"	open buds	*	3
	24	38	"	**	2
	38	70	"	***	1
	50	100	"	****	2

[†] in days after release from 3 weeks vernalization treatment below 5°C in the dark.

The two dates refer to the day on which the canes were cut from the field.

TABLE 8.2.1 Relationship of C^{14} label and oil accumulation in the bud

Experiment	Date $^{14}CO_2$ applied	Day	Bud Fresh weight (g)	Counts	C^{14} label x 10^4 counts/g buds	Level of bud oil	
						µl	µl/g buds
1	17/12/82	1	0.0900	3524	3.92	3.81	42.36
		5	0.0761	3559	4.68	1.25	16.36
		6	0.1413	3953	2.79	1.25	8.82
2	17/1/83	1	0.1432	355	2.48	9.91	69.18
		2	0.2928	705	2.41	3.15	10.75
		7	0.3027	449	1.48	4.87	16.09
		8	0.2690	201	1.34	4.04	15.00
3	24/1/83	1	0.0717	229	3.19	1.87	26.02
		2	0.1732	256	1.48	2.23	12.87
		4	0.1491	349	2.34	2.38	15.97
		7	0.1673	337	2.01	2.42	14.45
4	31/1/83	1	0.3345	190	0.57	2.88	8.61
		2	0.2308	280	1.21	3.13	13.57
		4	0.2991	245	0.82	4.24	14.17
		7	0.2719	509	1.88	3.51	12.93
5	7/2/83	1	0.0980	237	0.24	2.97	30.31
		2	0.1167	86	0.74	2.17	18.60
		4	0.0847	91	1.07	1.22	14.38
		7	0.1008	80	0.79	2.04	20.21

TABLE 8.2.2 Relationship of amount of C^{14} label, soluble carbohydrate and available polysaccharide

	Day	Location	Soluble Carbohydrate		Available Polysaccharide	
			MG/G Dry Matter	Counts/G Dry Matter	MG/G Dry Matter	Counts/G Dry Matter
Expt. 1	1	Stems	207.24	9333306	41.38	762279
		Leaves	332.08	173731	208.50	76848
	5	Stems	121.42	2814514	59.72	404246
		Leaves	273.84	246767	260.05	18218
	6	Stems	198.18	4543703	0.65	89075000
		Leaves	308.18	436261	324.14	58722
Expt. 2	1	Stems	48.52	1532935	38.72	78076
		Leaves	280.56	15211	308.55	2840
	2	Stems	70.05	547025	66.05	18839
		Leaves	248.70	4643	676.07	785
	7	Stems	106.73	208983	37.79	42380
		Leaves	277.32	1666	207.34	3831
	8	Stems	207.64	136603	71.65	37601
		Leaves	295.49	12125	347.41	2730
Expt. 3	1	Stems	191.84	82023	59.35	9602
		Leaves	305.53	2286	246.91	508
	2	Stems	57.91	696710	59.54	19371
		Leaves	335.13	3239	321.47	936
	4	Stems	67.74	95521	53.79	10966
		Leaves	246.32	243	247.45	639
	7	Stems	78.05	70531	178.07	2828
		Leaves	304.45	445	302.46	495
Expt. 4	1	Stems	70.76	21501	81.14	3000
		Leaves	334.41	728	385.46	500
	2	Stems	536.72	5672	114.03	5652
		Leaves	443.05	897	327.57	657
	4	Stems	79.29	117713	23.37	24251
		Leaves	52.63	23100	421.67	1991
	7	Stems	172.17	22184	20.32	21496
		Leaves	339.94	833	284.88	922
Expt. 5	1	Stems	89.21	59566	84.47	9098
		Leaves	327.39	1329	374.90	622
	2	Stems	84.85	43798	231.25	2132
		Leaves	99.60	3641	313.84	491
	4	Stems	226.95	19424	25.18	65961
		Leaves	347.82	1169	416.67	1039
	7	Stems	105.07	24441	312.23	2437
		Leaves	341.29	584	364.85	599

* see Table 8.2.1 for dates experiments commenced.

In addition to the monitoring of oil accumulation, the levels of
soluble carbohydrate and available polysaccharide were measured in
order to detect any relation between oil accumulation and carbohydrate
availability. This data, collated in Table 8.2.2, demonstrated that
the levels of both soluble carbohydrate, available polysaccharide and
the associated label counts vary widely without any apparent relation.

9. FIELD EXPERIMENTS

9.1 The Effect of Plant Density on Yield Factors

This experiment was carried out using a systematic fan design
(fully described Section III 10.1), for each plant the number, length,
fresh weight of canes, and the yield of handpicked buds were recorded for
the years 1981-1983.   In addition the basal girth of each cane was
measured in 1983.   The data collected are presented graphically in
Figures 9.1.1 to 9.1.8, while the means for the three replicate blocks
are tabulated in Appendix VIII 5.

Bud yield per plant (Figure 9.1.1) is shown to decrease with
increasing plant density, when expressed on a per area basis, bud yield
then increased with increasing density (Figure 9.1.2).   Total fresh
weight yield of canes per plant decreased with increasing plant density
(Figure 9.1.4), indicating the size of each plant decreased at higher
densities. This trend is confirmed by numbers of canes produced by
each plant, which also decreased with increasing plant density (Figure
9.1.5).   The length of extension growth of each cane that occurred
during the summer is reduced at both high and low planting densities
due to competition for resources or lack of competition for light res-
pectively. Further, basal cane girth, a more reliable measure of plant

FIGURE 9.1.1 The relationship of blackcurrant bud yield per plant and planting density
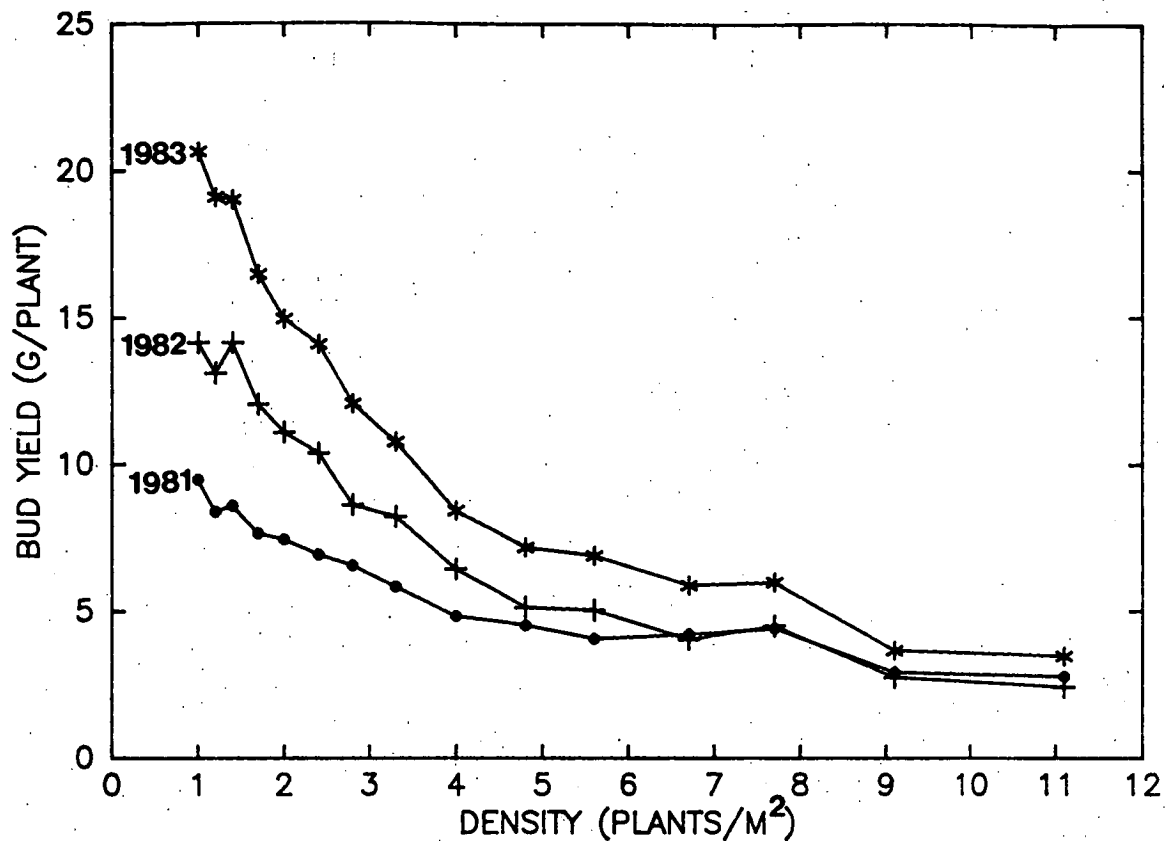
FIGURE 9.1.2 The relationship of blackcurrant bud yield per square metre and planting density
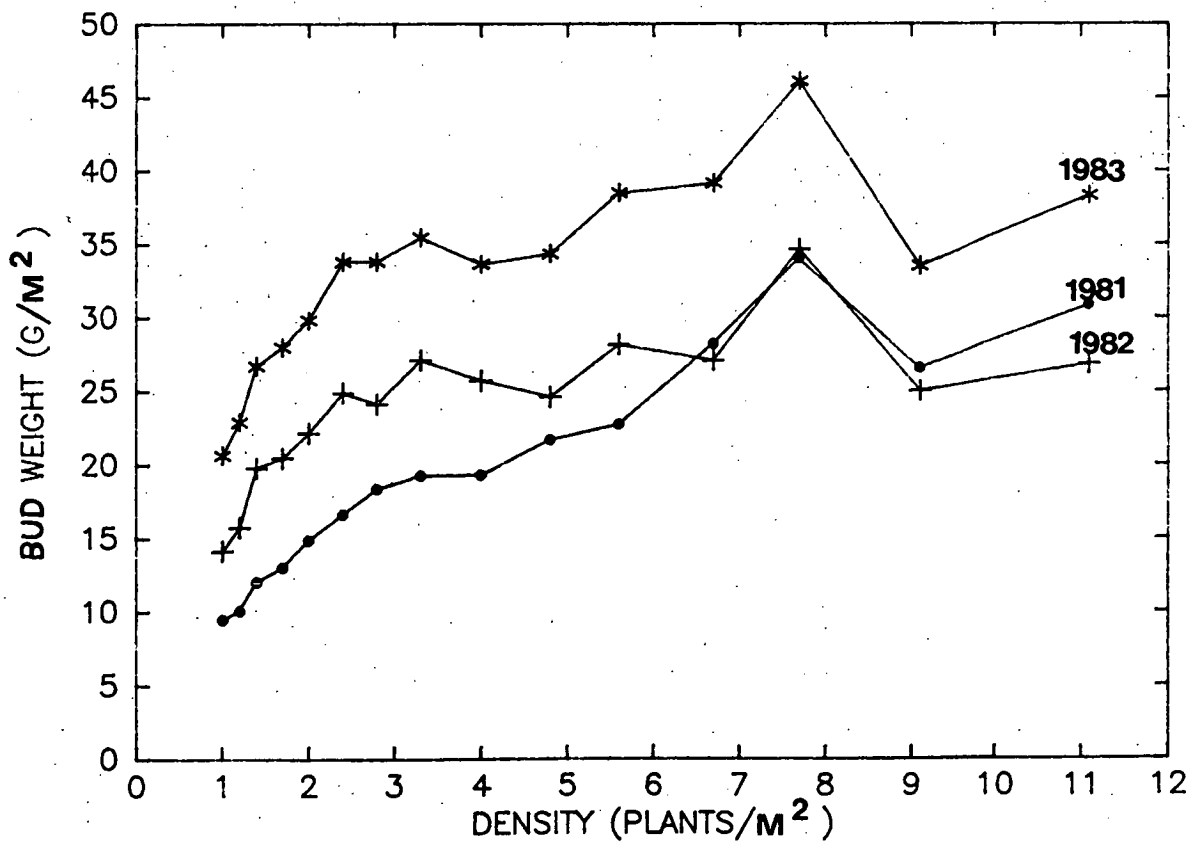
FIGURE 9.1.3 The relationship of plant density and bud yield per shoot
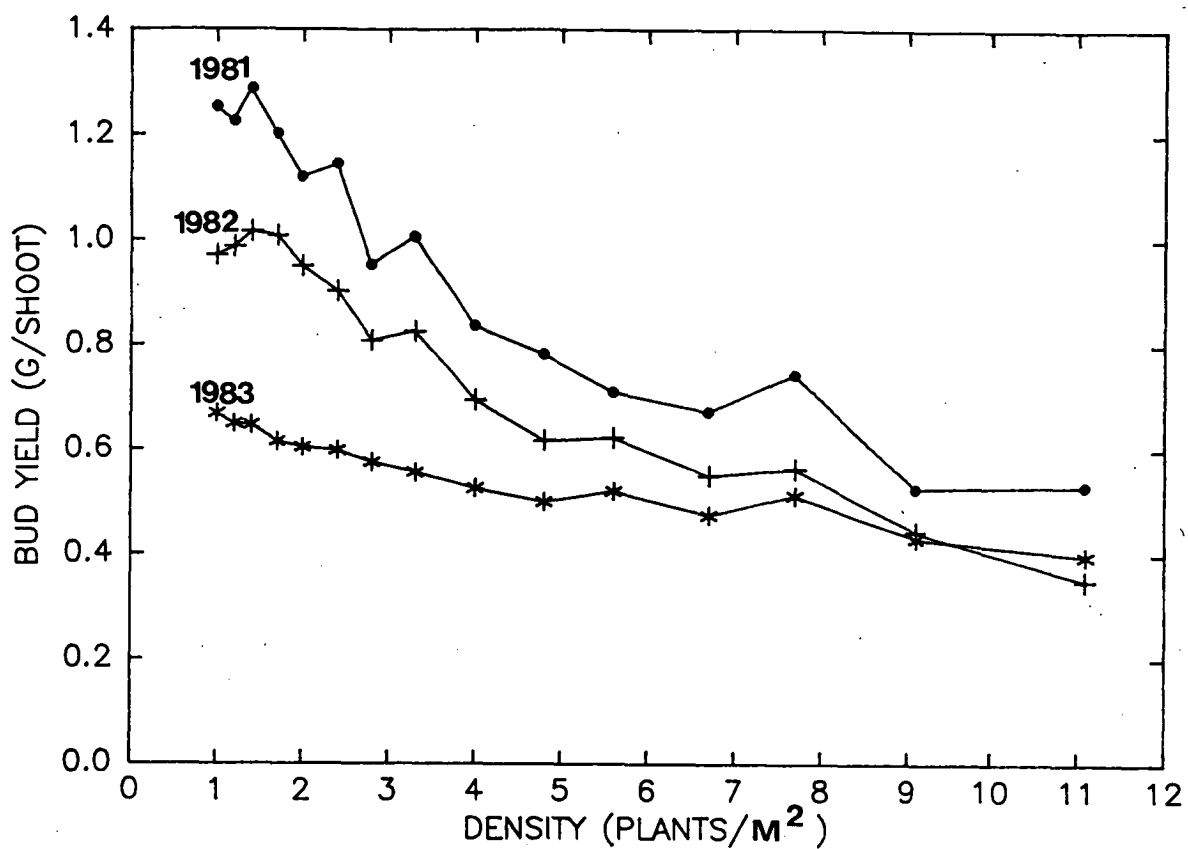
FIGURE 9.1.4 The relationship of plant density and total cane fresh weight per plant
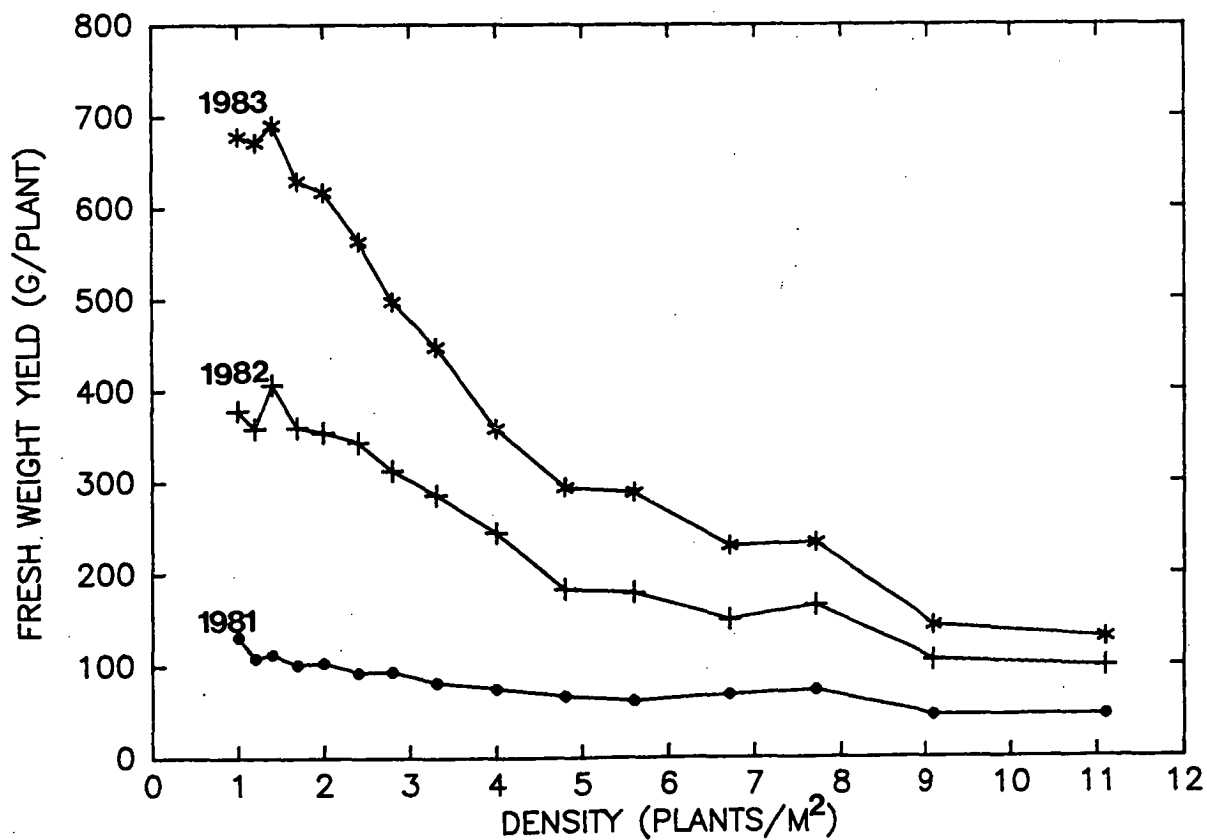
FIGURE 9.1.5 Relationship of plant density and shoot number per plant
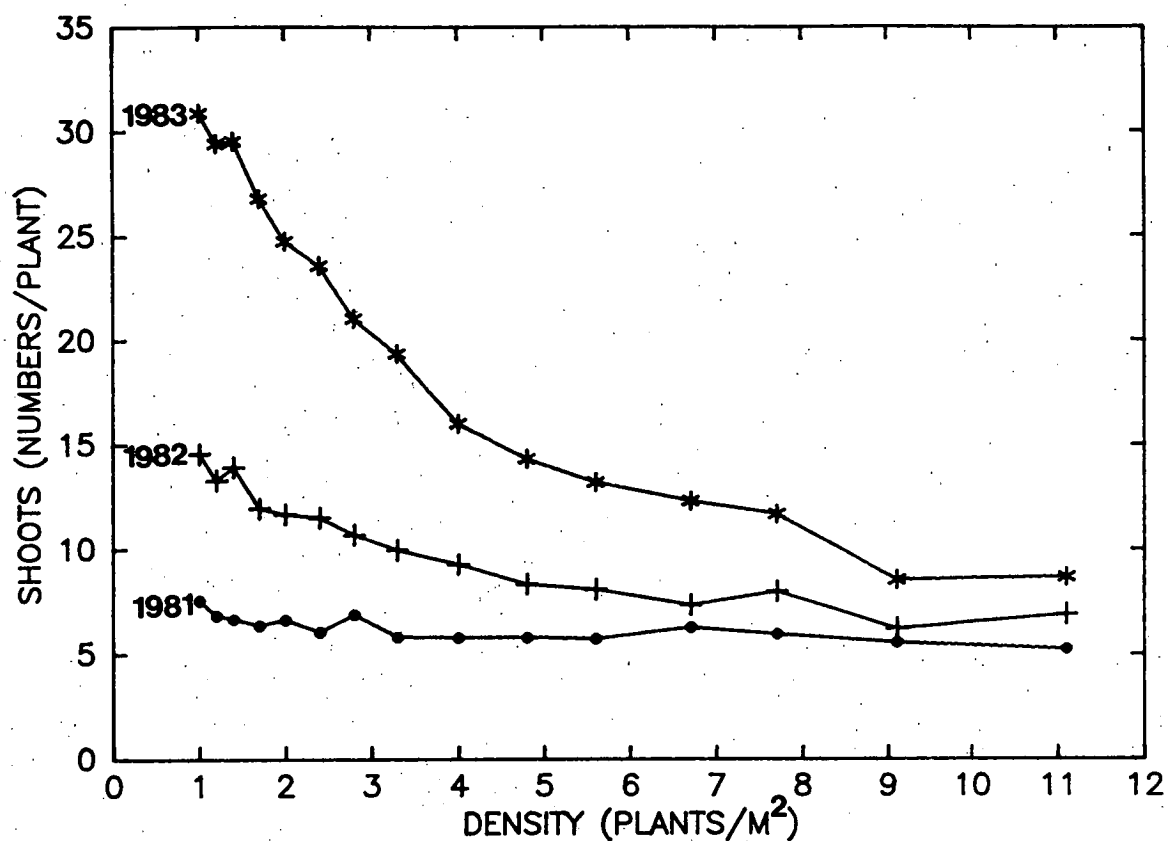
FIGURE 9.1.6 The relationship of plant density and cane length
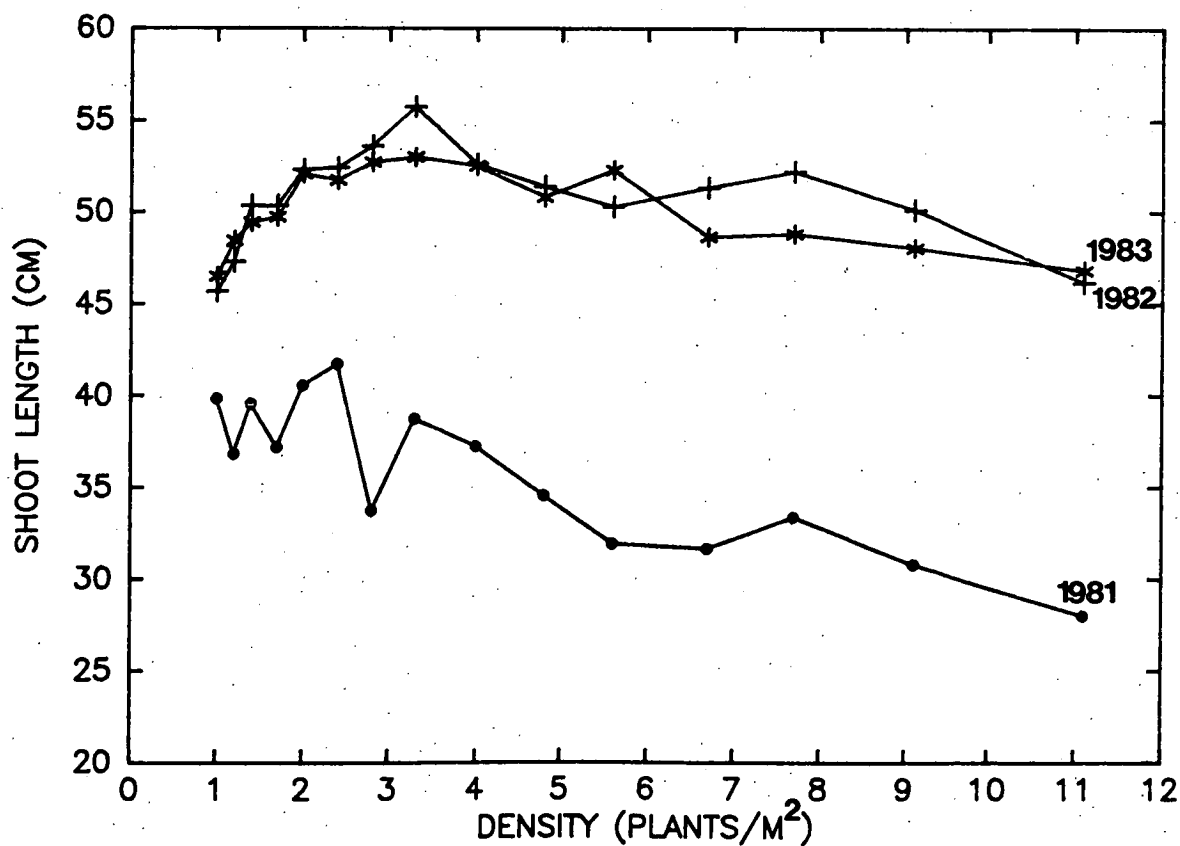
vigour, is shown to decrease with increasing plant density (Figure 9.1.7).
Mean basal cane girth was also shown to be a reliable estimator of plant
productivity, for plant densities ranging from 11.1 to 1.0 plants/m^2
(Figure 9.1.8). For bud yield the relationship is of the form, Y=82.86X
+ 67.12, r = 0.92; whereas for total fresh weight yield it has the form,
Y = 3088X - 2563, r = 0.96.

Analysis of the response curves obtained (Figures 9.1.1 and 9.1.4 to
9.1.7) was carried out by attempting to fit a polynomial (of up to the
third order) to each data set. A t-test utilizing the variance ratio,
produced from the analysis of variance table, was used to test the
hypothesis that the population regression behaved as the first, second
or third order polynomial being fitted (Zar 1974). The calculated t
values are presented in Table 9.1.1 and the analysis of variance tables
for each data set are included in Appendix 6.

Plots of the standard errors of each dependent variable against plant
density (Figures 9.1.9 to 9.1.13), show that the standard error of each
mean decreased with increasing density. This is a normal error situation
and suggests that a log transformation was not required. The data in
Table 9.1.1 demonstrates that a quadratic expression was the most approp-
riate fit for the relationship of bud yield, fresh weight and shoot number
to plant density. Basal stem girth was best explained as a linear
relationship with plant density, while cane length was highly variable
and its relationship was unclear. This was confirmed by the use of a
correlation regression analysis which extracted the percent variance
accounted for by fitting each polynomial (Table 9.1.2). The correlation
matrices and analysis of variance tables are attached in Appendix 7.
This analysis brings forth the conflicting suggestion that a higher per-
centage of the variance observed for basal stem girth can be explained
by a quadratic rather than linear expression.

FIGURE 9.1.7 The relationship of plant density and basal cane girth
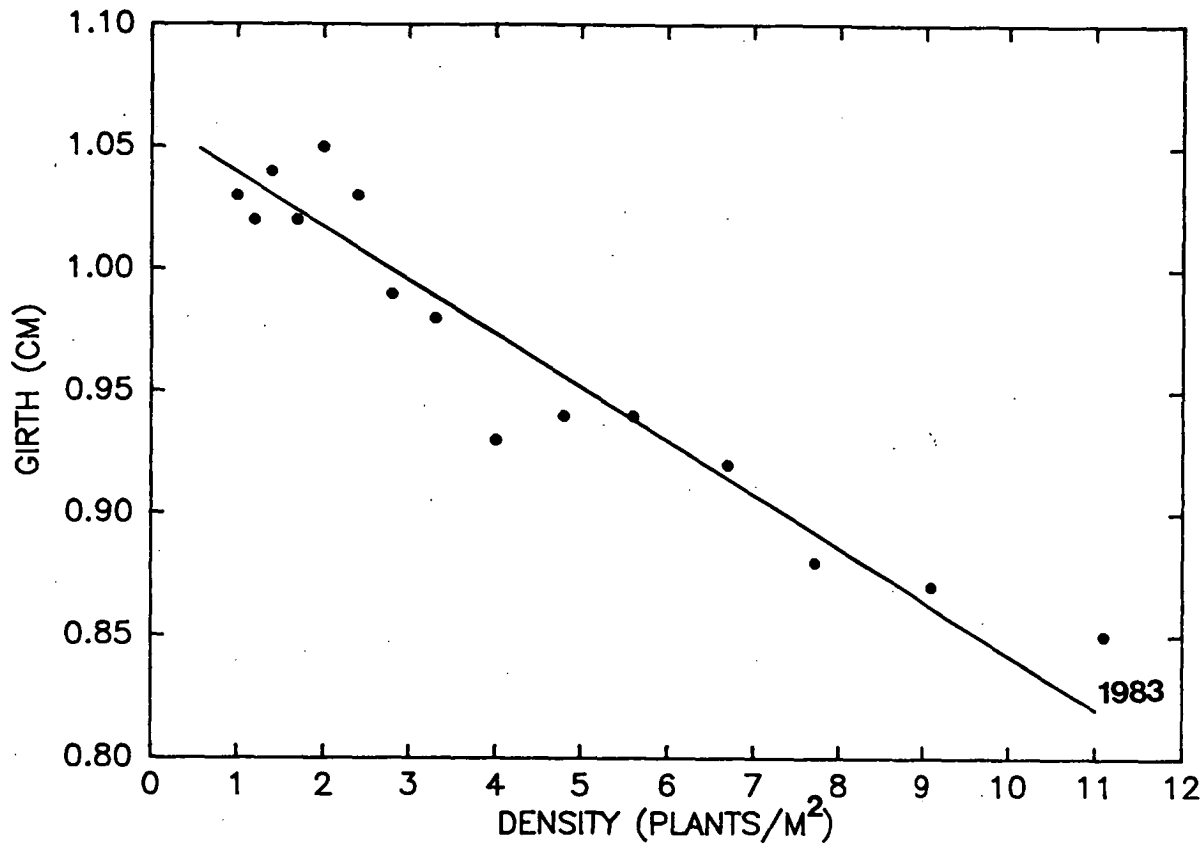
FIGURE 9.1.8 The relationship of both bud and cane fresh weight yields with basal cane girth
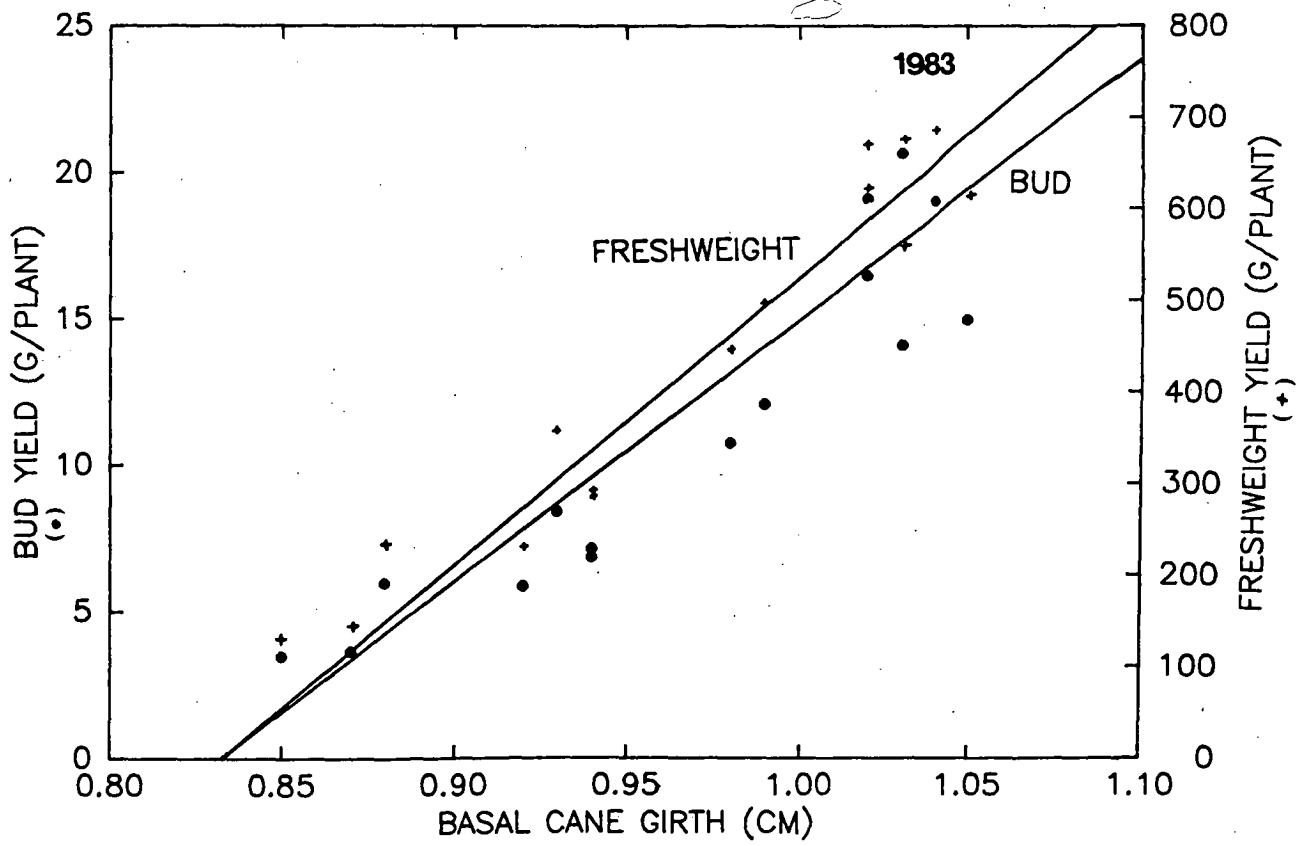
FIGURE 9.1.9 The relationship of bud yield per plant standard errors and plant density
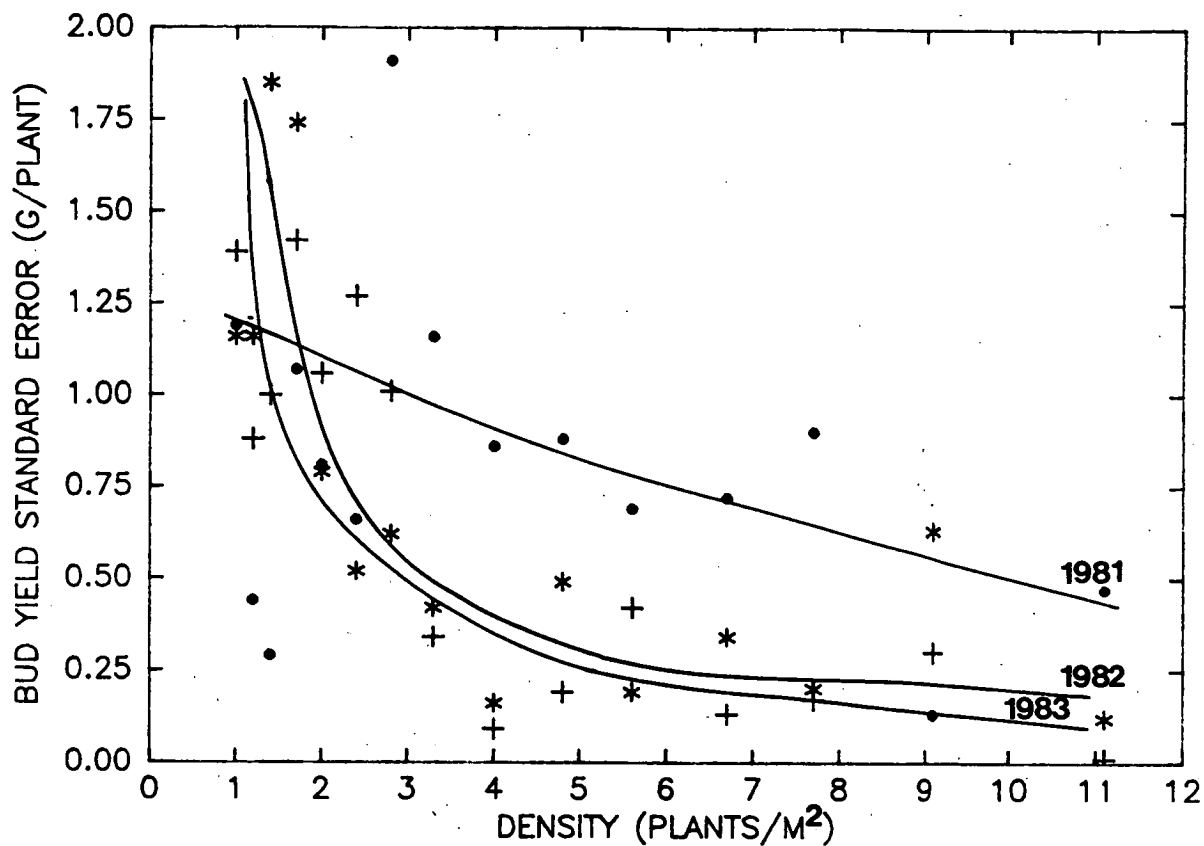
FIGURE 9.1.10 The relationship of freshweight yield standard errors and plant density
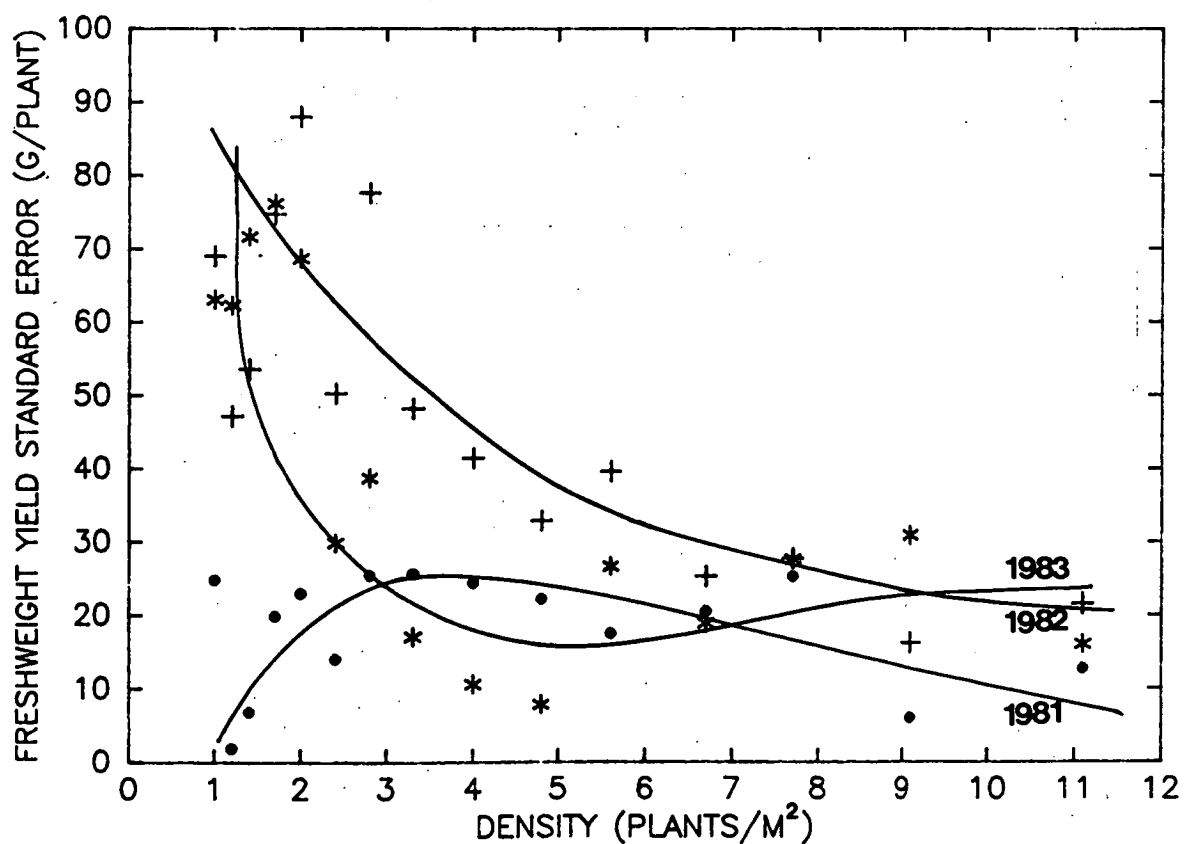
FIGURE 9.1.11 The relationship of shoot number standard errors and plant density
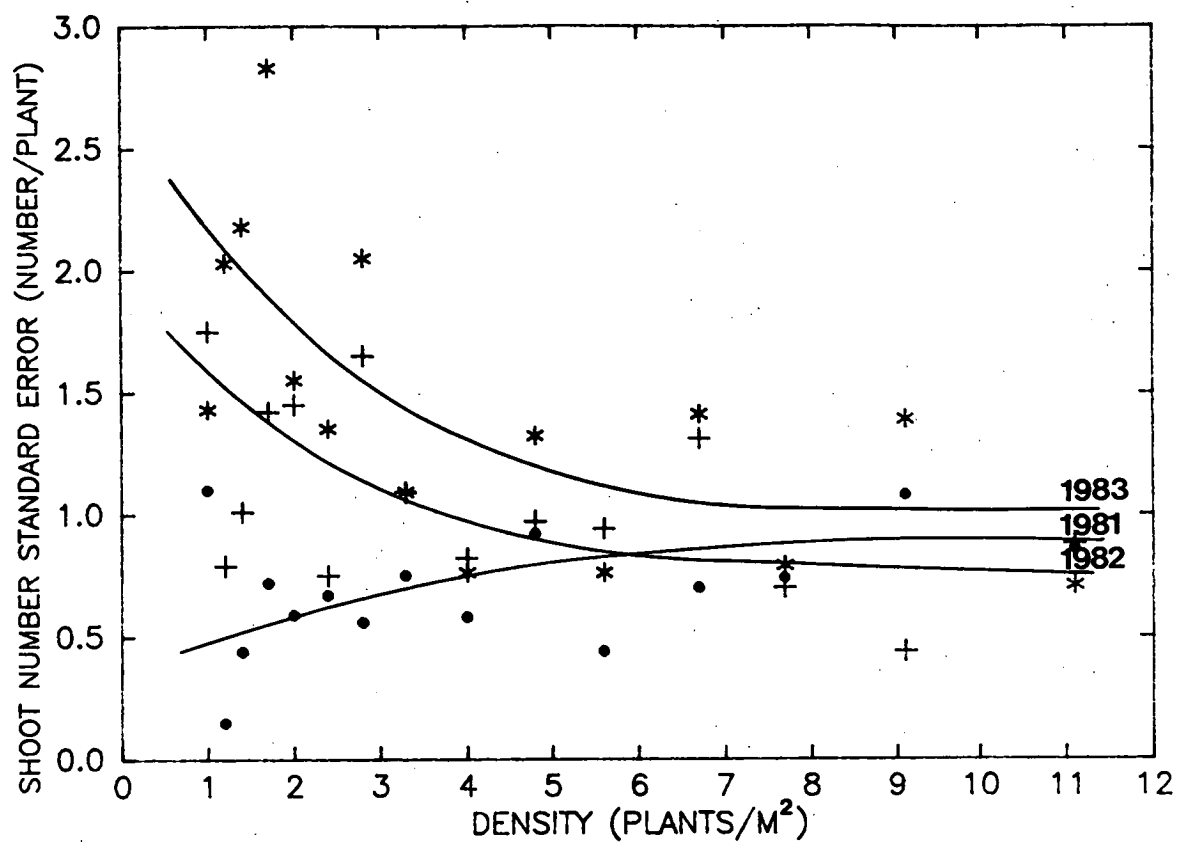
FIGURE 9.1.12 The relationship of cane length standard errors and plant density
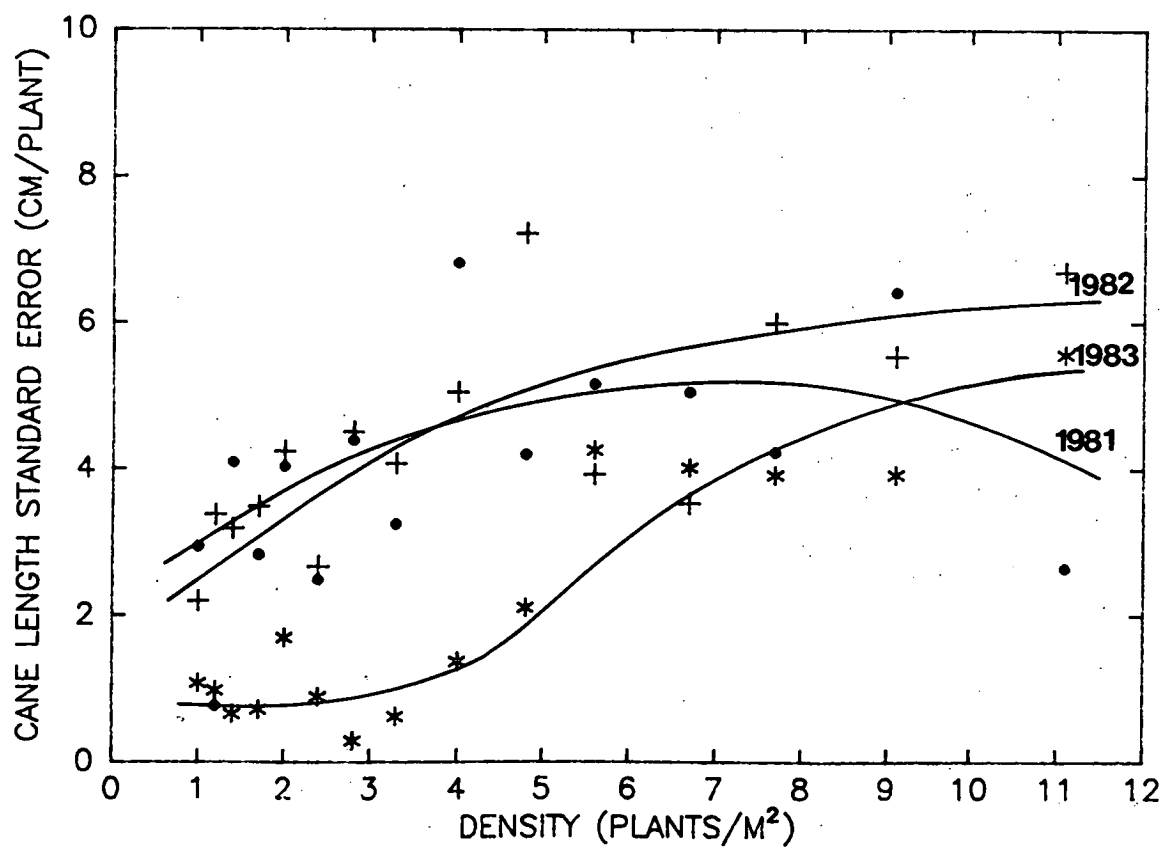
FIGURE 9.1.13 The relationship of basal cane girth standard error and plant density
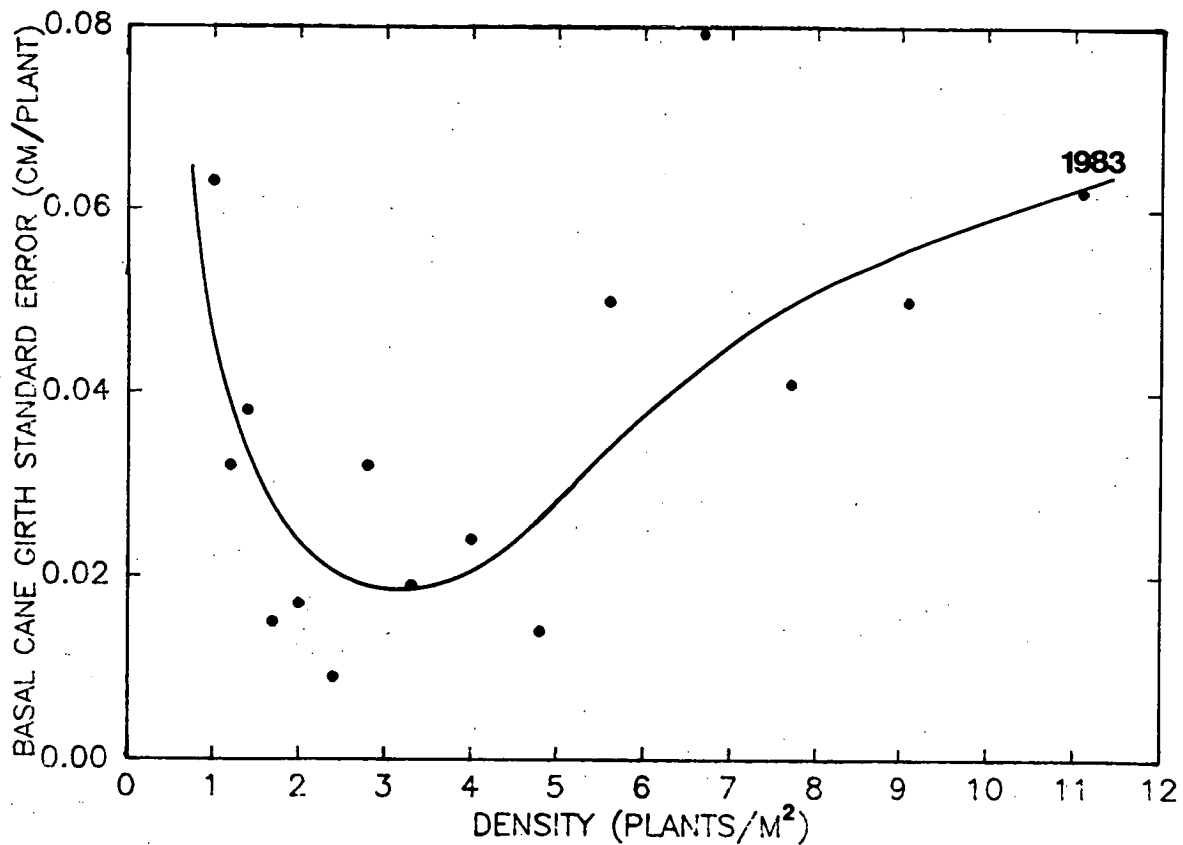
FIGURE 9.1.14

THE RELATIONSHIP OF PLANT DENSITY AND THE RECIPROCAL OF BUD YIELD
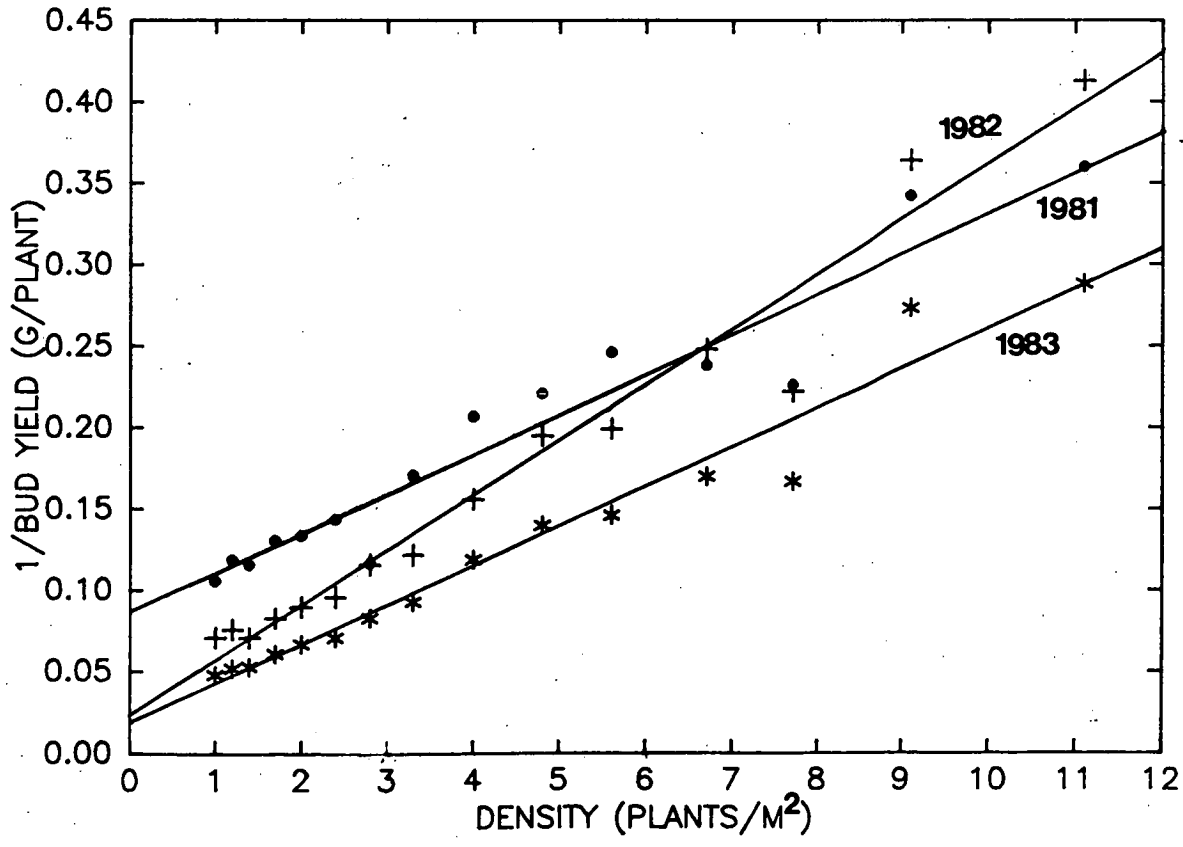
TABLE 9.1.1 Testing the population regression fits a first, second or third order polynomial (calculated t values at df and P = 0.05)

Y	Polynomial	1981	1982	1983
		Y	Y	Y
bud yield	linear	433.43**	620.84***	1,288.585***
	quadratic	29.262**	57.46**	203.162***
	cubic	1.746 ns	0.03 ns	34.107 ns
fresh-weight yield	linear	206.25***	314.719***	1,361.632***
	quadratic	17.18*	20.964 ns	216.004***
	cubic	9.71 ns	0.071 ns	36.165*
shoot number	linear	38.96**	334.32***	1,513.997***
	quadratic	1.91 ns	18.85*	21.408*
	cubic	12.66 ns	1.14 ns	0.247 ns
cane length	linear	41.53**	5.20 ns	1.690 ns
	quadratic	17.58*	29.21**	21.46*
	cubic	5.77 ns	10.02 ns	31.683**
basal stem girth	linear	-	-	149.394***
	quadratic	-	-	6.865 ns
	cubic	-	-	0.358 ns

ns = not significant
* = significance level 1
** = significance level 2
*** = significance level 3

In addition, the bud yield-density relationship was analysed to determine if the reciprocal of bud yield bore a linear relationship to plant density. The analysis of variance performed is summarized in Table 9.1.3, and dictates a strong linear response as illustrated in

TABLE 9.1.2 Percent variance accounted for by fitting a first, second or third order polynomial

Y	Polynomial	1981	1982	1983
		Y	Y	Y
bud yield	linear	69.3	78.5	89.2
	quadratic	73.5	89.9	94.5
	cubic	73.5	91.1	94.3
fresh weight yield	linear	39.4	59.4	89.2
	quadratic	41.9	62.6	94.5
	cubic	42.5	61.7	94.3
shoot number	linear	16.0	60.3	83.3
	quadratic	15.0	70.2	91.2
	cubic	19.2	70.2	91.0
cane length	linear	19.5	N.A.	0.5
	quadratic	27.1	4.5	10.0
	cubic	28.5	4.5	11.6
basal stem girth	linear	-	-	38.0
	quadratic	-	-	49.8
	cubic	-	-	49.5

Figure 9.1.14 for this relationship. The full analysis of variance tables are attached in Appendix 8.

TABLE 9.1.3 Variance ratio values for the reciprocal of bud yield
          plant density relationship

| Source of Variation | DF | Variance Ratio | | |
		1981	1982	1983
Linear	1	116.038***	48.693***	185.418***
Quadratic	1	3.611 ns	2.787 ns	0.183 ns
Cubic	1	1.704 ns	2.139 ns	0.180 ns

ns = not significant
*** = highly significant

This linear response suggests strongly the applicability of an asymptotic model, thus an attempt was made to fit the yield density models previously described in Section II 3.1.

9.1.2 *Yield-Density Models for Blackcurrant Bud Yield Data*

There are in general two types of yield-density response, viz.:

(i)   the "asymptotic", where the yield per area approaches an asymptote as the density increases towards infinity;

(ii) the "parabolic" where the yield per area rises to a maximum value as the density is increased beyond that value.

There is extensive literature cover of yield-density models, for example Willey and Heath (1969). As they, and other authors (Section II 3.1) have pointed out, it is customary to formulate yield-density models in terms of yield per plant (Figure 9.1.1). For the asymptotic responses, the applicable model is:

(1)
$$Y = \frac{1}{\alpha + \beta X}$$

where X is the plant density and Y the yield per plant, with α and β being parameters. For the parabolic response, several models have been proposed, the statistical properties of which have been studied by Ratkowsky (1983). His conclusions strongly suggested the suitability of the Holliday (1960) model;

$$(2) \qquad Y = \frac{1}{\alpha + \beta X + \gamma X^2}$$

where γ is an additional parameter. It was found that other alternative models, such as the Bleasdale-Nelder (1960) and the Farazdaghi-Harris (1968), had undesirable statistical properties in least-squares estimation and should not be used.

For each of the three years, 1981, 1982 and 1983, there were three replicate blocks. The mean bud yield per plant Y, averaged over the three replicates are given in the following table, the set of plant densities X being the same for each year:

Plant Density (plants/m^2)	Bud Yield (g/plant)		
X	1981 Y	1982 Y	1983 Y
1.0	9.480	14.14	20.65
1.2	8.383	13.12	19.10
1.4	8.593	14.14	19.10
1.7	7.653	12.04	16.47
2.0	7.440	11.09	14.93
2.4	6.929	10.38	14.09
2.8	6.561	8.621	12.08
3.3	5.839	8.221	10.74
4.0	4.830	6.430	8.410
4.8	4.521	5.129	7.156
5.6	4.061	5.030	6.870
6.7	4.210	4.040	5.840
7.7	4.419	4.500	5.987
9.1	2.920	2.751	3.680
11.1	2.780	2.420	3.450

Graphs of the variance of Y (Figure 9.1.9), obtained using the three replicates, for each X, show that the variance of Y increases with decreasing plant density X, suggesting that a "multiplicative" error term may be appropriate. This is consistent with the finding of Nelder (1963) that log Y, rather than Y, has constant variance for a given X. With this error assumption the asymptotic model may now be written as:

$$(3) \qquad E(\log Y) = -\log(\alpha + \beta X),$$

and the Holliday model as:

$$(4) \qquad E(\log Y) = -\log(\alpha + \beta X + \gamma X^2),$$

where E denotes the expectation operator. Models (3) and (4) can now be fitted by standard methods used for non-linear regression modelling (see Ratkowsky, 1983, Appendix 2.A). The following residual sums of squares (RSS) are obtained for the data for each year:

Bud Yield Data	1981	1982	1983
RSS (model 3)	0.085373	0.125638	0.086092
RSS (model 4)	0.081352	0.092591	0.072266
$F_{1,12}$	0.593^{ns}	4.28^{ns}	2.30^{ns}

Thus, use of model 4, which incorporates an additional parameter, results in only a small additional valuation in the residual sum of squares. The reduction can be formally tested for each year by using

$$F_{1,12} = \frac{RSS[Model\ (3)] - RSS[Model\ (4)]}{RSS[Model\ (4)]/\nu_2}$$

where ν_2 is the residual degrees of freedom (in this case 12) for model (4).

The statistic $F_{1,12}$ has an F-distribution with 1 and 12 degrees of freedom and its values are recorded in the above table. For each year, the decrease in the residual variance due to the extra parameter is seen to be non-significant. Hence, the conclusion must be that the data are consistent with the asymptotic model. Graphs that have been prepared of yield per area, (i.e. XY; Figure 9.1.2), tend to bear this out; the yield per area appears to approach an asymptote for each year rather than reaching a maximum for some optimum value of X.

The following values for the least squares estimates of α and β, and their standard errors, were obtained for the asymptotic model [Model (3)]:

| | | Parameter | |
		α	β
	1981	0.08563 ± 0.00548	0.02509 ± 0.00163
Year	1982	0.03296 ± 0.00461	0.03062 ± 0.00172
	1983	0.02266 ± 0.00275	0.02269 ± 0.00104

The magnitudes of the parameter estimates and their standard errors demonstrate that α (and β as well) is not a constant from year to year.

There are obviously other factors operating, in this perennial crop, which prevent α from coming out to be a constant, as if often does for annual crops of the same species or variety (Bleasdale 1967a; Frappell 1979).

The asymptotic model has very good statistical properties. Values are given for the asymmetry measure of non-linearity of Lowry and Morton (1983) and for the intrinsic (IN) and parameter-effects (PE) curvature measures (see Ratkowsky (1983) for a discussion of these measures.)

The Lowry-Morton measures are closely related to the asymmetry measure
of bias that is discussed in Section 2.9.; IN and PE are discussed in
Section 2.4 of Ratkowsky (1983).

	Bud Yield Data		
	1981	1982	1983
Asymmetry measures			
for: α	0.001	0.001	0.001
β	0.001	0.001	0.001
Rule-of-thumb decision value	0.01	0.01	0.01
Curvature measures:			
IN	0.019	-0.028	0.024
PE	0.061	0.065	0.053
Critical value $1/(2\surd F)$	0.256	0.256	0.256

As the asymmetry measures are much less than the rule-of-thumb decision
value of 0.01, and IN and PE are much less than their critical values
for statistical significance, Model (3) exhibits close-to-linear behaviour.
Hence, although Model (3) is a non-linear regression model, its behaviour
in estimation is very similar to that of a linear model.

As previously discussed (Section II 3.1), $\frac{1}{\alpha}$ is considered to be a
measure of the genetic potential of the crop, and $\frac{1}{\beta}$ a measure of the
environmental potential. In order to understand the variation of α
and β, displayed in the data examined, the dependent variable Y was trans-
formed to a bud yield expressed as grams per shoot, rather than the yield
per plant. The data set is as follows:

Plant Density (plants/m²)	Bud Yield Data		
X	1981 (g/shoot) Y	1982 (g/shoot) Y	1983 (g/shoot) Y
1.0	1.254	0.971	0.669
1.2	1.226	0.987	0.649
1.4	1.288	1.016	0.647
1.7	1.203	1.008	0.615
2.0	1.120	0.950	0.604
2.4	1.145	0.902	0.598
2.8	0.952	0.807	0.575
3.3	1.005	0.825	0.556
4.0	0.836	0.694	0.526
4.8	0.782	0.617	0.500
5.6	0.711	0.623	0.521
6.7	0.673	0.551	0.475
7.7	0.744	0.564	0.513
9.1	0.527	0.444	0.431
11.1	0.532	0.351	0.398

This data set is represented graphically (Figure 9.1.3) and displays an asymptotic response. From the asymptotic model the following least squares estimates of the parameters (and their standard errors) were obtained:

	α	β
1981	0.6536 ± 0.0301	0.1187 ± 0.0081
1982	0.7778 1 0.0353	0.1576 ± 0.0097
1983	1.453 ± 0.0302	0.0921 ± 0.0068

Again the data demonstrates that α and β are not constant from year to year. It may well be that the plants have not yet attained an

equilibrium condition within the density experiment, and this is what is preventing α and β from remaining constant (although more variation in β would be expected due to seasonal variation). This is borne out by Figure 9.1.1, where the incremental increase in yield at each successive density was decreasing, suggesting the approach to an equilibrium situation.

The fit of the asymptotic yield-density model to the above data was a good one in each of the three years; note that the anomalous yields obtained for a density of 7.7 plants/m² apply to the above data as well as to the yield per plant data previously considered. Caution needs to be exercised before accepting this anomalous data as a peak yield, particularly considering the good fit attained with the asymptotic, but not the parabolic model. The anomaly may simply be due to the fact that the same plants were sampled each year. However, there is also the consideration that the data is real with a sound physiological basis (refer to section V).

9.2   The Effect of Harvest Date and Plant Density on Oil Yield and Composition

The amount of volatile oil present in blackcurrant buds was measured at various harvest dates throughout the growing season, over seven plant densities. As the data tabulated (Table 9.2.1 and Appendix 13) shows the level of oil in the buds remains at a low but steady level, from early November through until late December. At this time (23/12) there is a rapid increase in the amount of oil present, indicating an increase in rate of oil synthesis in late December and early January. From then on there is a steady rise in the level of volatile oil measured in the buds, until the end of the growing season. Planting density appears to have effect on the period of most rapid oil accumulation.

The yield concrete was measured at the beginning of June, the traditional harvest period for bud material, over the full range of density treatments. Table 9.2.2 demonstrates that the variation in percent concrete yield recorded at each planting density showed no relation with plant density. The variation observed must therefore be due to variation in the bud material and the extraction method. When the bud yields for 1983 are used to calculate an expected concrete yield, the relationship of plant density with concrete yield shows the same pattern as with bud yield. This confirms that plant density has little or no effect on the percentage yield of concrete.

Figures 9.2.1 to 9.2.10 demonstrated that the levels of particular terpenes do vary at final harvest across a range of plant densities (raw data attached as Appendix 13). Despite the amount of variation observed in composition, no difference in organoleptic quality of extracts was observed.

TABLE 9.2.1   Amount of volatile oil present in blackcurrant buds at different times during the growing season

Plant Density (plants/m²)	(μℓ/g bud fresh weight)						
	1.1	1.6	2.2	3.0	5.2	7.2	10.1
Harvest Date							
12/11	3.84	1.79	2.44	3.35	2.68	4.38	2.59
19/11	3.31	2.28	2.17	3.15	4.14	3.50	2.55
3/12	4.40	4.21	2.02	2.77	3.03	2.45	2.88
10/12	3.09	4.05	3.10	4.07	3.48	3.00	2.88
17/12	3.41	3.65	2.97	2.44	1.93	3.03	3.82
23/12	7.69	4.65	5.08	4.70	7.43	4.65	8.39
6/1	6.66	3.68	6.09	5.79	5.80	-	-
20/1	7.20	6.43	5.12	8.21	6.81	8.12	8.30
3/2	6.97	6.10	7.19	6.93	7.81	7.32	10.21
18/3	8.94	6.92	8.76	8.86	6.86	10.13	12.80
8/4	12.14	10.59	10.84	9.53	7.89	7.27	13.80
29/4	12.86	9.59	9.24	11.46	9.26	8.76	11.68

FIGURE 9.2.1 The relationship of alpha thujene (•) and alpha pinene (*) with plant density in oils extracted from dormant buds
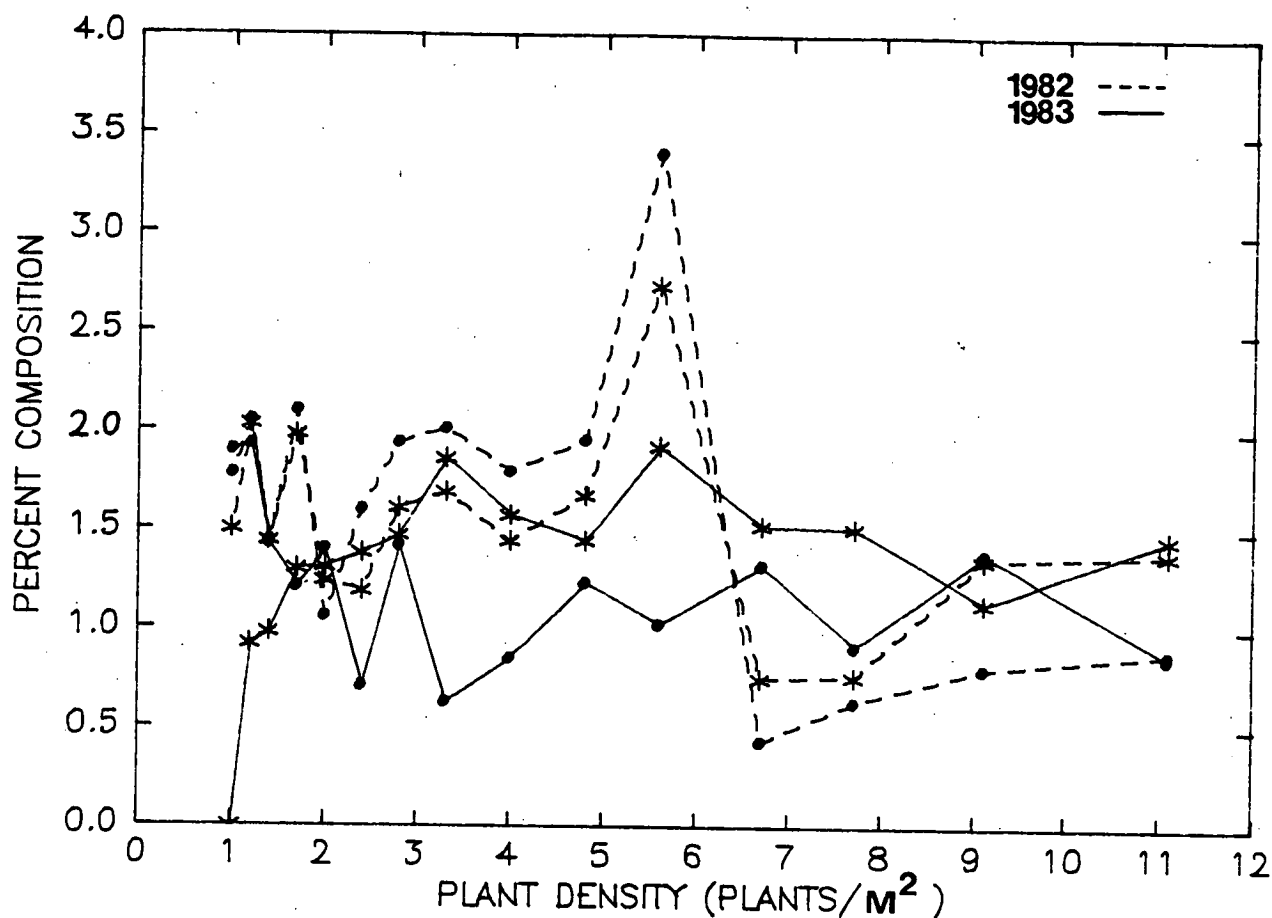
FIGURE 9.2.2 The relationship of myrcene (•) and alpha phellandrene (*) with plant density in oils extracted from dormant buds
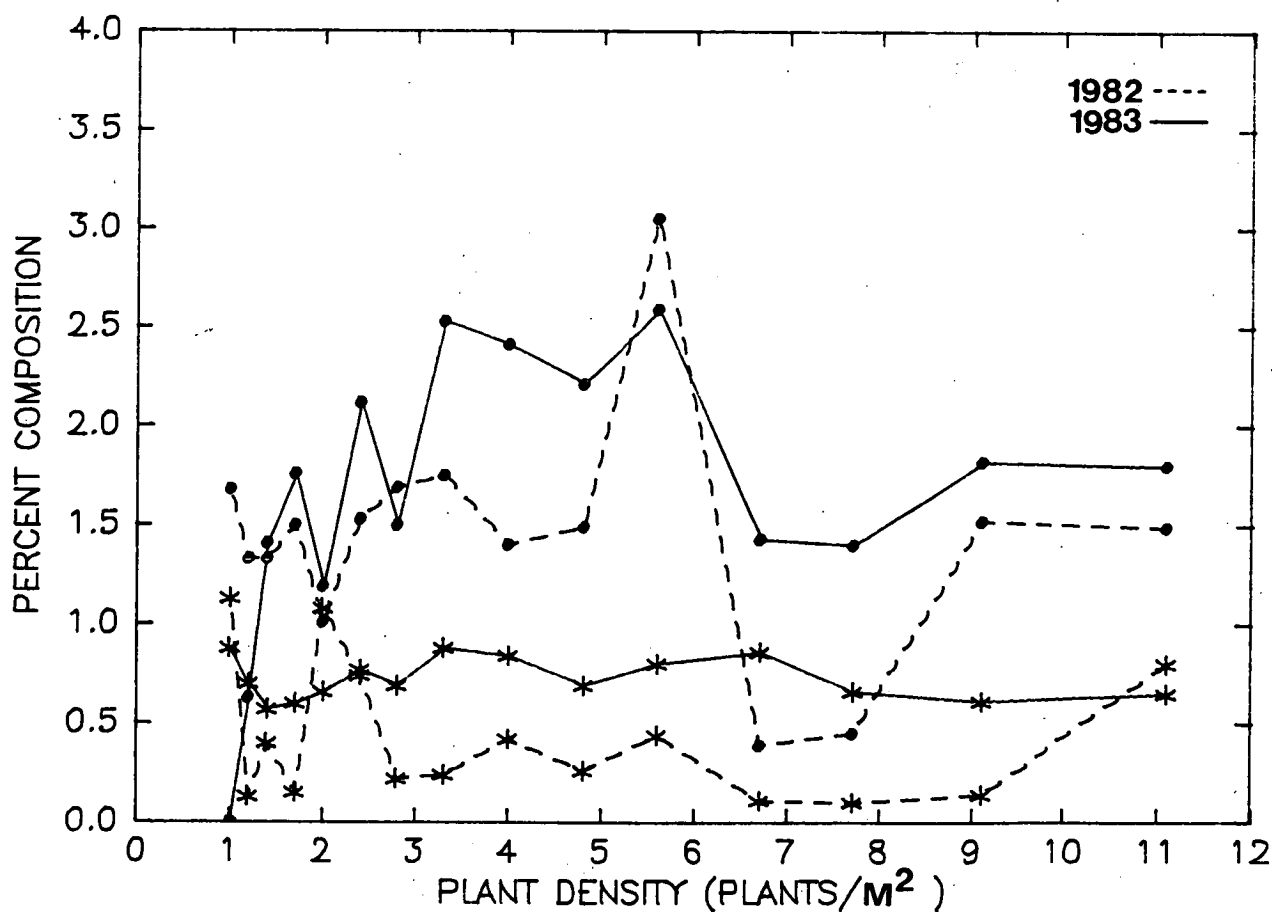
FIGURE 9.2.3 The relationship of sabinene (•), delta-3-carene (*) and alpha-terpinolene (O) with plant density in oils extracted from dormant buds
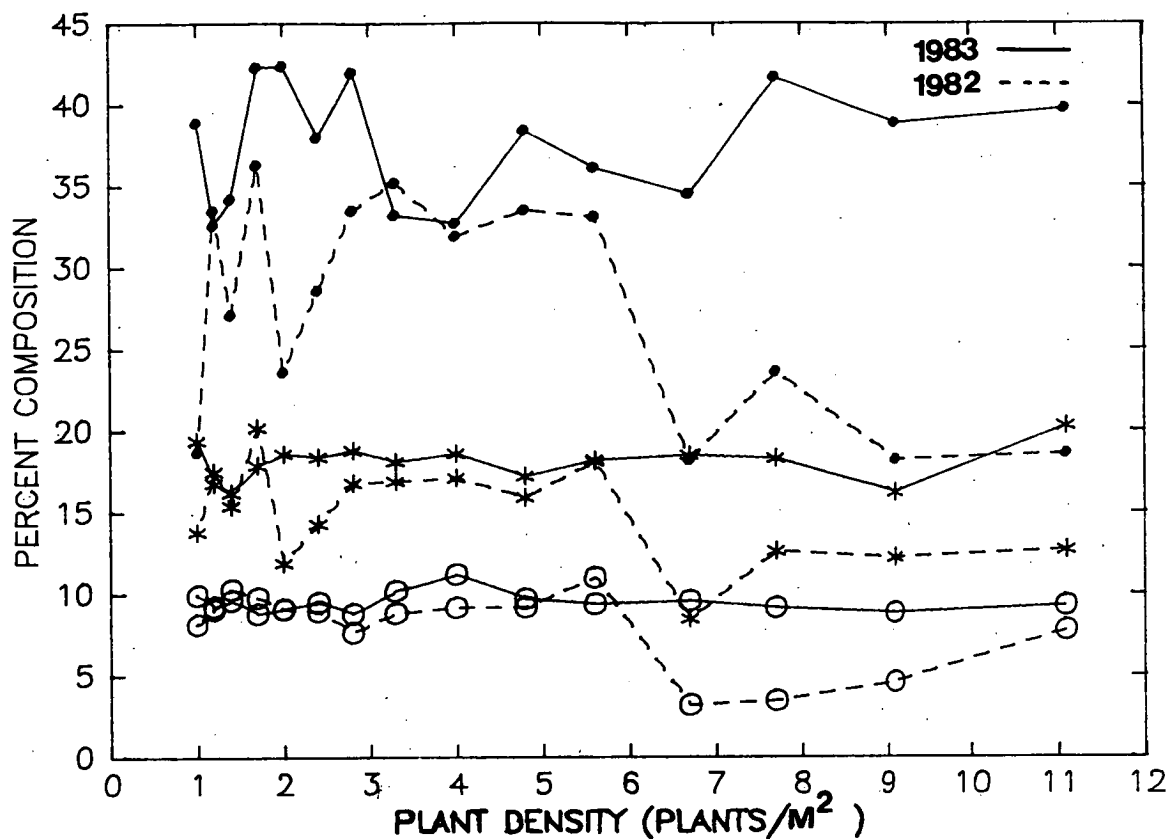
FIGURE 9.2.4 The relationship of limonene (•) and gamma terpinene (*) with plant density in oils extracted from dormant buds
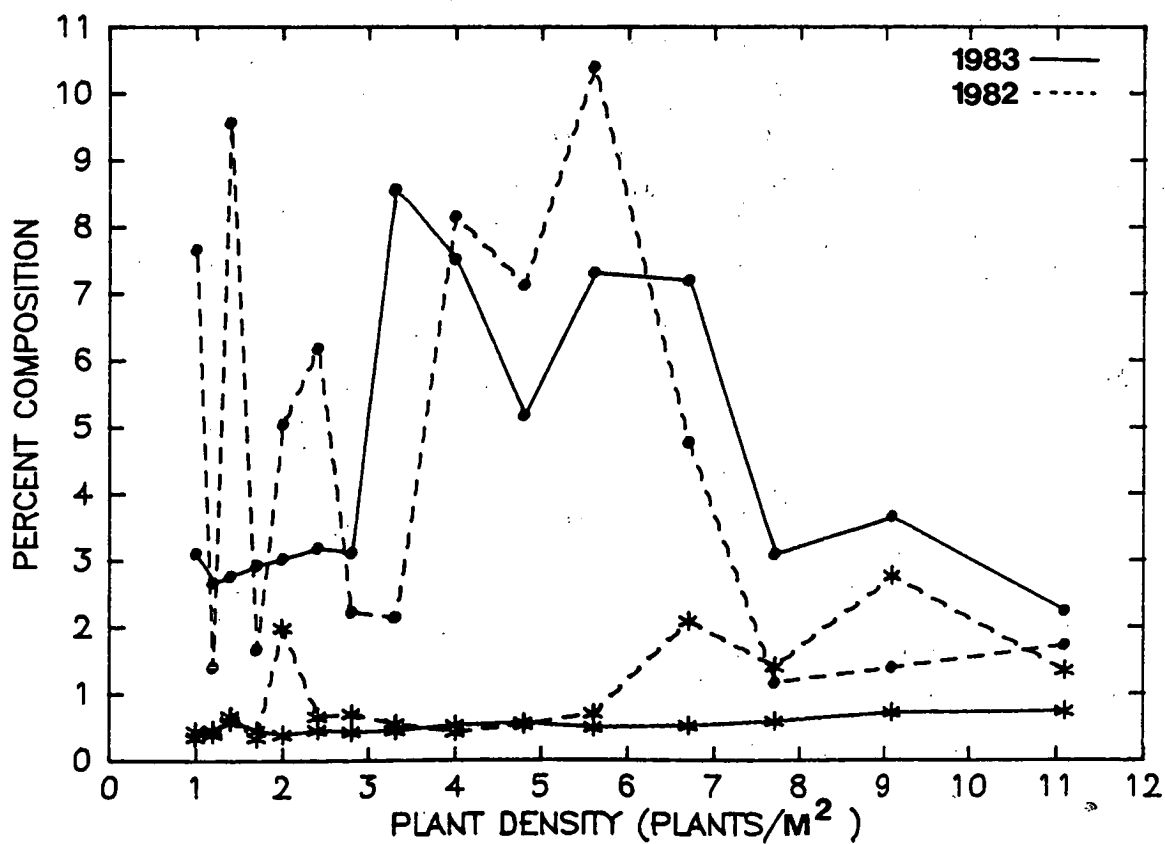
FIGURE 9.2.5 The relationship of beta phellandrene (•), cisbeta ocimene (*) and trans beta ocimene
(O) with plant density in oils extracted from dormant buds
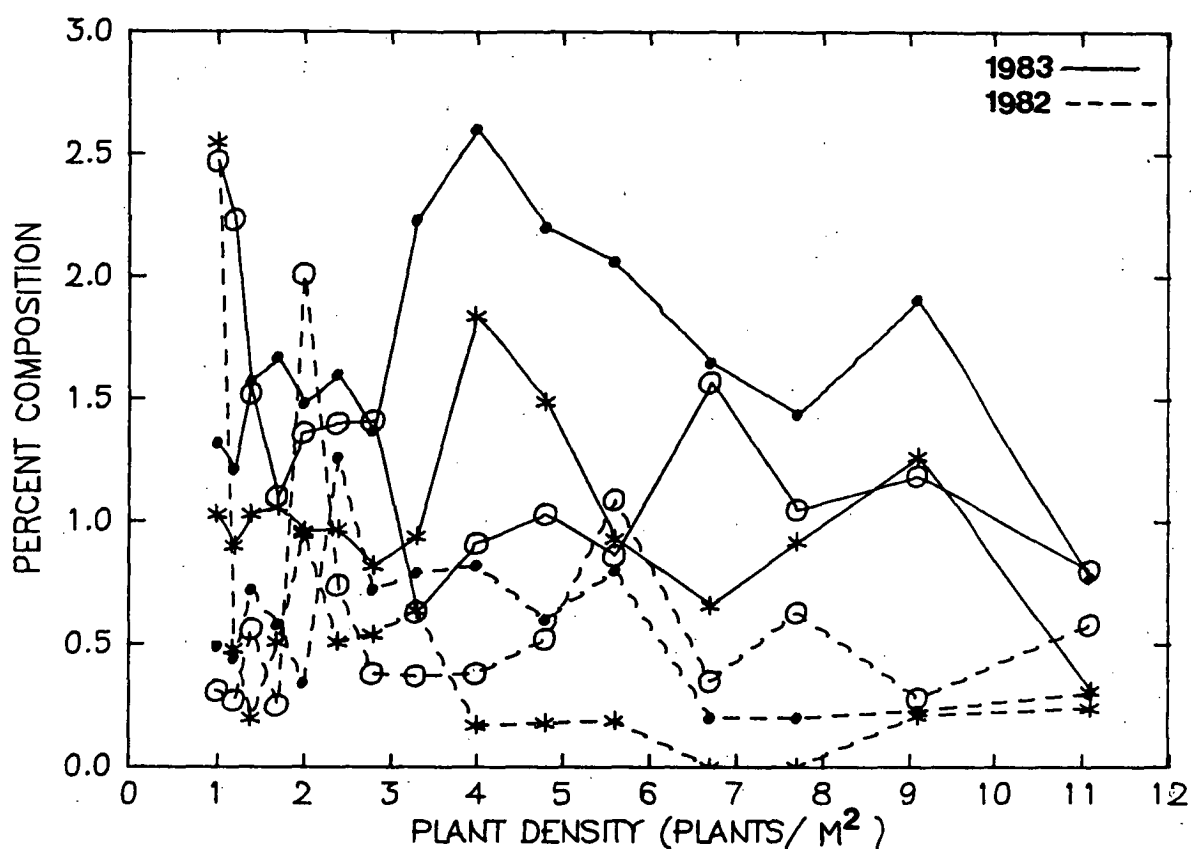
FIGURE 9.2.6 The relationship of non-an-2-one (•), an unknown MW 152 (*) and terpinen-4-ol (O)
with plant density in oils extracted from dormant buds

trans-
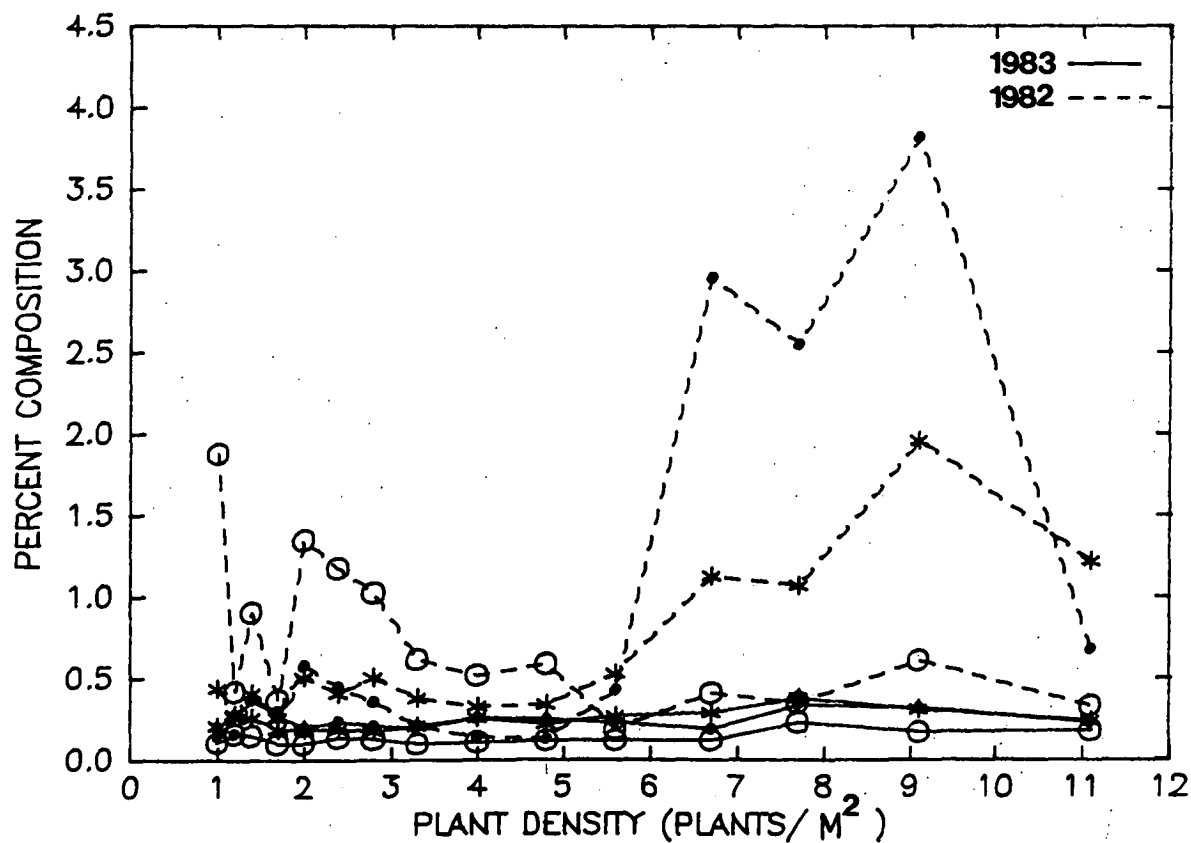
FIGURE 9.2.7 The relationship of alpha terpineol (•), piperitol (*) and carvone (O) with plant density in oils extracted from dormant buds
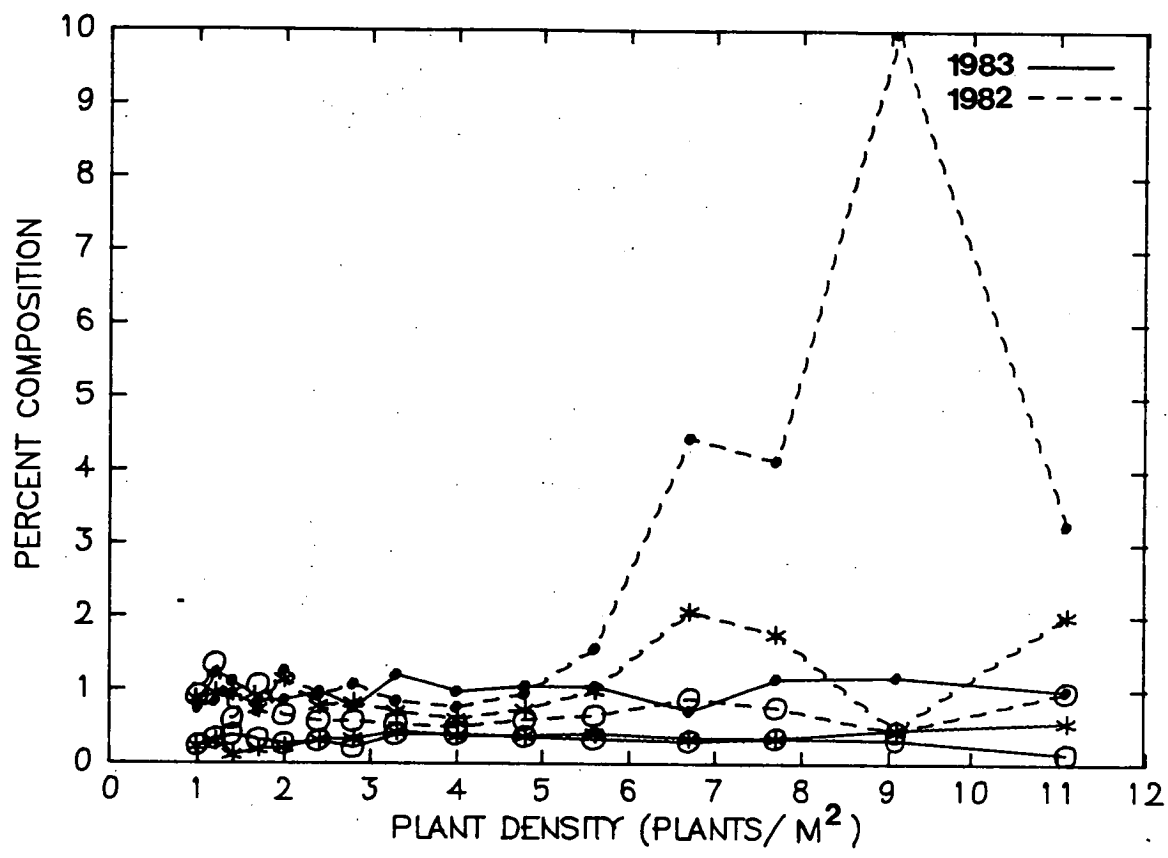
FIGURE 9.2.8 The relationship of beta caryophyllene (•), humulene (*) and germacrene-D (O) with plant density in oils extracted from dormant buds
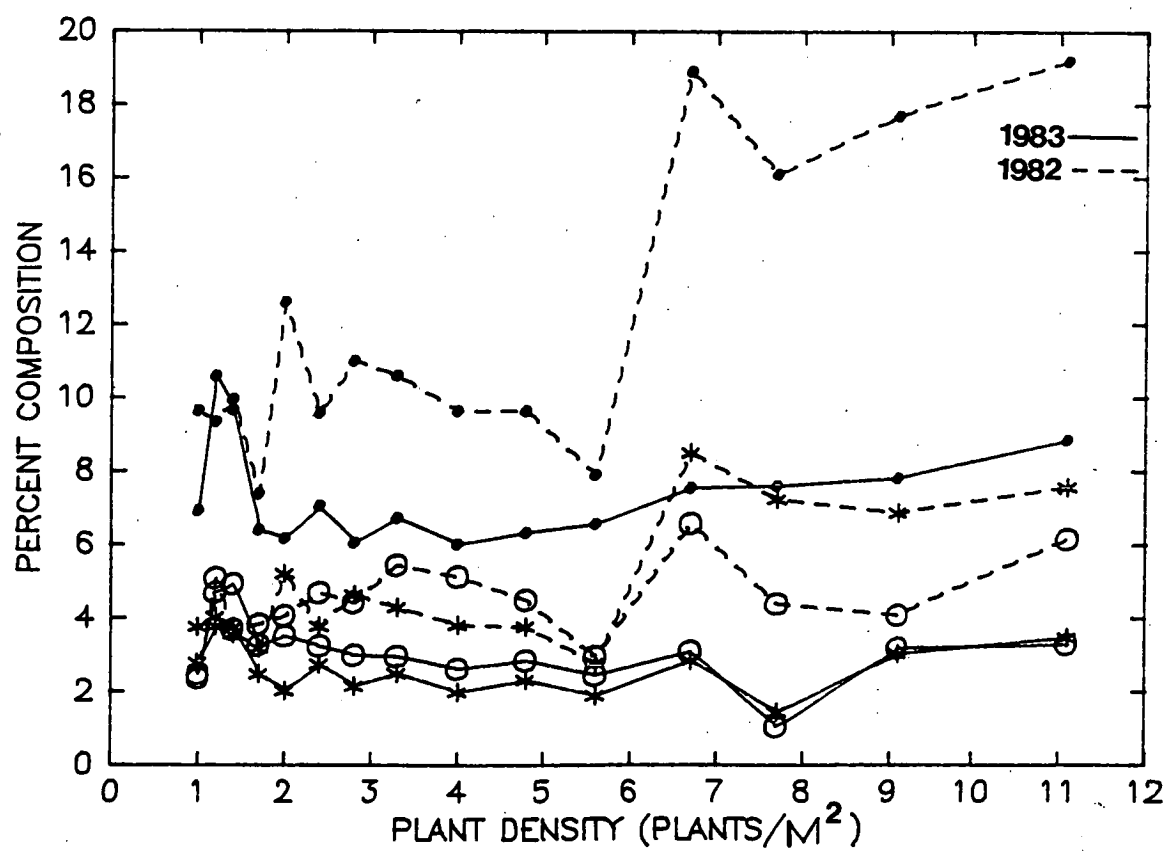
FIGURE 9.2.9 The relationship of beta elemene (●) and gamma elemene (*) with plant density in oils extracted from dormant buds
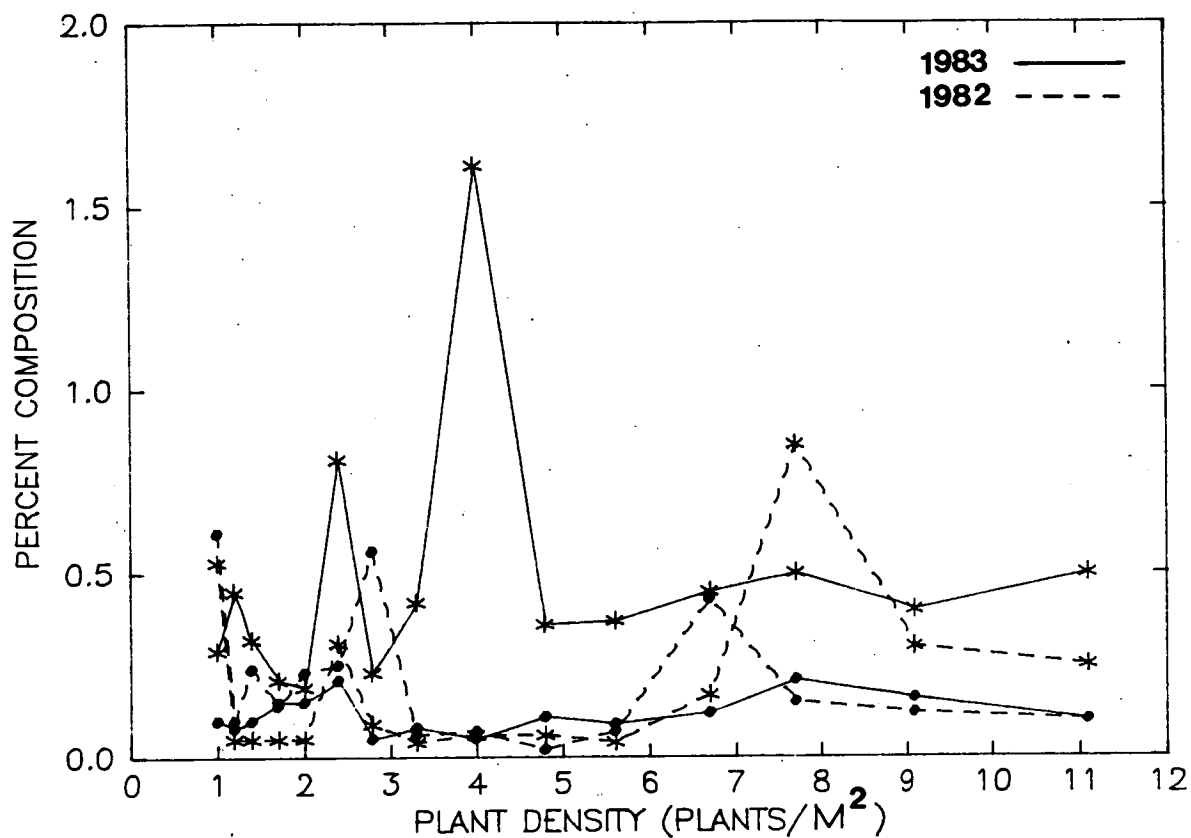
FIGURE 9.2.10 The relationship of caryophyllene epoxide (●) and humulene epoxide (*) with plant density in oils extracted from dormant buds
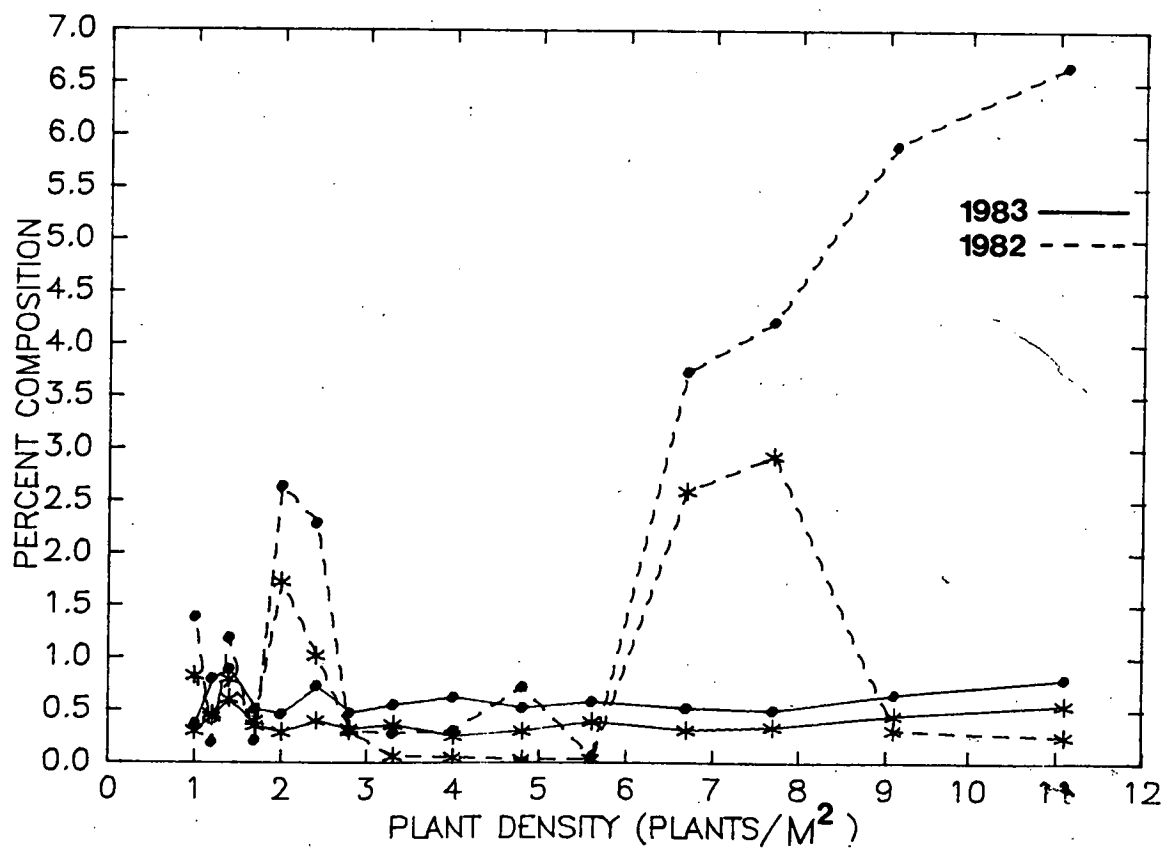
TABLE 9.2.2   Yields of concrete from blackcurrant buds at various
              planting densities

Plant density (plants/m²)	Percent Yield Concrete			Calculated Concrete g/plant	Yield of 1983 g/m²
	1982	1983	Av.		
11.1	2.84	2.16	2.50	8.68	95.75
9.1	1.94	3.54	2.74	10.03	91.76
7.7	2.21	2.91	2.56	15.31	118.02
6.7	3.17	4.28	3.73	21.90	145.95
5.6	1.96	2.32	2.14	14.70	82.33
4.8	2.41	2.52	2.47	17.69	84.84
4.0	2.83	2.98	2.91	24.47	97.89
3.3	1.96	3.25	2.61	28.08	92.52
2.8	2.11	2.78	2.45	29.57	82.86
2.4	2.09	2.62	2.36	33.25	79.82
2.0	2.41	2.09	2.25	33.64	67.16
1.7	2.84	2.46	2.65	43.65	74.20
1.4	2.36	2.30	2.33	44.32	62.26
1.2	2.44	3.15	2.80	53.48	64.18
1.0	2.85	3.16	3.01	62.16	62.16
		overall mean	2.63		

9.3   The Effect of Bud Burst on Oil Quality and Yield

The effect of bud burst on the yield of concrete is shown in the
table of means below, and represented graphically as Figure 9.3.1. The
expanded data table is included in Appendix 9.

TABLE 9.3.1

Harvest Date	Bud Nos/10 g	Percent buds opened	Percent concrete yield		Adjusted yield
12/8	241	0	3.59		3.59
19/8	189	7.3	2.25	2.25 x 241	2.87
26/8	92	42.1	1.72	189	4.51
29/8	83	54.7	1.72		5.06
30/8	82	62.3	1.48		4.35
2/9	45	94.2	1.12		6.00

FIGURE 9.3.1 The relationship of concrete yield and percentage open buds during bud burst
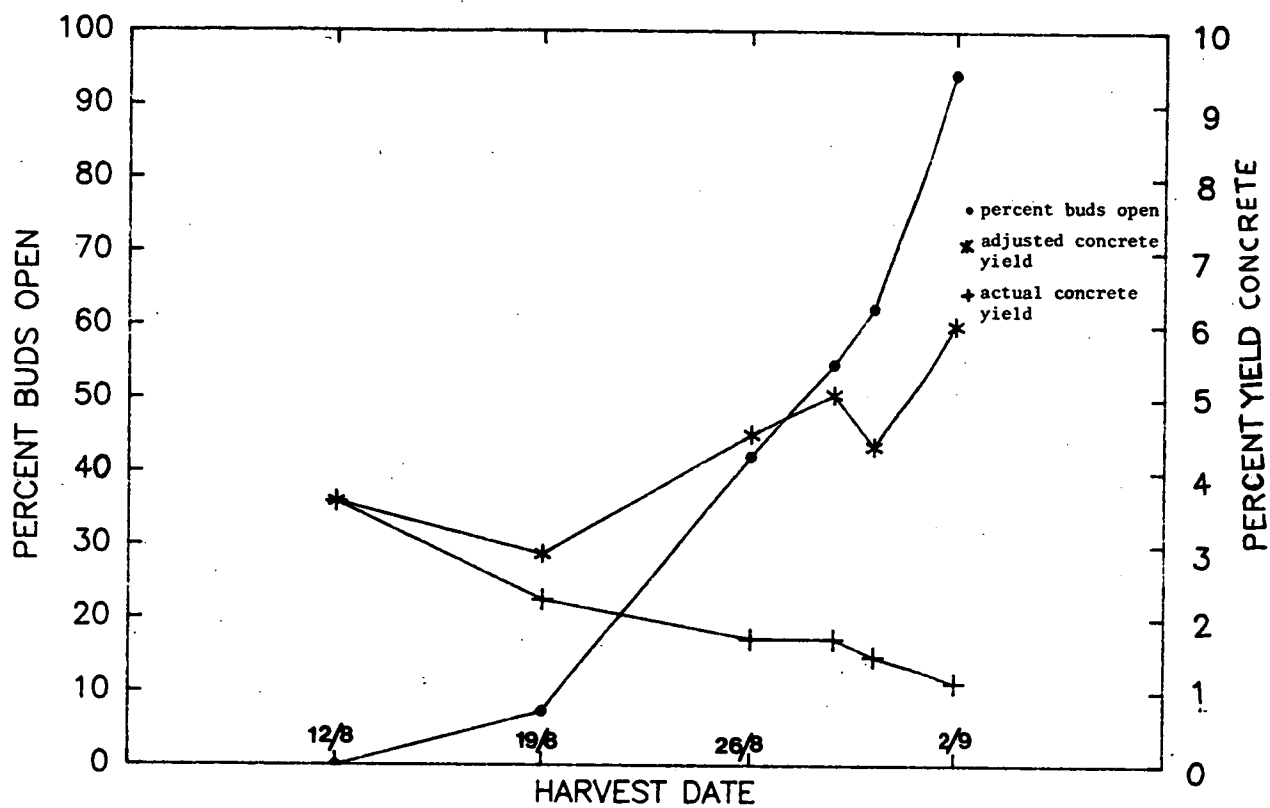
FIGURE 9.3.2 The relationship of selected oil components with harvest date during bud burst
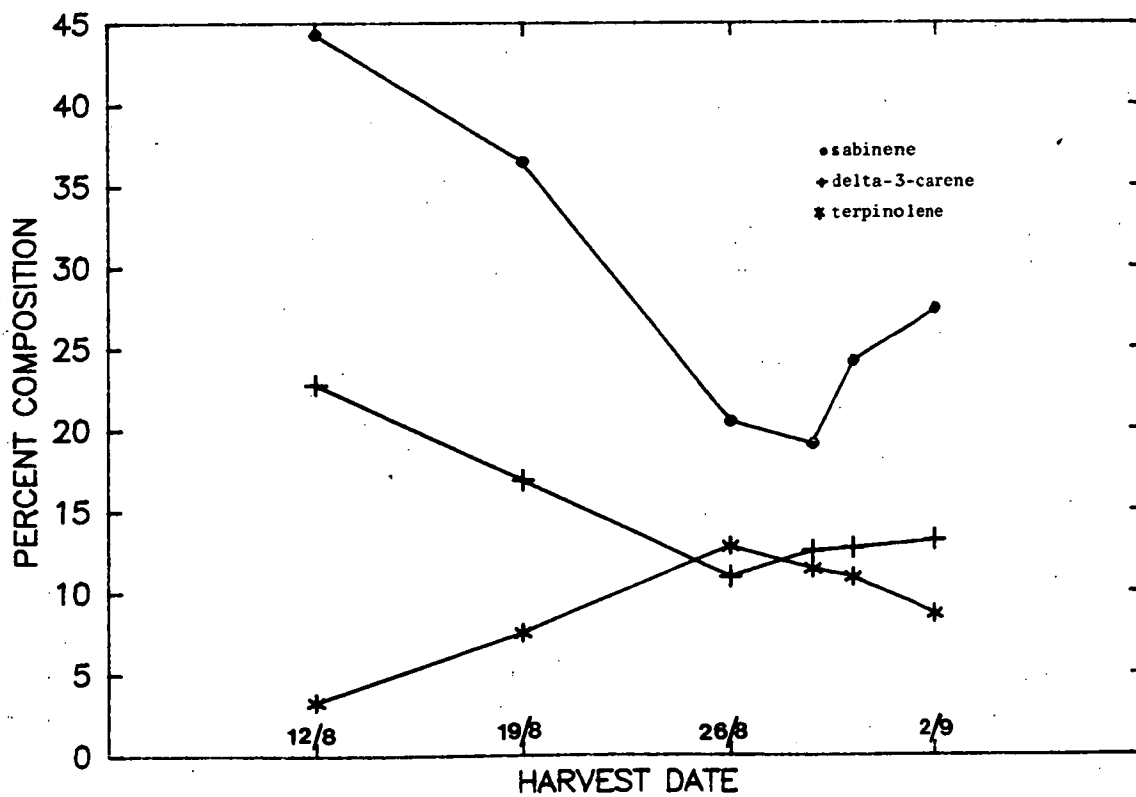
This data demonstrated that as more buds opened the yield of concrete declined, on a fresh weight basis, as was expected due to the rapid increase in weight of leaf material present. However, when the yield was adjusted proportionally to take into account the decrease in numbers of buds contributing to the sample, this yield increased; this demonstrated that the actual oil yield per bud was increasing at bud burst.

The relative composition of the oil was determined by gas chromatography at each harvest date; this data has been presented grahpically and is tabulated in Appendix 9. The major monoterpenes sabinene and delta-3-carene were observed to decrease over the three week harvest period while alpha-terpinolene increased (Figure 9.3.2). Alpha thujene increased to a peak and then declined; the sharp rise observed on the 29/8 is difficult to explain when compared to the relative level measured on the 30/8 but was reproducible. The relative concentration of both alpha and beta pinene decreased until the 29/8 then increased slowly, while for myrcene and alpha-phellandrene the converse is true (Figure 9.3.3).

Beta phellandrene and cis beta ocimene continued to increase throughout the harvest period, whereas trans beta ocimene, gamma terpinene and limonene rose to peaks at different stages then fell to a plateau level (Figure 9.3.4). Non-an-2-one, terpinen-4-ol and carvone do not change significantly, but the unknown MW 152 (17) declined with a small subsidiary rise at the 29/8 (Figure 9.3.5). Alpha terpineol levels fell sharply then climbed steadily to a higher level. Trans piperitol, on the other hand, rose from a very low level to peak around the time fifty percent of the buds were open before declining (Figure 9.3.5). The sesquiterpene hydrocarbons beta-elemene, the unknown MW 204 (39) and alloaromadrene were present in small amounts throughout this period (Figure 9.3.6). Beta terpinyl acetate and gamma cadinene rose to sharp peaks on the 29/8 and then declined, while gamma elemene reached a peak earlier on the 26/8 (Figure 9.3.6).

FIGURE 9.3.3 The relationship of selected oil components with harvest date during bud burst

Legend:
- ✷ beta pinene
- ● alpha thujene
- ✛ alpha pinene
- ◎ myrcene
- ✕ alpha phellandrene
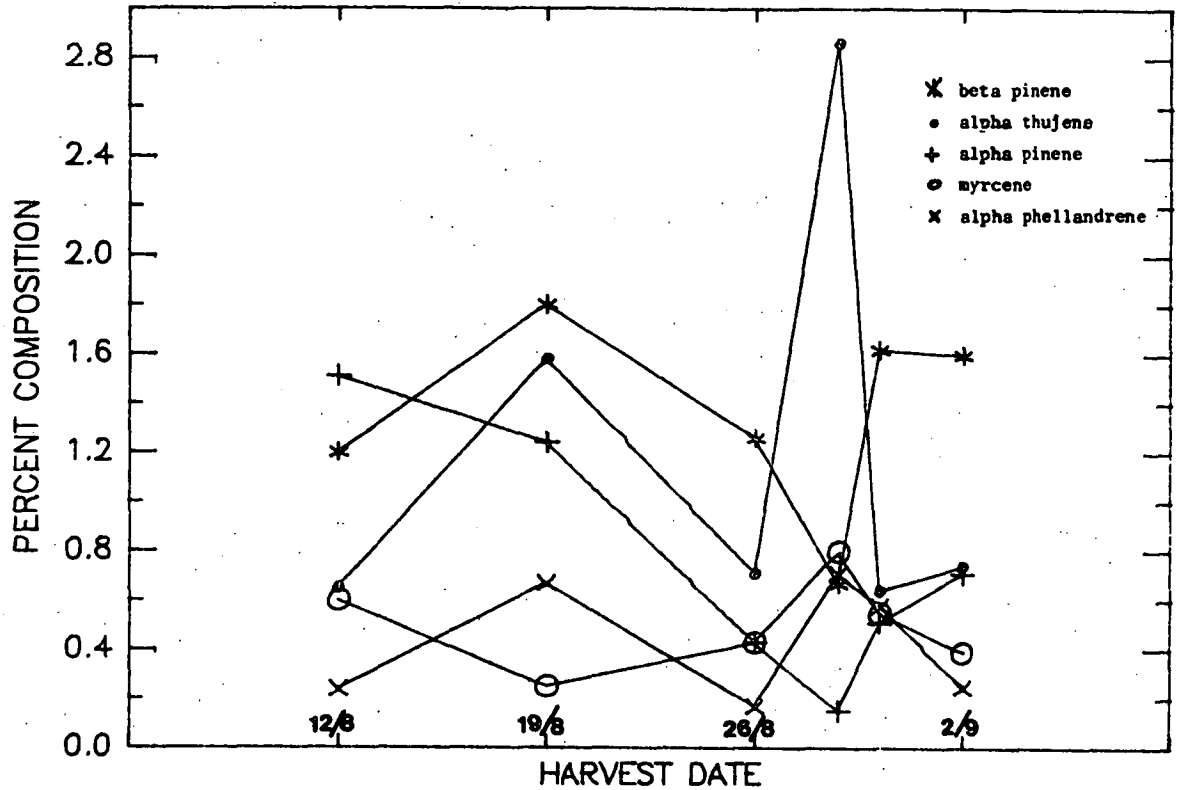
FIGURE 9.3.4 The relationship of selected oil components with harvest date during bud burst

Legend:
- ✛ limonene
- ● beta phellandrene
- ✷ cis beta ocimene
- ◎ transbeta ocimene
- ✕ gamma terpinene
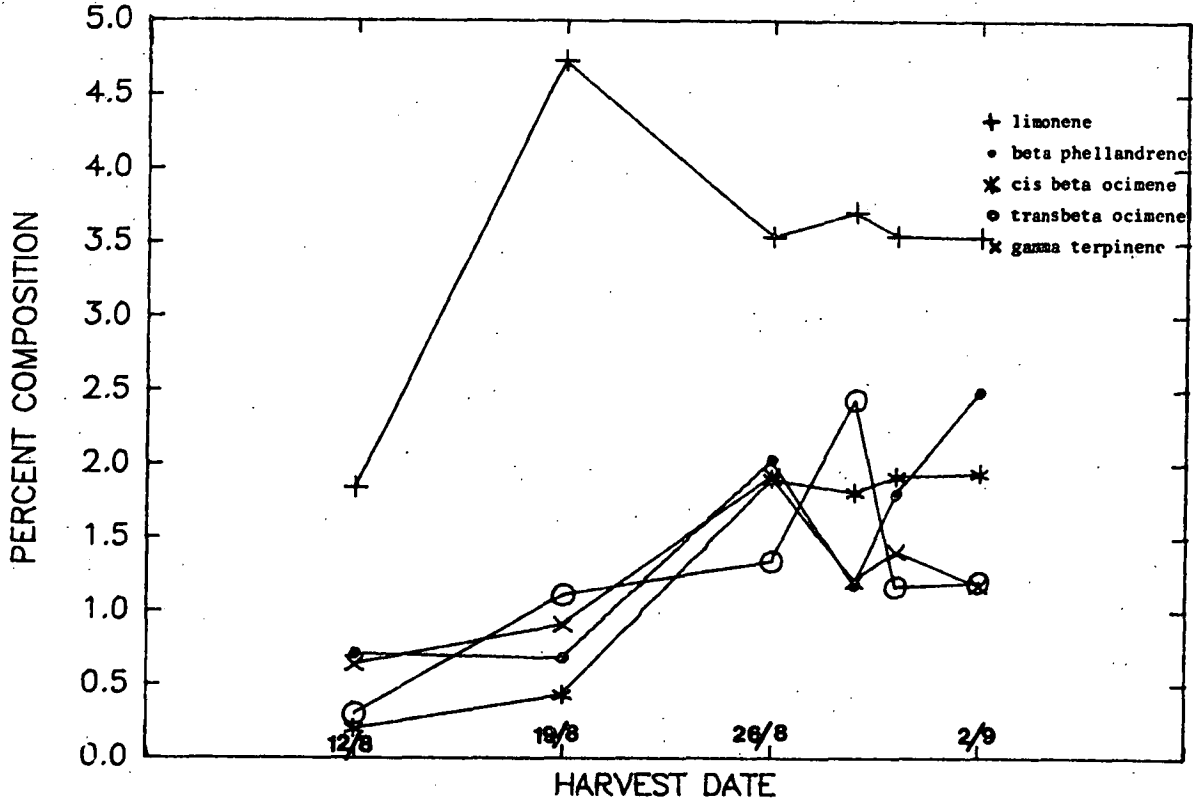
FIGURE 9.3.5 The relationship of selected oil components with harvest date during bud burst
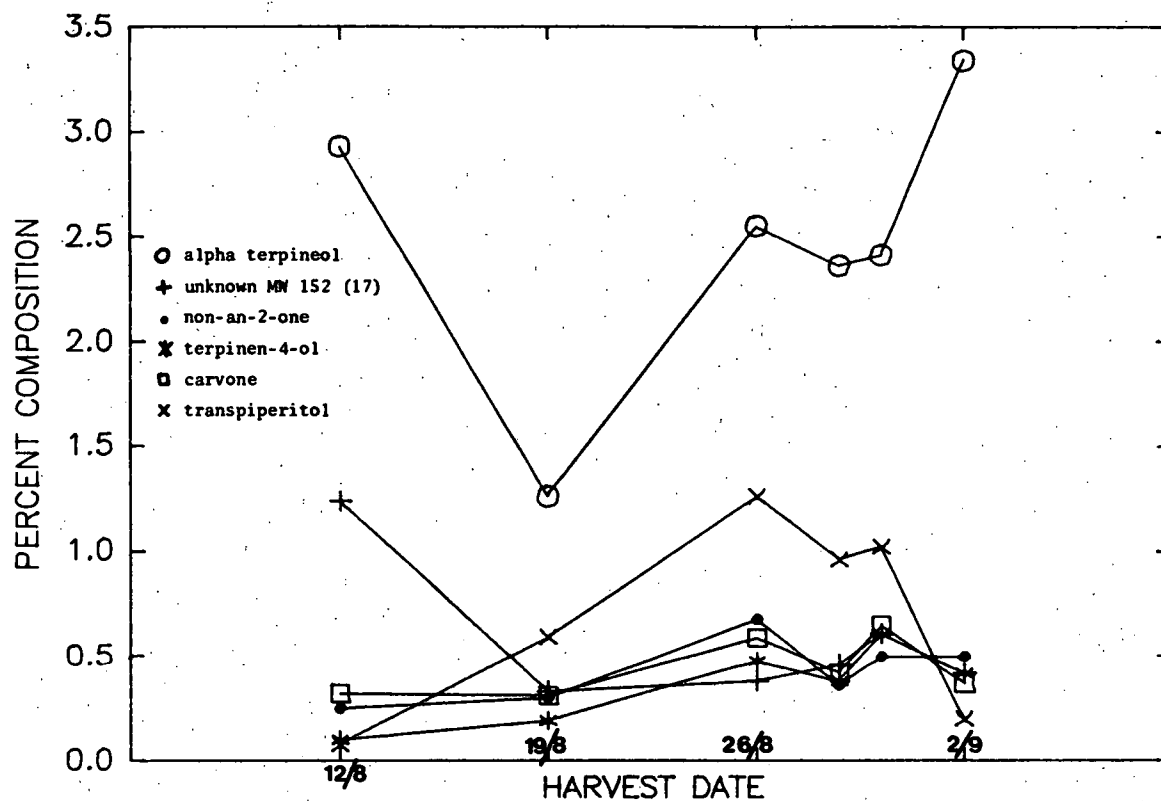
FIGURE 9.3.6 The relationship of selected oil components with harvest date during bud burst
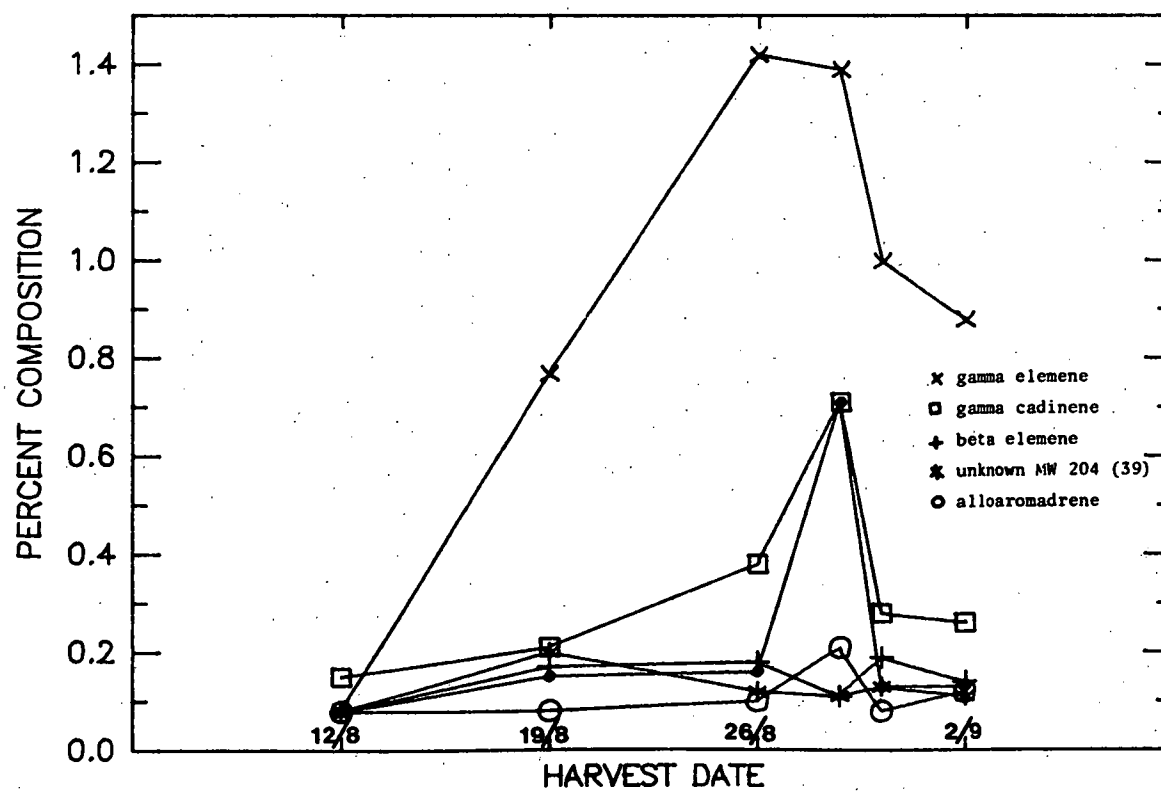
Similarly, beta caryophyllene reached a peak on the 26/8 and then decreased, whereas humulene and germacrene D both rose steadily to an equilibrium level (Figure 9.3.7). The epoxide of caryophyllene behaved as the beta form, while humulene epoxide declined steadily (Figure 9.3.8). The other two unknown sesquiterpene types remained at fairly constant levels.

For an organoleptic comparison of these samples, the triangle test described by Larmond (1977) was used. Each of the three replicates were individually compared to two samples of Tasmanian concrete produced from the White Bud selection growing at Bushy Park, Southern Tasmania. The results of this analysis are collated in Table 9.3.2. The standard samples are well balanced quality concretes possessing a reasonably strong catty aroma with a background blackcurrant fruit impression. As the samples approach fifty percent bud burst (29/8), the strength of the catty note increases without affecting the blackcurrant fruit after impression. However, once a high proportion (>90%) of buds are open (2/9) the catty note is overpowering and unpleasant without any blackcurrant fruit aroma.

TABLE 9.3.2 Organoleptic comparison* of bud burst oil samples

| | Harvest Date | | | | | | | | | | | |
| | 12/8 | | 19/8 | | 26/8 | | 29/8 | | 30/8 | | 2/9 | |
Sample	C	BF	C	BF	C	BF	C	BF	C	BF	C	BF
Standard	2	2	2	2	2	2	2	2	2	2	2	2
Rep. I	2	2	3	2	4	2	4	2	4	2	5	0
Rep. II	2	2	3	2	4	2	4	2	4	2	5	0
Rep. III	2	2	3	2	4	2	4	2	4	2	5	0

* based on a strength rating of 0 to 5 for the catty (C) and blackcurrant fruit (BF) notes

FIGURE 9.3.7 The relationship of selected oil components with harvest date during bud burst
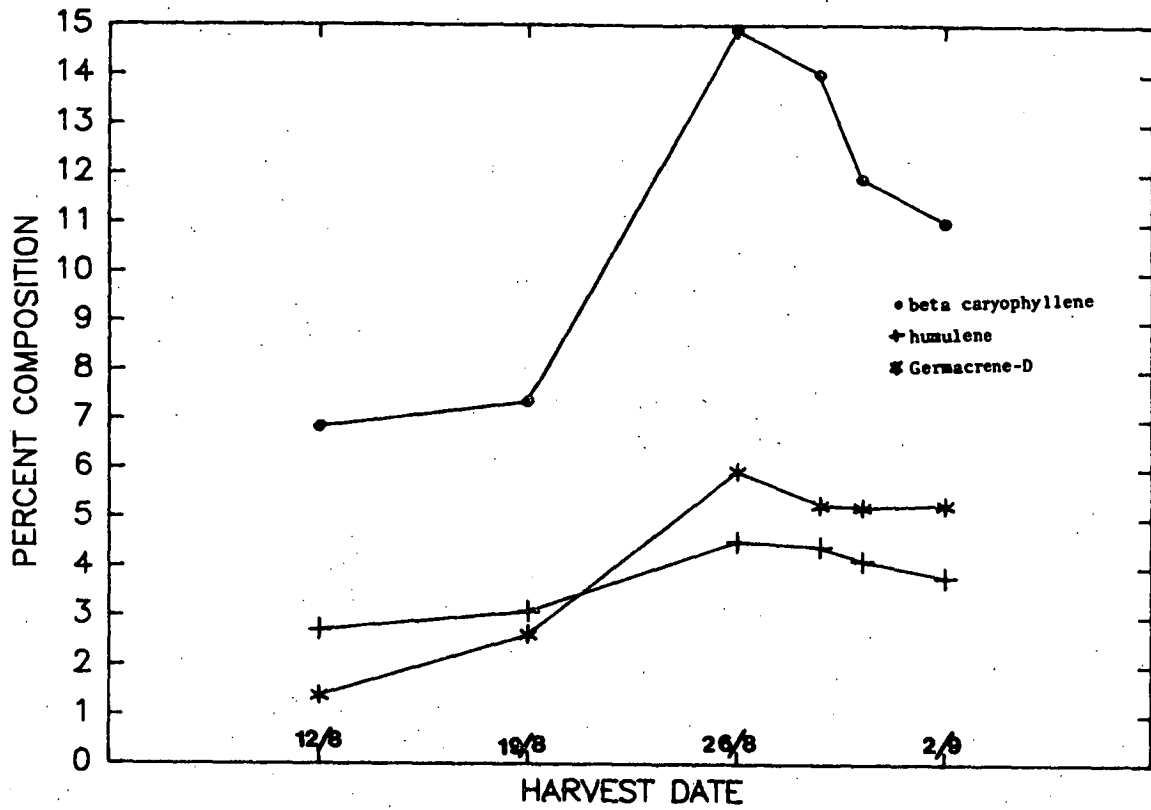
FIGURE 9.3.8 The relationship of selected oil components with harvest date during bud burst
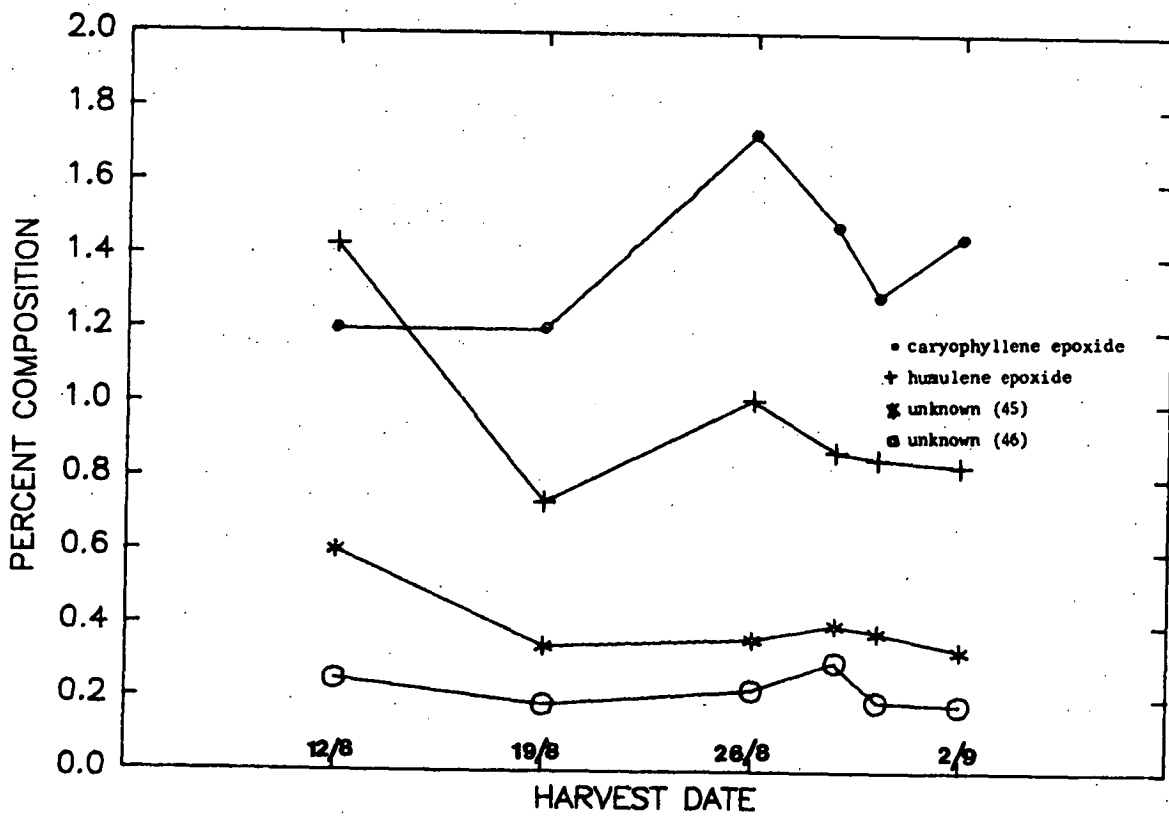
9.4 Light Interception, Utilization and Relationship to Planting Density

Transmission of light through the blackcurrant canopy was shown to be closely related to the percent of total leaf cover present at each planting density. Figures 9.4.1 and 9.4.2 show how the amount of incident light transmitted (I/I_o) to the plantation alleyways declined to a minimum in the middle of the growing season and then increases again. This was due to the direct relation between leaf area and transmitted light as demonstrated with the rise of percent leaf cover to a maximum during the middle of the growing season (Figures 9.4.3 and 9.4.4). Appendix 10 contains the observed and mean values with which the graphs were constructed. For those densities that reach a complete canopy (10.1 to 4.4 plants/m^2) there was little variation in the amount of transmitted light within that range of densities (Figures 9.4.1 and 9.4.2. However, as the maximum canopy falls below 85%, then considerable variation due to stray light from the alleyways was observed.

The relationship of transmitted light to percent leaf cover was shown to be linear (Figures 9.4.5 and 9.4.6) for blackcurrants across a range of plant densities.

The effect of plant density on the relationship of fractional transmitted light (I/I_o) to percent leaf cover is to cause a decrease in the slope of the linear regression equation (Table 9.4.1). Thus indicating at lower plant densities that a greater proportion of incident light is transmitted to the plantation floor per unit of ground cover than at higher plant densities. A cohabitant decrease in the correlation coefficient (Table 9.4.1) is also evident, indicating an increase in the observed variation at low planting densities.

At high densities (10.1-4.4 plants/m^2) the slope of the relationship falls as planting density decreases; rising again at 3.3 plants/m^2 before

FIGURE 9.4.1

LIGHT TRANSMISSION IN BLACKCURRANTS—THE EFFECT OF PLANT DENSITY

FIGURE 9.4.2

LIGHT TRANSMISSION IN BLACKCURRANTS—THE EFFECT OF PLANT DENSITY

FIGURE 9.4.3

LEAF COVER IN BLACKCURRANTS—THE EFFECT OF PLANT DENSITY

FIGURE 9.4.4

LEAF COVER IN BLACKCURRANTS—THE EFFECT OF PLANT DENSITY

FIGURE 9.4.5

RELATIONSHIP OF TRANSMITTED LIGHT TO LEAF COVER—THE EFFECT OF PLANT DENSITY

FIGURE 9.4.6

RELATIONSHIP OF TRANSMITTED LIGHT TO LEAF COVER—THE EFFECT OF PLANT DENSITY
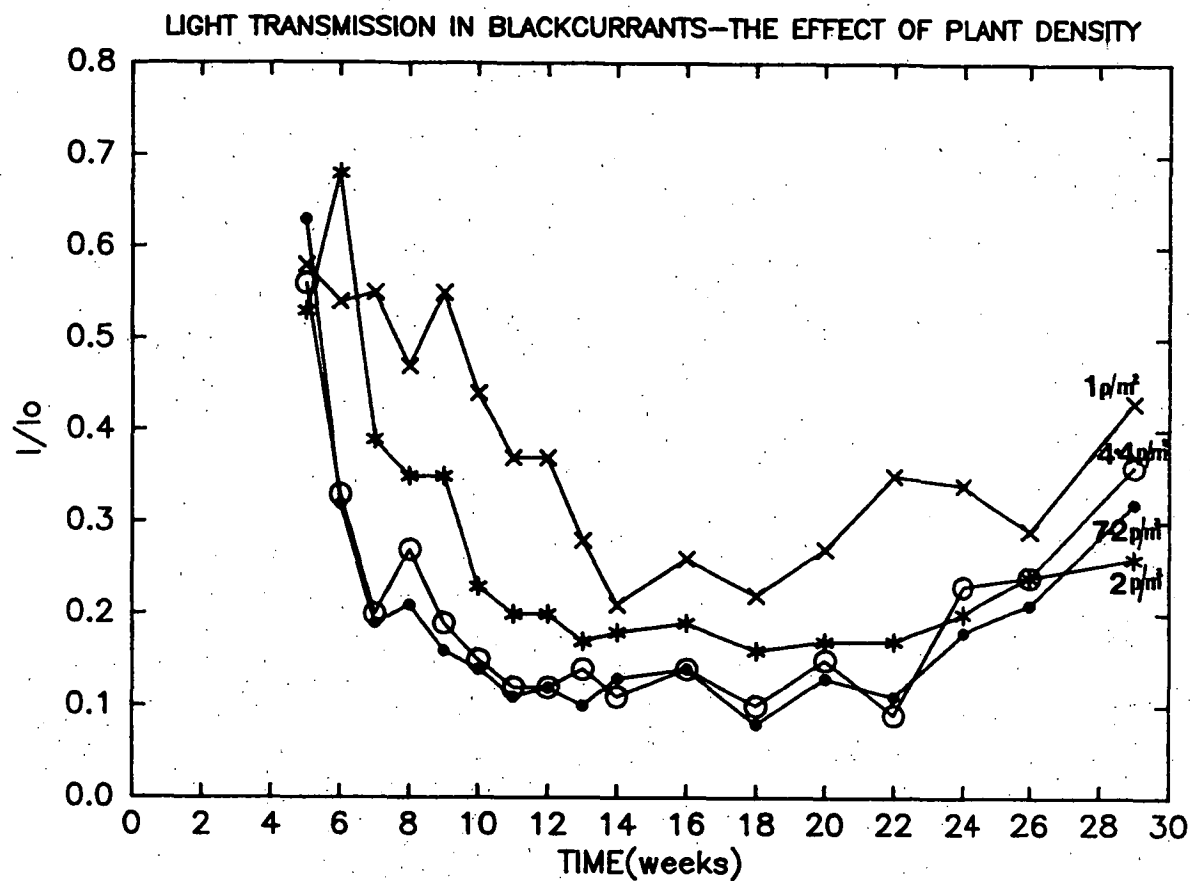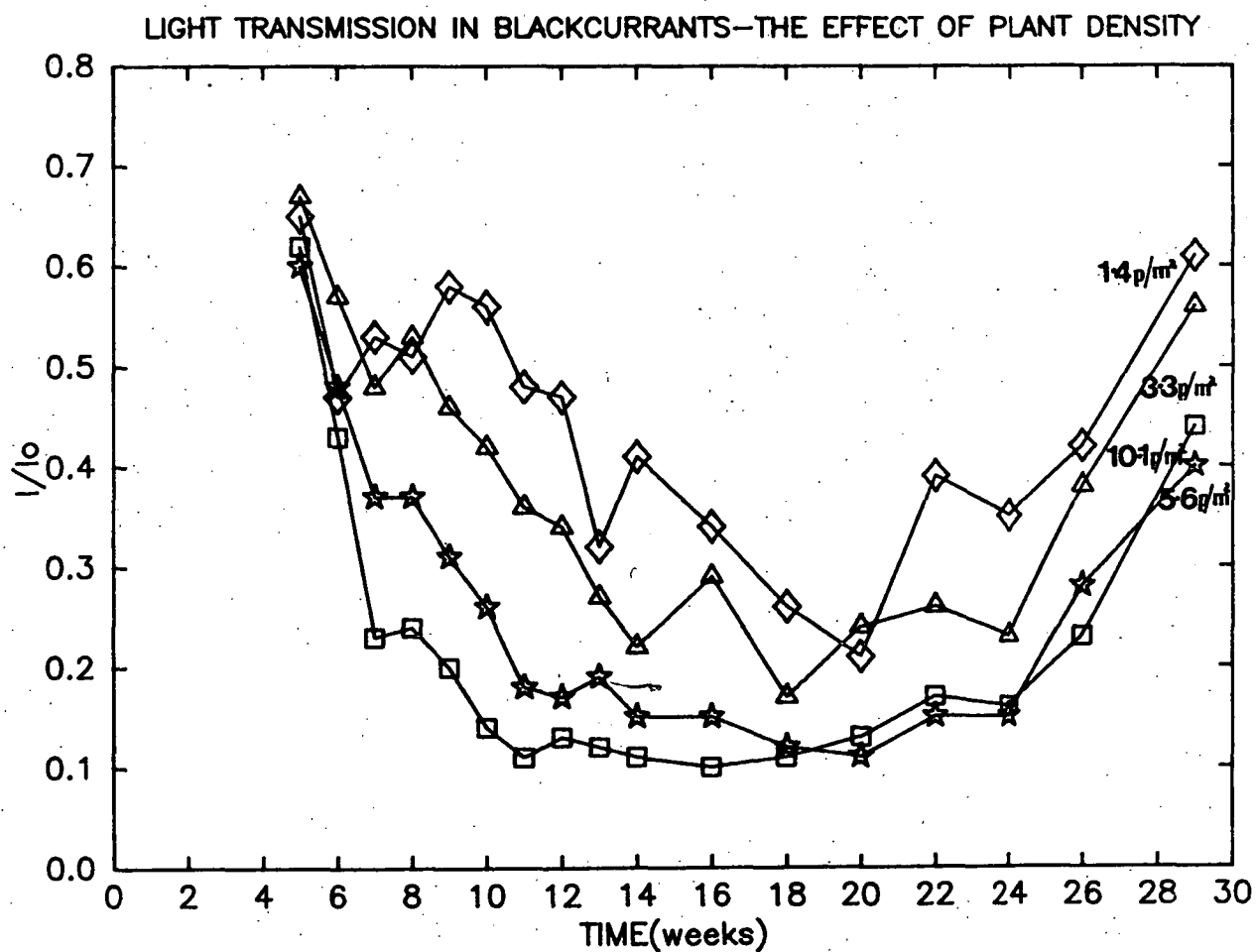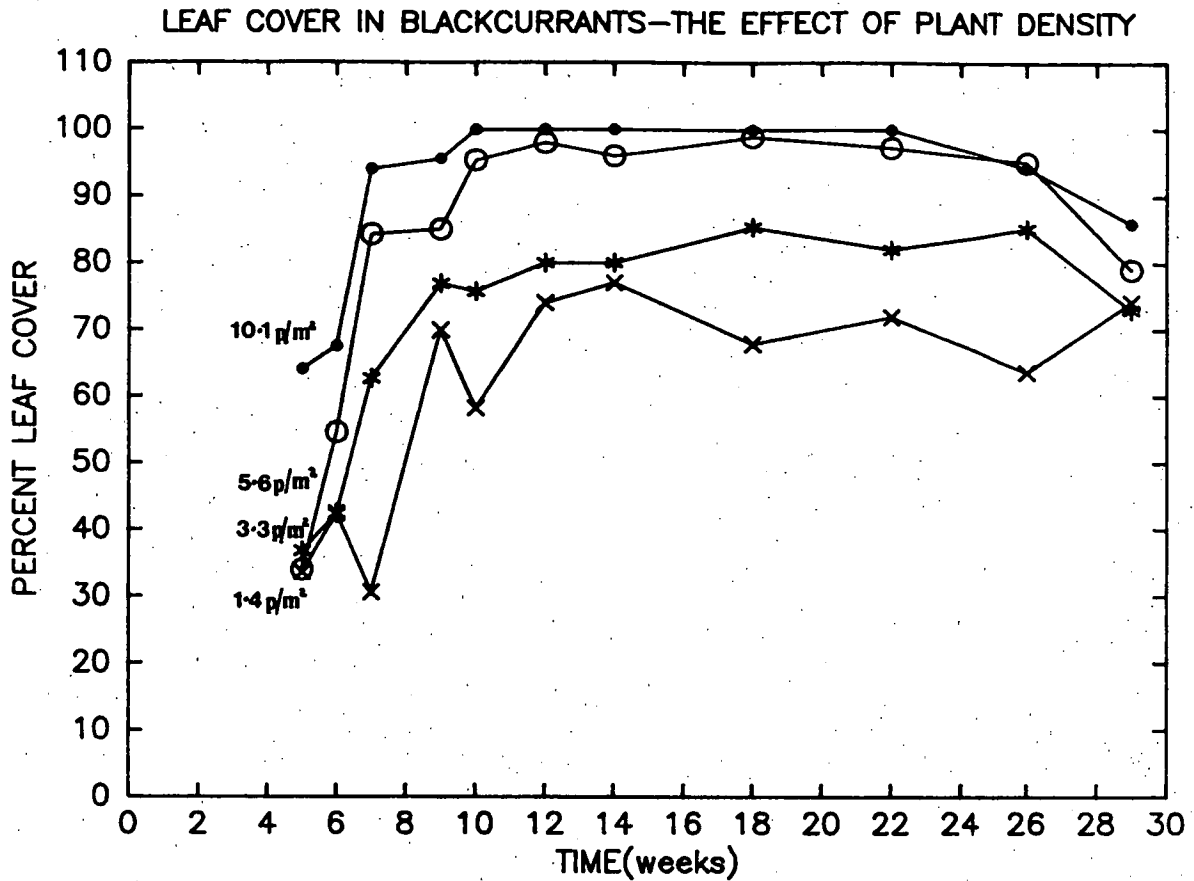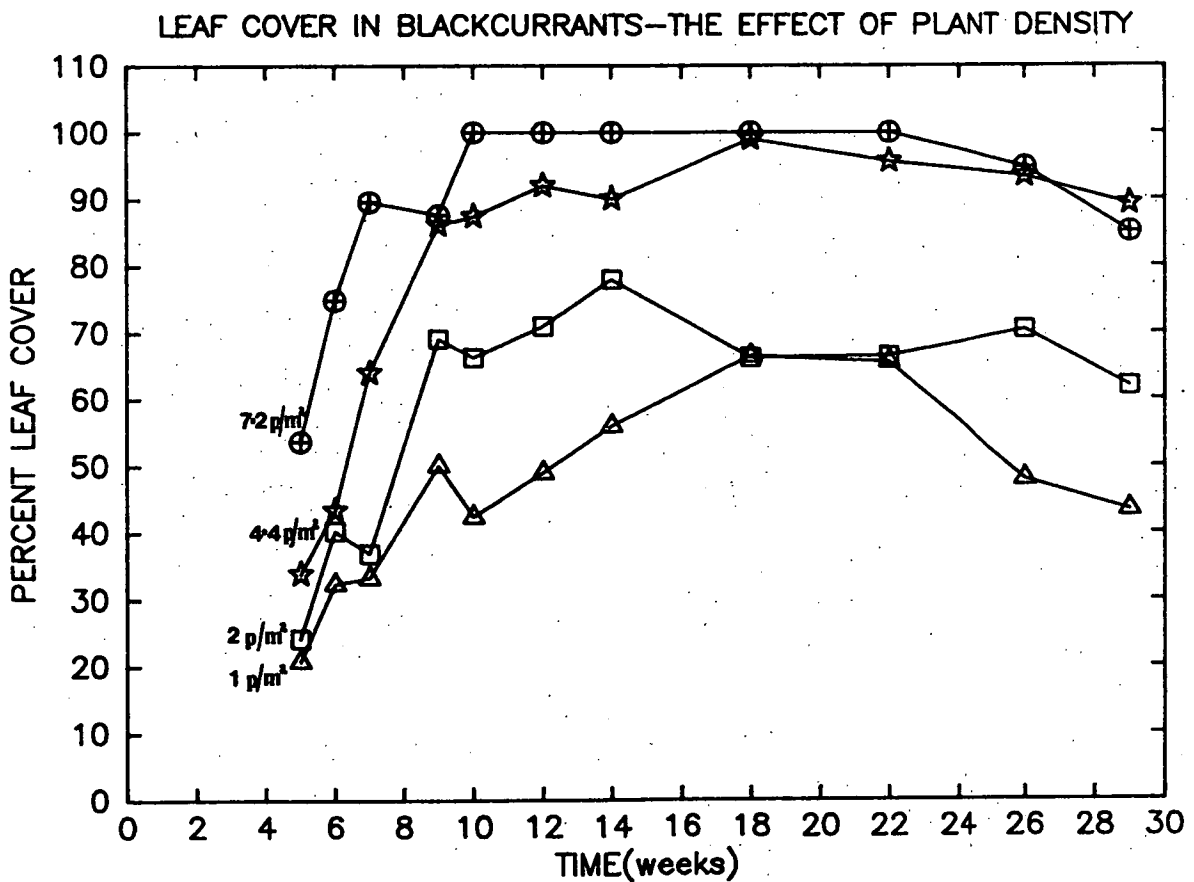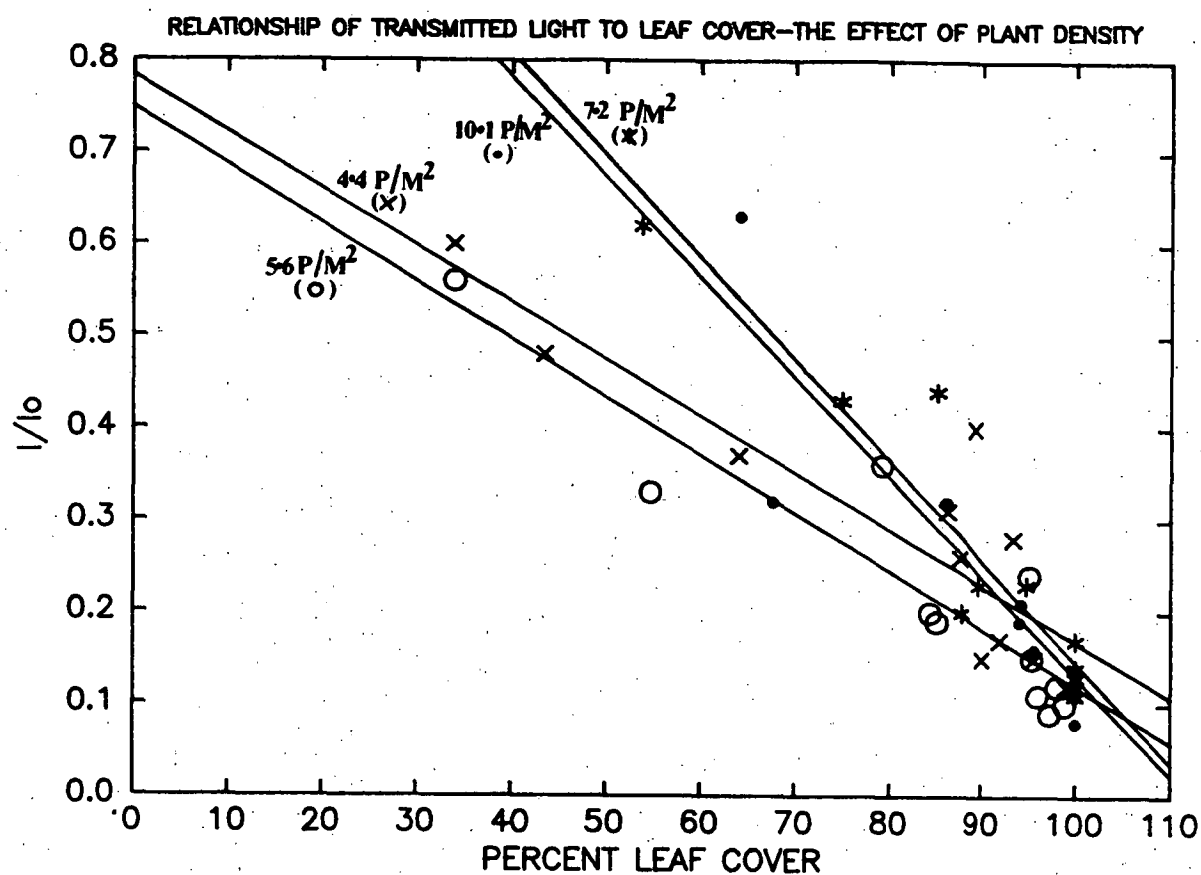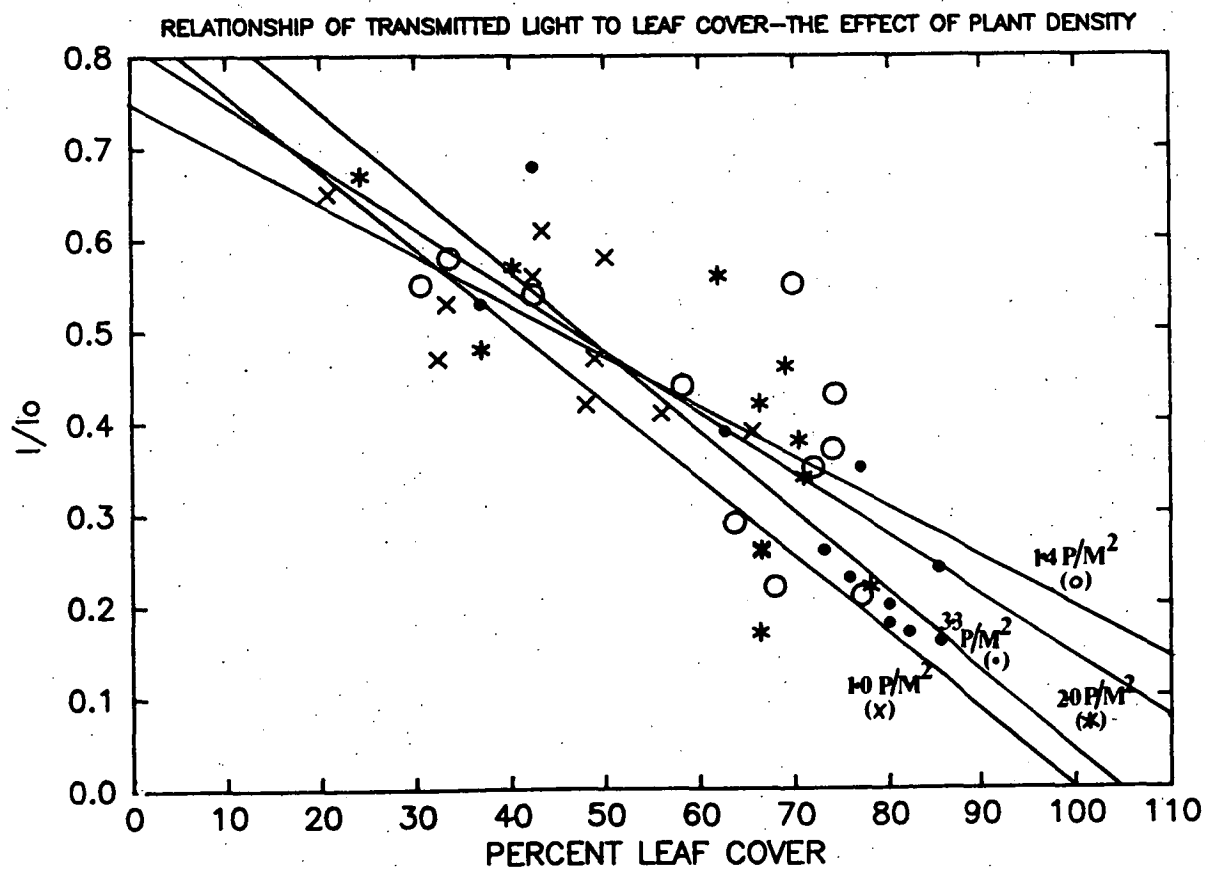
falling again with increased variation at low densities. This bimodal distribution suggests the presence of two distinct canopy types within the experimental plots - at high densities a continuous canopy and at low densities a discontinuous canopy subject to clumping and shading effects. This relationship was confirmed by observation of the canopy type at all planting densities.

TABLE 9.4.1 Values for a, b and r for a regression equation of the general form, Y = aX + b, relating transmitted light to percent leaf cover, as affected by plant density

Plant density (plants/m²)	a	b (slope)	r (correlation coefficient)
10.1	-0.0109	1.21	-0.91
7.2	-0.0109	1.24	-0.95
5.6	-0.0063	0.75	-0.92
4.4	-0.0061	0.78	-0.88
3.3	-0.0088	0.92	-0.87
2.0	-0.0068	0.82	-0.75
1.4	-0.0056	0.75	-0.69
1.0	-0.0084	0.84	-0.63

The total irradiance incident on the three plots was also measured throughout the growing season (Table 9.4.2 and Figure 9.4.7). The total solar input at each density has been estimated (Appendix 10) by calculating the percentage energy input during each time interval from the known irradiance (Table 9.4.2) and percentage of light transmitted through the canopy (Appendix 10):

Estimated Solar Input = interval irradiance x (1-% transmittance)

The estimated cumulative solar energy input has been plotted (Figure 9.4.8) on a per plant and a per m² basis. On a per plant basis the solar energy

TABLE 9.4.2 Total irradiance measured during the growing season

Week ending	Weeks	Interval Irradiance		Cumulative irradiance (MJ/m²)	Weeks after bud burst*
		(MJ/m²)	(MJ/m²/day)		
23/9-22/10/82	4	521	17.97	522	5
29/10/82	1	135	19.29	657	6
5/11/82	1	163	23.29	818	7
12/11/82	1	161	23.00	979	8
19/11/82	1	114	16.29	1,094	9
26/11/82	1	150	21.43	1,245	10
3/12/82	1	158	22.57	1,403	11
10/12/82	1	110	15.86	1,514	12
17/12/82	1	163	23.29	1,677	13
23/12/82	1	127	18.14	1,804	14
6/1/83	2	313	22.34	2,117	15
20/1/83	2	277	19.79	2,395	18
3/2/83	2	308	22.00	2,703	20
17/2/83	2	279	20.00	2,983	22
4/3/83	2	235	15.67	3,218	24
18/3/83	2	226	16.21	3,445	26
8/4/83	3	245	11.67	3,690	29
29/4/83	3	166	7.90	3,857	32

* bud burst 14/9/82

FIGURE 9.4.7

CUMULATIVE INCIDENT RADIATION AT BUSHY PARK TASMANIA 1982/83

FIGURE 9.4.8

CUMULATIVE SOLAR ENERGY INPUT FOR BLACKCURRANTS
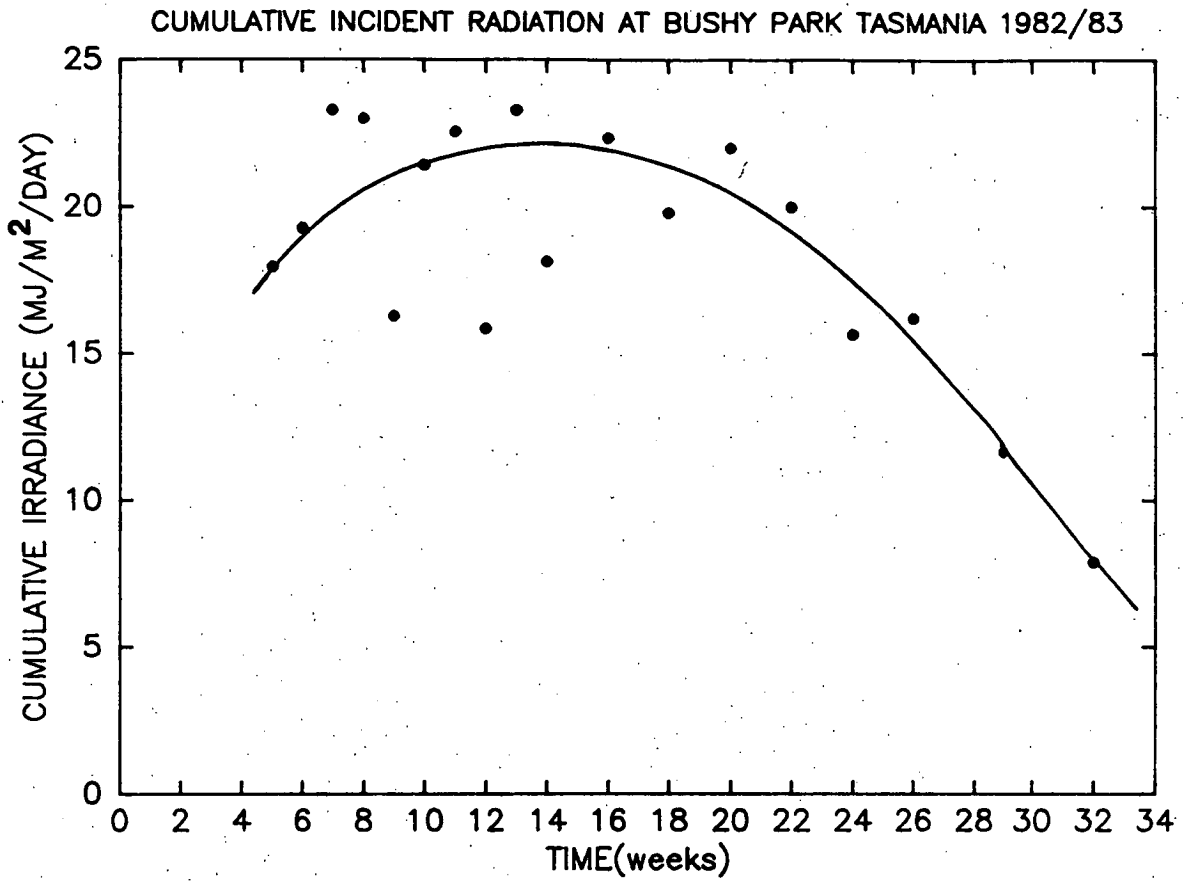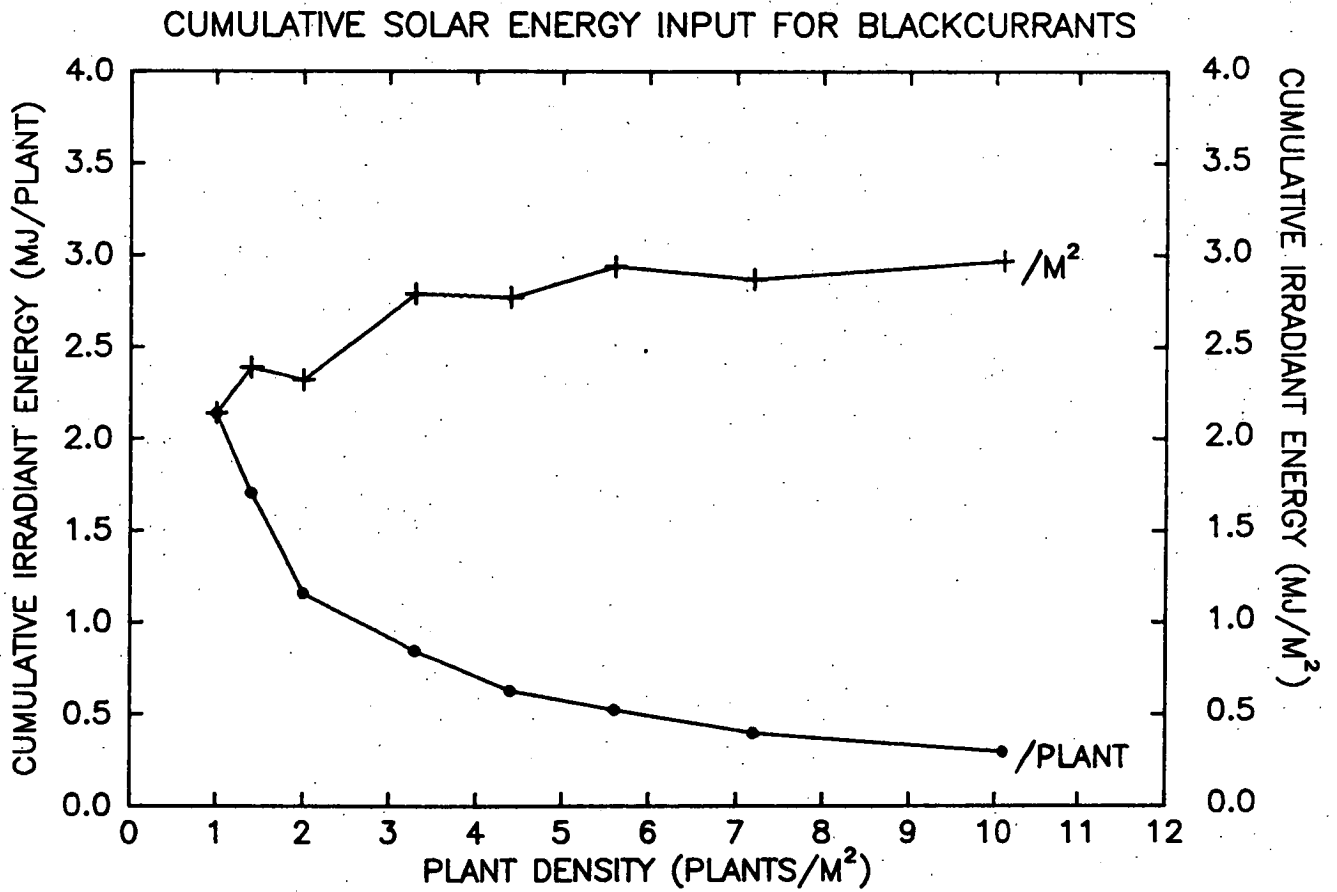
input declines with increasing plant density as would be expected. However, on a per m^2 basis the reverse was true, due to a greater percentage of transmitted light reaching the plantation floor. The cumulative values are summarized in Table 9.4.3 with total cane fresh weight (an estimate of total plant productivity) and bud yield in 1983. Figure 9.4.9 shows clearly that both bud yield and total cane fresh weight per plant are increased as the amount of cumulative irradiant energy increases. Total cane fresh weight appears to have reached a maximum at 1,500 MJ/plant, whereas bud yield is still increasing at an energy input of 2,000 MJ/plant. When these figures are expressed as yields per solar energy input (Figure 9.4.10), it is clear at low densities that both bud yield and cane fresh weight per solar input are depressed. This could be due to shading as a result of the discontinuous canopy and changing sun angles. Both yields then rise steadily with a slight peak at 7.7 plants/m^2 before decreasing. This decrease is probably due to increased competition between plants at higher densities, with the peak indicating the density enabling the most efficient conversion of solar energy to yield in a continuous canopy.

The data also demonstrates that there is a linear relationship between cumulative solar energy input (Appendix 10) and percent leaf cover (Appendix 10). This relationship takes the form (Figure 9.4.11):

$$Y = 4.05 - 0.0426X$$

with a correlation coefficient, $r = -0.96$; a very good fit.

The monthly minimum and maximum temperatures were obtained from the Meteorological Bureau, Hobart, Tasmania for Bushy Park during the 1982/83 growing season and are plotted here (Figure 9.4.12) for discussion later.

TABLE 9.4.3 Estimated solar energy input and yield/solar input (per plant basis)

Plant Density (plants/m^2)	Cumulative Solar Energy Input		Bud Yield (1983) (g/plant)	Bud Yield/Solar Input (mg/MJ)	Cane Fresh weight (g/plant)	Cane Fresh weight/solar input (g/MJ)
	(MJ/m^2)	(MJ/plant)				
10.1	2,970	294	3.56	12.11	136.53	0.46
7.2	2,870	397	5.92	14.91	231.79	0.58
5.6	2,940	525	6.87	13.09	288.06	0.55
4.4	2,770	630	7.78	12.35	325.64	0.52
3.3	2,790	846	10.76	12.72	446.90	0.53
2.0	2,320	1,160	14.95	12.89	616.87	0.53
1.4	2,390	1,707	19.02	11.14	690.06	0.40
1.0	2,140	2,140	20.65	9.65	678.05	0.32

FIGURE 9.4.9

RELATIONSHIP OF YIELD TO SOLAR ENERGY INPUT
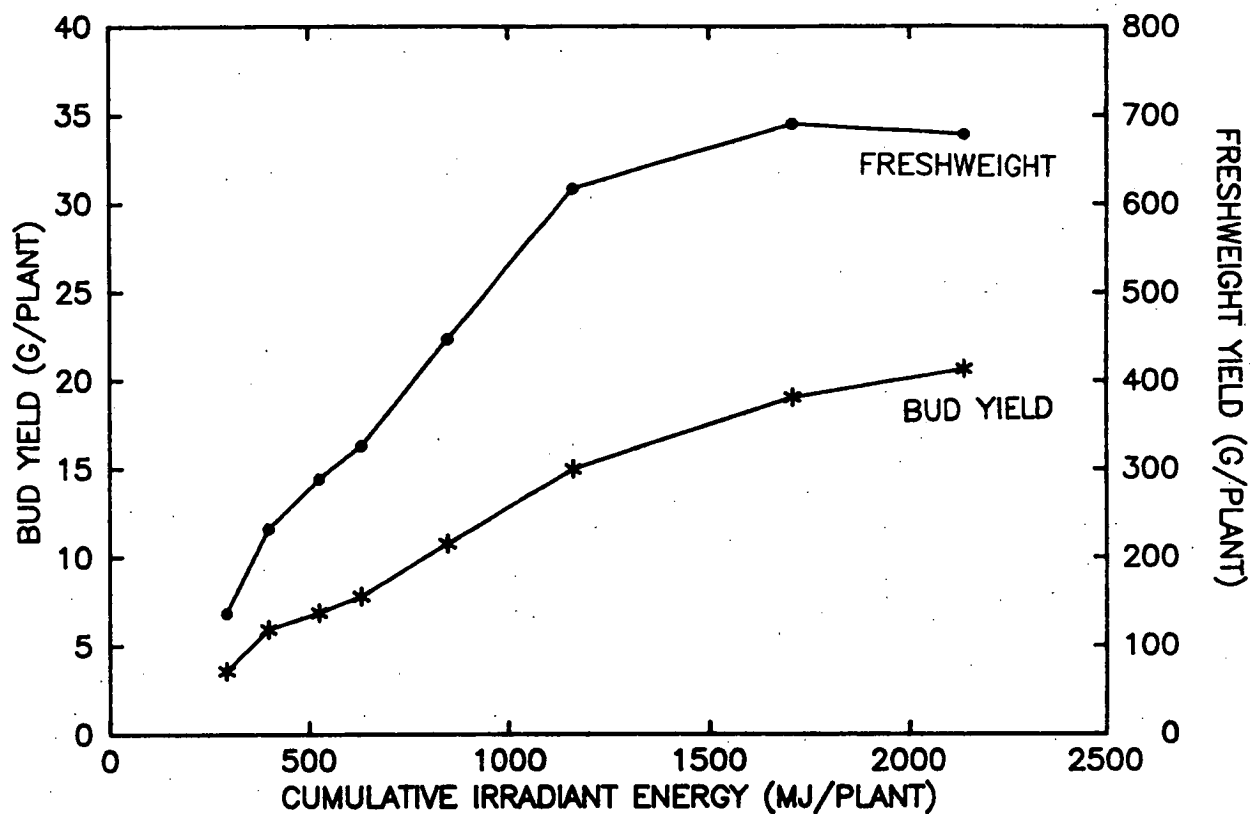
FIGURE 9.4.10

RELATIONSHIP OF DENSITY AND YIELD PER PLANT PER SOLAR INPUT

FIGURE 9.4.11

RELATIONSHIP OF CUMULATIVE SOLAR ENERGY INPUT AND LEAF COVER

FIGURE 9.4.12

MEAN MONTHLY MAXIMUM AND MINIMUM TEMPERATURES AT BUSHY PARK 1982/83
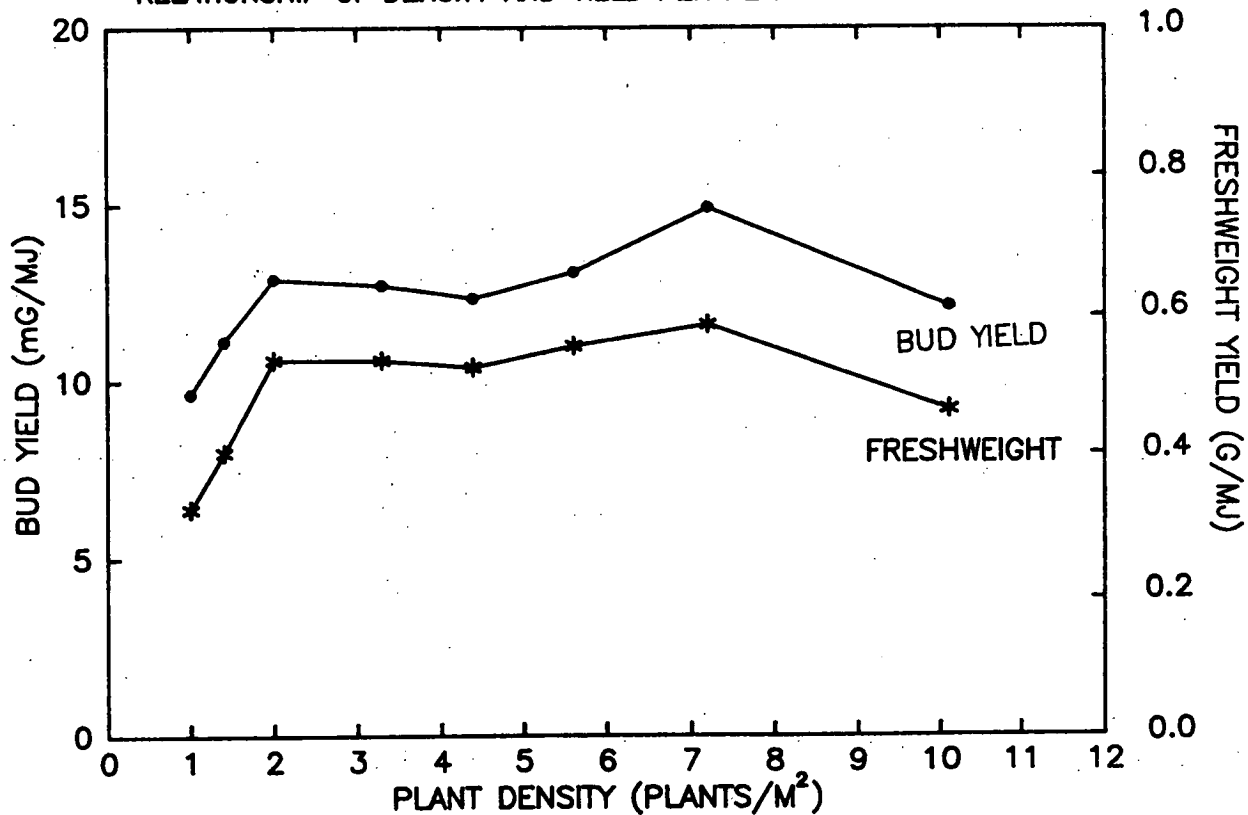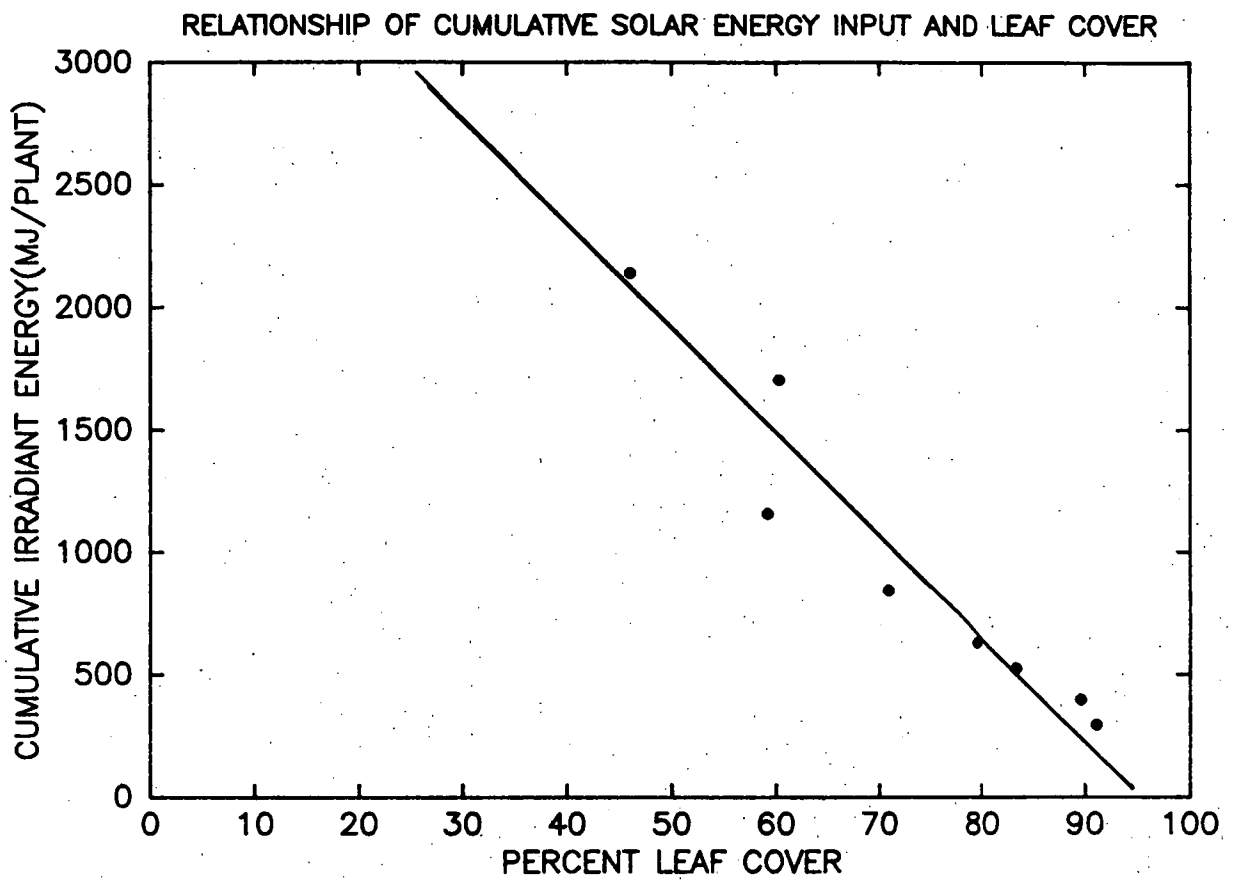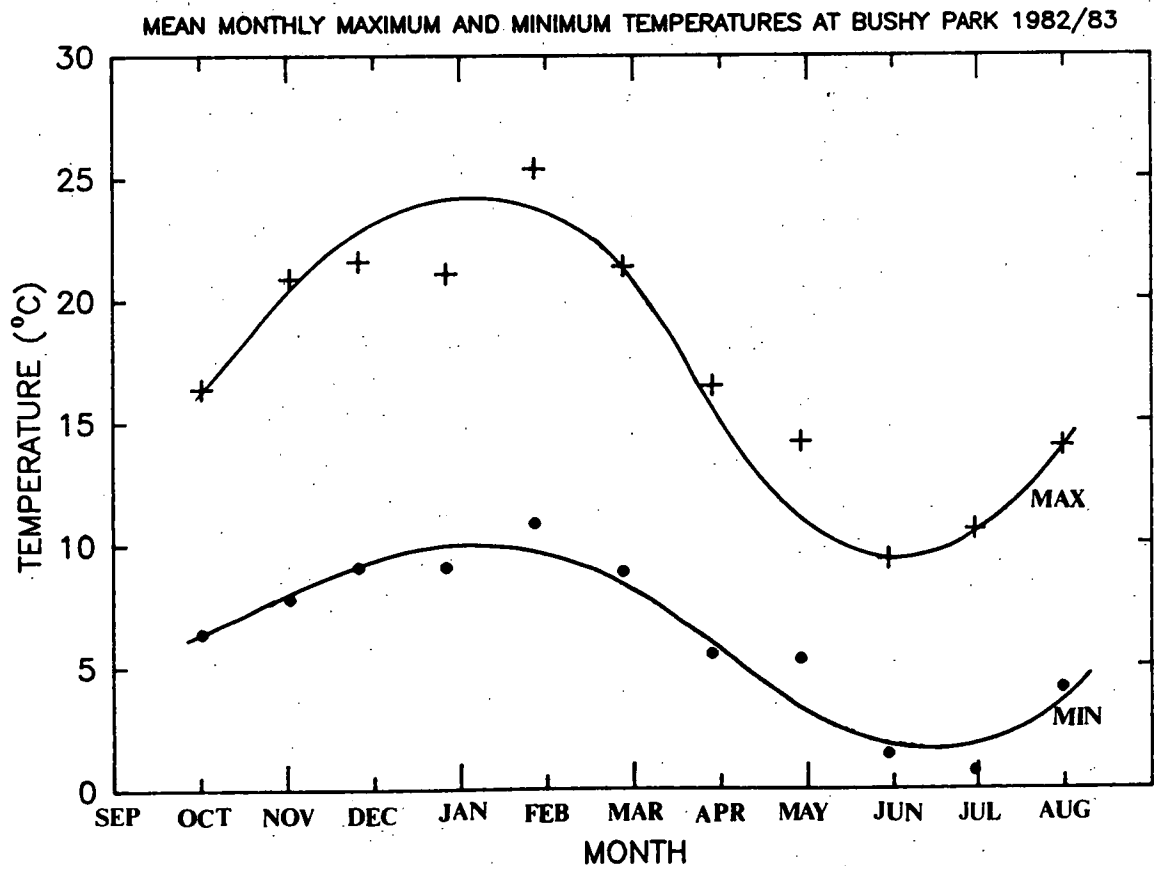
9.5 Varietal Differences in Oil Quality

The varietal differences in oil yield are contained in Table 9.5.1.
White Bud, the main local variety, was fairly high yielding as was the
Super C selection.   However, the Grahams No. 1 selection was not as
productive.   All these selections were more prolific producers than the
parent variety Baldwin (for a full discussion of variety interrelationships
see Section II 1.3).   Other high yielding varieties which may be important
were Boskoop Giant, Lees Prolific, Magnus and Goliath.

Commercial acceptance of various varietal concretes has already been
discussed (refer to Section IV 5.1).   Table 9.5.2 contains an organoleptic
description of each variety compared to the two French products (refer to
Section III 6.1).   From this comparison, a preference for White Bud
selections (Super C, White Bud) and closely related varieties (Baldwin,
Goliath, Hatton Black) was shown as indicated.   The Grahams White Bud
selection does not produce a quality oil and some question was raised as
to whether it is true to type.   The less desirable oils were those, such
as Lees Prolific and Boskoop, which appeared to be enriched in monoterpene
constituents and lacked a strong catty impression.

Compositional data was obtained for blackcurrant bud oils from ten
varieties grown at three sites in Southern Tasmania.   The ten varieties
were related in Todd's classification (1962) as shown in Table 9.5.3.
The compositional data, which is found in Appendix 11, was analysed by
principal co-ordinate analysis.   This technique, due to Gower (1966),
requires the user to define a similarity matrix between sampling units,
which in this case were 37 blackcurrant bud oils with various location and
variety attributes.   This similarity matrix is a matrix of similarity
coefficients between pairs of units and has been described in part by
Gower (1971).   The similarity coefficient is a number between 0 and 1
that is defined in terms of a set of variates; in this case individual

TABLE 9.5.1 Varietal concrete yields (% fresh weight basis)

| Variety | Horticultural Research Centre | | | | Huon Research Station | | | Marion Bay | Overall |
	1980	1982	1983	Mean	1982	1983	Mean	1980	mean
White Bud	2.55	-	3.61	3.08	-	3.77	3.77	3.34	3.32
Grahams WB No.1	-	2.40	3.06	2.73	-	3.06	3.06	-	2.84
Super C	2.71	-	2.06	2.39	4.00	4.24	4.12	-	3.25
Baldwin	3.06	2.60	2.25	2.64	-	-	-	-	2.64
Goliath	3.28	3.00	2.65	2.98	4.93	3.16	4.05	2.40	3.24
Boskoop Giant	3.21	2.70	3.25	3.05	7.26	2.49	4.88	-	3.78
Lees Prolific	-	3.30	2.43	2.87	5.14	2.80	3.97	-	3.42
Hatton Black	-	-	3.65	3.65	-	1.92	1.92	-	2.79
Magnus	-	-	-	-	3.81	2.72	3.27	-	3.27
Kerry	1.41	3.50	2.42	2.44	3.55	3.17	3.37	-	2.81

TABLE 9.5.2 Organoleptic description of varietal concretes produced in 1983

Variety	Preference Mark	Description
White Bud	1	Typical blackcurrant product, fresh top notes with a reasonably strong catty impression. Does not possess the peppery, spicy top notes of French standards.*
Super C	2	A sweeter sample, strength of catty and fruit aromas not as powerful as above.
Goliath	3	Fresh top notes with reasonably strong fruit and catty aroma impressions; well balanced.
Baldwin	4	Typical blackcurrant fruit and catty aromas, top notes are a little flat; also a green background note.
Magnus	5	Good balance of blackcurrant fruit and catty aromas.
Hatton Black	6	Good blackcurrant fruit impression, not as well balanced, lacking in strong catty note although gives a fresh impression.
Grahams No. 1 White Bud	7	Lacks any fresh top notes, heavy green note that distracts from fruit and catty aromas.
Kerry	8	Heavy first impression of cooked blackcurrants.
Lees Prolific	9	Lacks a catty impression, sweet fruit background possibly rich in monoterpene hydrocarbons.
Boskoop Giant	10	Lacks strong catty note, balance is fruit notes with sweeter terpene aroma. Obviously richer in monoterpene hydrocarbons.

TABLE 9.5.3   Variety interrelationships (from Todd 1962)

Group	AI stems & peptides green	AII stems & peptides red	AIII stems green, peptides red
Subgroups			
B1 Cotswold Group		B1 Baldwin Group e.g. Baldwin, White Bud, Super C, Grahams No. 1, Hatton Black, Magnus, Kerry	B1 French Group e.g. Lees Prolific
B2 Goliath Group e.g. Goliath		B2 Wellington Group	B2 Boskoop Group e.g. Boskoop Giant

terpene components of the oils.   It takes the value 1 if all variate values are the same for both units, and 0 if all variate values are as different as possible.   The principal coordinate analysis used was that available with the GENSTAT Statistical Package (Mark 4.03 Rothamsted Experiment Station, England 1980).

Initially the analysis was carried out using the 34 oil components measured for each variety.   The latent vector coordinates are summarized in Appendix 11, along with the percentage of the observed variance accounted for by each coordinate.   The coordinates produced by the analysis have been plotted in four dimensions, taking into account 60.4% of the variation observed.

Figures 9.5.1, 9.5.2 and 9.5.3 display the variation accounted for by the combination of the first and second, first and third, and first and fourth coordinates, respectively.   Figure 9.5.1 shows three groupings of sample varieties, which show a close relationship of Baldwin and Goliath Groups irrespective of location and year of harvest.   In addition, three other groupings show a close relationship between the French and Boskoop Groups.   One of these latter was confounded by the Super C varietal

selection sampled at Grove in 1982 and 1983, suggesting this may not be true to type. Grahams No. 1 associates readily with other White Bud selections tending to discount the early suggestion that it is not true to type.

Figures 9.5.2 and 9.5.3 confirm these associations.

The principal coordinate analysis was repeated using only the twelve monoterpene and sesquiterpene hydrocarbons that Latrasse and Lantin (refer to Section II 2.5) used in their classification of varieties into distinct phenotypes. The latent vector coordinates produced by the analysis were again plotted in four dimensions, taking into account 78% of the observed variation (the data set is attached in Appendix 11).

This analysis presents a less complex pattern of associations within the oils sampled, suggesting that the similarity matrix shows a bias towards those compounds present in higher percentages. Such analysis should therefore be treated with caution as components present in only small amounts may well point to characteristic varietal differences.

Figure 9.5.4 shows several associations of oils from the Goliath and Baldwin Groups which suggest that although these groups were separated morphologically (Table 9.5.3), they were closely related oil phenotypes. In addition, Boskoop Giant and Lees Prolific frequently associate closely together. The Boskoop and French Groups on this evidence are related to each other but not to the Goliath and Baldwin Groups. Figures 9.5.5 and 9.5.6 confirm this as well as demonstrating that the Super C selection sampled at Grove was quite different from the White Bud selection. Indeed this Super C selection was different from all the White But selections suggesting that either it is not true to type, or it is not a White Bud progeny as is widely thought (refer to Section II 1.3).

The separation of oils from the various White But selections and the closely related Goliath Group into distinct groupings appears to be due to environmental variation. For example, the varietal selections

Key to ordination diagrams.   Figures 9.5.1 to 9.5.6.

		Horticultural Research Centre			Grove Research Station
1983	1	White Bud	1983	22	White Bud
	2	Grahams No. 1 White Bud		23	Super C
	3	Super C		24	Goliath
	4	Baldwin		25	Hatton Black
	5	Goliath		26	Lees Prolific
	6	Kerry		27	Magnus
	7	Boskoop Giant		28	Boskoop Giant
	8	Hatton Black		29	Kerry
	9	Lees Prolific			
1982	10	Grahams No. 1 White Bud	1982	30	Goliath
	11	Goliath		31	Boskoop Giant
	12	Baldwin		32	Kerry
	13	Boskoop Giant		33	Magnus
	14	Kerry		34	Lees Prolific
	15	Lees Prolific		35	Super C
1980	16	White Bud			Marion Bay
	17	Baldwin	1980	36	White Bud
	18	Boskoop Giant		37	Goliath
	19	Super C			
	20	Goliath			
	21	Kerry			

FIGURE 9.5.1

ORDINATION DIAGRAM — VARIETAL DIFFERENCES
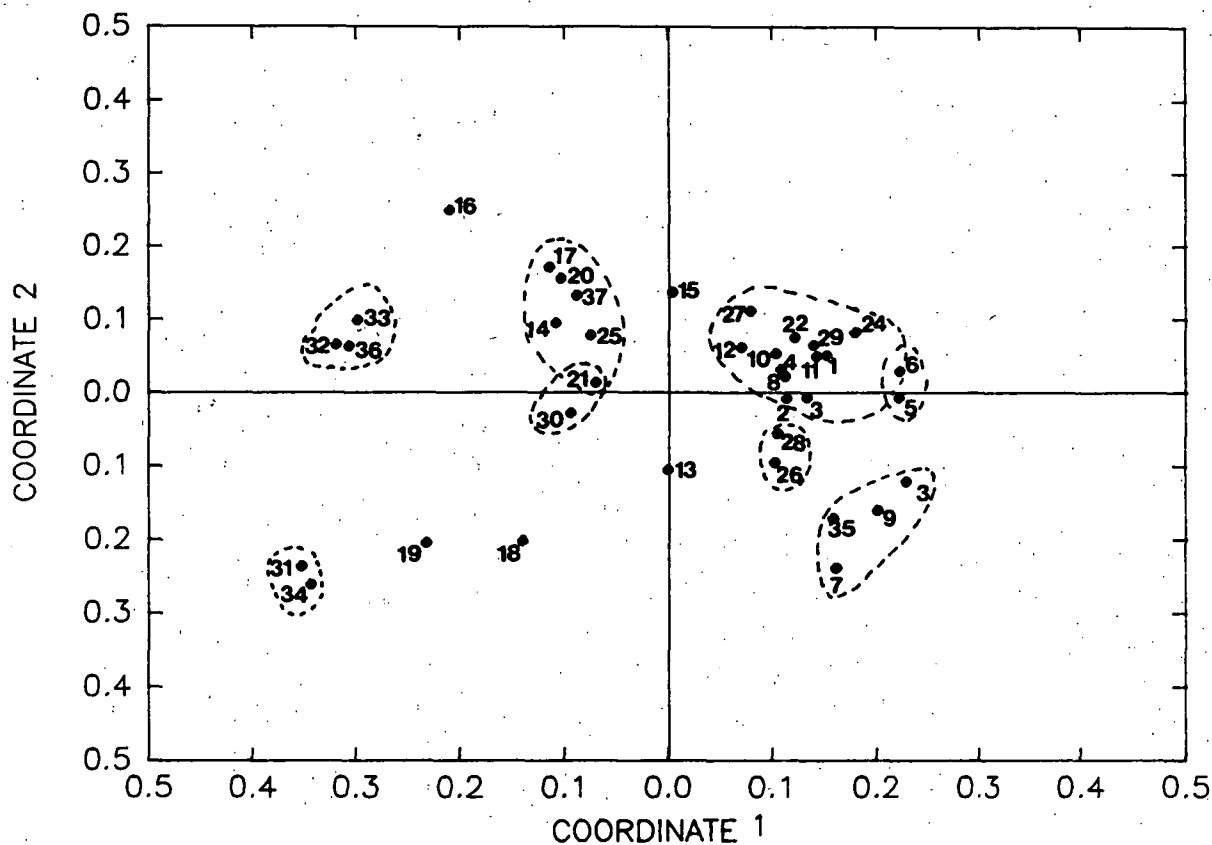
FIGURE 9.5.2

ORDINATION DIAGRAM — VARIETAL DIFFERENCES

FIGURE 9.5.3

ORDINATION DIAGRAM — VARIETAL DIFFERENCES

FIGURE 9.5.4

ORDINATION DIAGRAM — VARIETAL DIFFERENCES
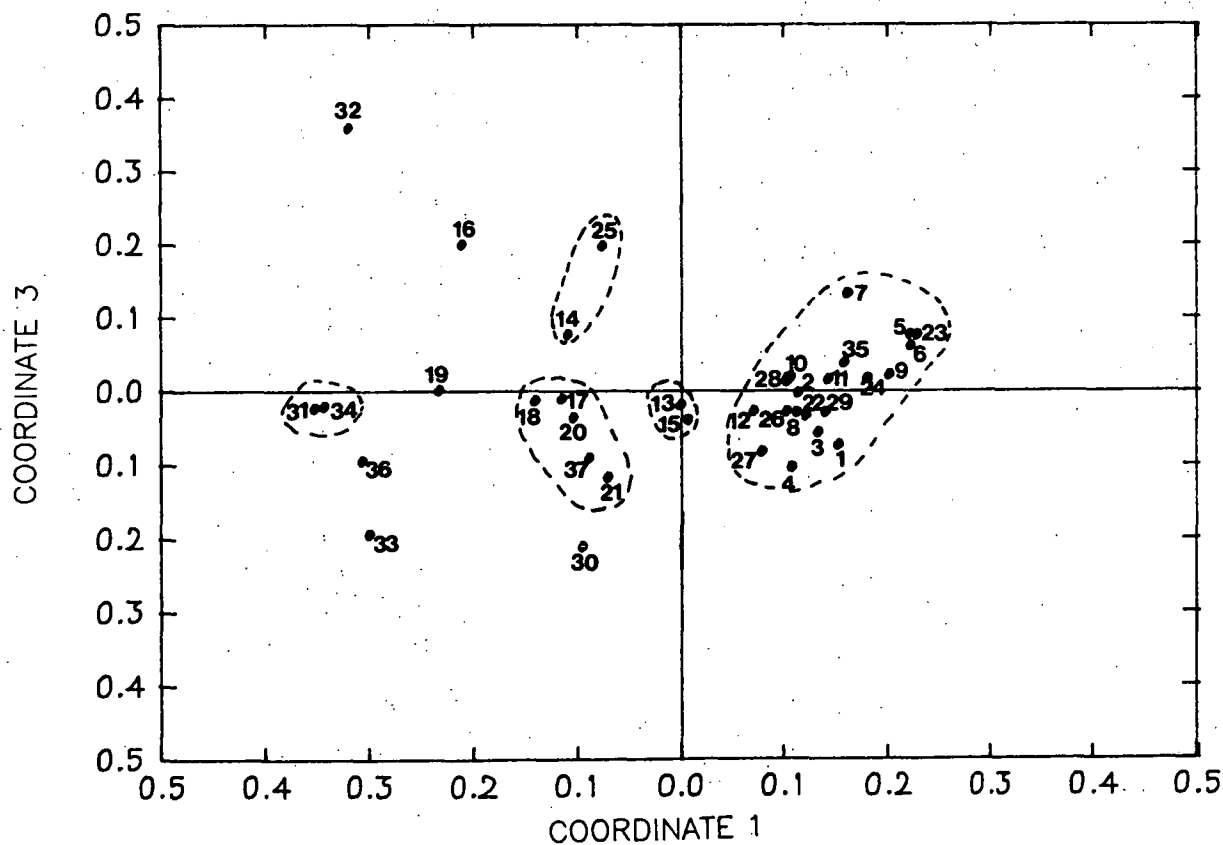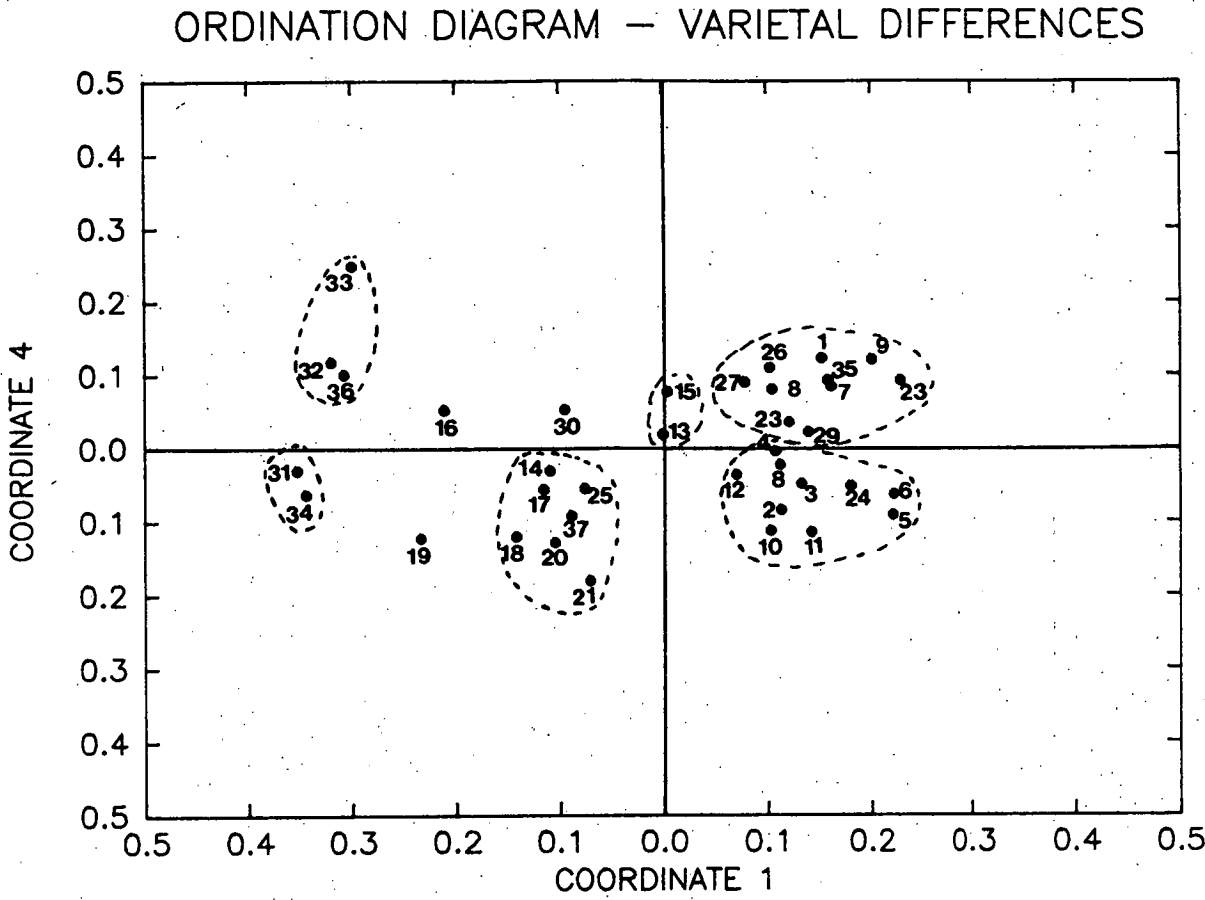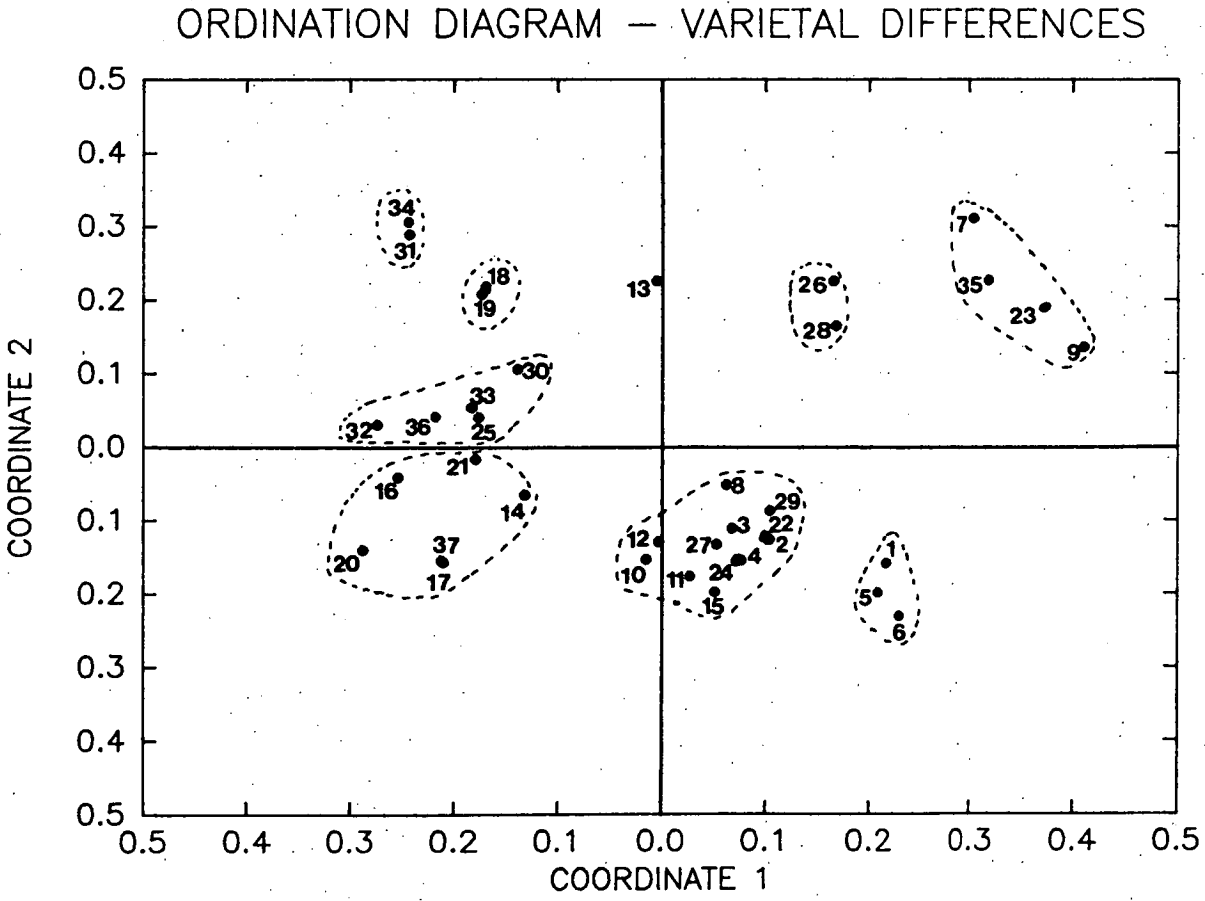
FIGURE 9.5.5

ORDINATION DIAGRAM — VARIETAL DIFFERENCES
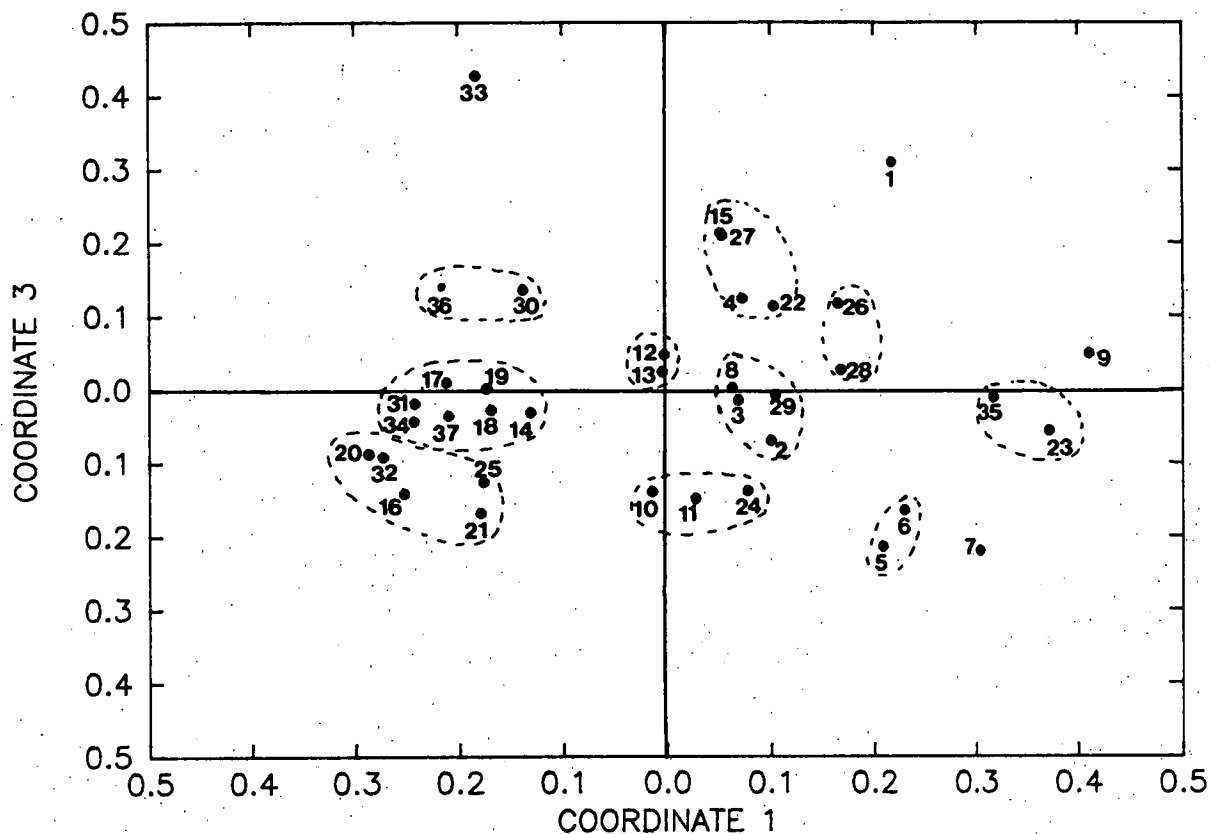
FIGURE 9.5.6

ORDINATION DIAGRAM — VARIETAL DIFFERENCES
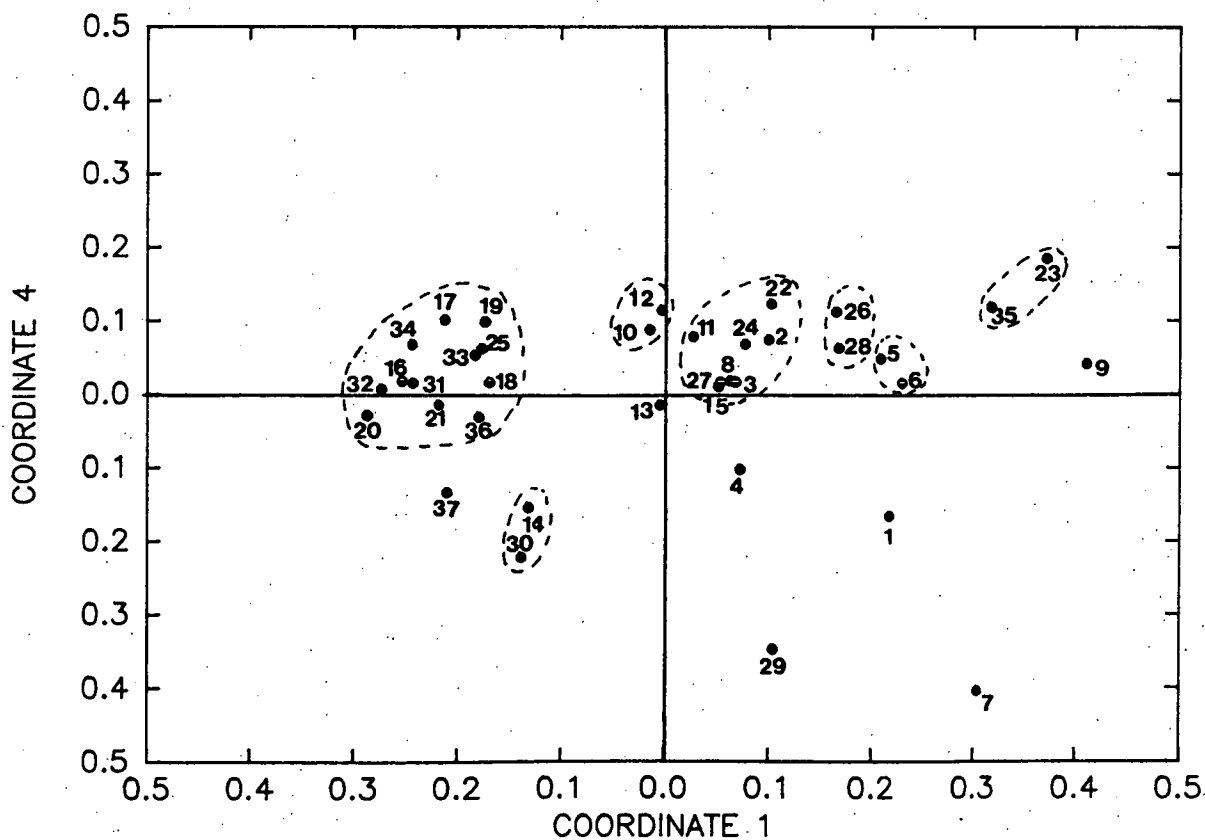
White Bud, Baldwin, Goliath and Kerry growing at the Horticultural Centre grouped together in 1980 (16,17,20,21, Figure 9.5.4), and again in 1983 (1,5,6), with the exception of Baldwin. However, in 1982 Baldwin was grouped with Grahams No. 1 White Bud and Goliath, and again with the Grahams in 1983. This pattern was repeated for the varieties White Bud, Goliath and Kerry grown at Grove Research Station (Figure 9.5.4). Similarly, Boskoop Giant and Lees Prolific are associated in 1982 and 1983 at both sites (Figures 9.5.4 to 9.5.6). The pattern of these associations on the axes demonstrate that the environmental variation had both a seasonal and a location component for all selections examined.

10. ECONOMIC ANALYSIS OF BLACKCURRANT BUD OIL PRODUCTION

A development budget has been prepared for essential oil production from blackcurrants in Tasmania. This budget was based on a number of price assumptions and actual costs associated with the operation of a blackcurrant fruit plantation, which are included in Appendix 12.

The investment situation is assumed to be a low capital investment, where only land and irrigation equipment are purchased outright. The establishment costs are those variable costs which are incurred during the first year of the blackcurrant crop. Annual operating costs are those variable costs incurred in each subsequent year.

An investment analysis approach (as opposed to one of gross margin) has been adopted because of the relatively large capital outlay involved and the perennial nature of the blackcurrant plant. From an investment viewpoint the problem is to see whether the returns from blackcurrants over an assumed number of years (in this case a planning horizon of ten years has been used) justify the initial capital costs.

A sensitivity analysis was undertaken to examine the effect of planting density and price on the profitability of both a manual and a machine harvested operation. A budget sheet has been prepared for both these situations ÷ Table 10.1.1 - manual harvest, and Table 10.1.2 - machine harvest. The sensitivity analysis first considered the effect of plant density on profitability of the enterprise, at a fixed market price of $1000 Australian per kilogram of concrete. This analysis, Table 10.1.3 and Figure 10.1.1, demonstrated that a low planting density (2.8 plants/m^2) with its consequential lower bud yield, provided a more than profitable return for the capital invested in the enterprise, than the planting density with the maximum achieveable yield (7.7 plants/m^2). This data strongly supports the conclusions of Saville (1983) that a realistic approach to experimental yield data is to convert the yield index (in this case bud yield) to an economic yield.

Following this conclusion, another sensitivity analysis was carried out to examine the effect of price on the internal rate of return at a planting density of 2.8 plants/m^2. As Table 10.1.4 demonstrates a price of $700 or greater provides an acceptable return for the machine harvested situation, whereas a price of greater than $900 is required in the hand harvested situation (using 15% as the decision-making value for investment to proceed).

TABLE 10.1.1. BLACKCURRANT DEVELOPMENT BUDGET - MANUAL HARVEST

AREA(HA)= 10

PRICE($/KG)= 1000

PLANTING DENSITY= 2.8(PLANTS/SQM)

YEAR	1	2	3	4	5	6	7	8	9	10
ACTUAL BUD YIELD(KG/HA)	74.36	125.06	226.46	338	338	338	338	338	338	338
HARVESTED BUD YIELD(KG/HA)	55.77	93.795	169.845	253.5	253.5	253.5	253.5	253.5	253.5	253.5
YIELD CONCRETE(KG) (4%/KG BUDS)	22.308	37.518	67.938	101.4	101.4	101.4	101.4	101.4	101.4	101.4
TOTAL REVENUE	22308	37518	67938	101400	101400	101400	101400	101400	101400	101400

CAPITAL COSTS

LAND	20000									
IRRIGATION	20000									
TOTAL CAPITAL COSTS	40000									

ESTABLISHMENT COSTS

LAND PREPARATION	1270
PLANTING MATERIAL	16800
PLANTING LABOUR	9240
SHELTER	2000
FENCING	1400
DRAINAGE	1000
FERTILIZERS	7630
WEED CONTROL	1300
DISEASE CONTROL	1240
TRACTOR OPERATING COSTS	1000
R&M EQUIPMENT	2700
IRRIGATION RUNNING COSTS	1000
TOOLS AND CONSUMABLES	1000
TOTAL ESTABLISHMENT COSTS	47580

OPERATING COSTS

	1	2	3	4	5	6	7	8	9
FERTILIZERS		7630	7630	7630	7630	7630	7630	7630	7630	7630
WEED CONTROL		1880	1880	1880	1880	1880	1880	1880	1880	1880
DISEASE CONTROL		1240	1240	1240	1240	1240	1240	1240	1240	1240
TRACTOR OPERATING COSTS		1000	1000	1000	1000	1000	1000	1000	1000	1000
R&M EQUIPMENT		2700	2700	2700	2700	2700	2700	2700	2780	2780
IRRIGATION RUNNING COSTS		1000	1000	1000	1000	1000	1000	1000	1000	1000
TOOLS & CONSUMABLES		3000	3000	3000	3000	3000	3000	3000	3000	3000
TOTAL OPERATING COSTS		18450	18450	18450	18450	18450	18450	18450	18530	18530

HARVEST & EXTRACTION COSTS

MANUAL HARVESTING COSTS	5148	8658	15678	23400	23400	23400	23400	23400	23400	23400
TRANSPORT & STORAGE	278.85	468.975	849.225	1267.5	1267.5	1267.5	1267.5	1267.5	1267.5	1267.5
EXTRACTION LABOUR	1784.64	3001.44	5435.04	8112	8112	8112	8112	8112	8112	8112
QUALITY CONTROL	223.08	375.18	679.38	1014	1014	1014	1014	1014	1014	1014
SOLVENT LOSS	267.696	450.216	815.256	1216.8	1216.8	1216.8	1216.8	1216.8	1216.8	1216.8
HIRE OF EXTRACTOR	646.932	1088.022	1970.202	2940.6	2940.6	2940.6	2940.6	2940.6	2940.6	2940.6
TOTAL EXTR/HARVEST COSTS	8349.198	14041.83	25427.10	37950.9	37950.9	37950.9	37950.9	37950.9	37950.9	37950.9

SALES OVERHEADS

PACKAGING & FREIGHT	669.24	1125.54	2038.14	3042	3042	3042	3042	3042	3042	3042
ADVERTISING	446.16	750.36	1358.76	2028	2028	2028	2028	2028	2028	2028
ADMINISTRATION	223.08	375.18	679.38	1014	1014	1014	1014	1014	1014	1014
R & D	446.16	750.36	1358.76	2028	2028	2028	2028	2028	2028	2028
AGENTS COMMISSION	1115.4	1875.9	3396.9	5070	5070	5070	5070	5070	5070	5070
TOTAL SALES COSTS	2900.04	4877.34	8831.94	13182	13182	13182	13182	13182	13182	13182

TOTAL OPERATING COSTS	98829.24	37369.17	52709.04	69582.9	69582.9	69582.9	69582.9	69582.9	69662.9	69662.9
CASH FLOW	-76521.2	148.827	15228.96	31817.1	31817.1	31817.1	31817.1	31817.1	31737.1	31737.1

IRR	.242
NET PRESENT VALUE	-18.6384

TABLE 10.1.2. BLACKCURRANT DEVELOPMENT BUDGET - MACHINE HARVEST

AREA(HA)=   10

PRICE($/KG)=   .1000

PLANTING DENSITY=   2.8(PLANTS/SQM)

YEAR	1	2	3	4	5	6	7	8	9	10
ACTUAL BUD YIELD(KG/HA)	74.36	125.06	226.46	338	338	338	338	338	338	338
HARVESTED BUD YIELD(KG/HA)	59.488	100.048	181.168	270.4	270.4	270.4	270.4	270.4	270.4	270.4
YIELD CONCRETE(KG) (4%/KG BUDS)	23.7952	40.0192	72.4672	108.16	108.16	108.16	108.16	108.16	108.16	108.16
TOTAL REVENUE	23795.2	40019.2	72467.2	108160	108160	108160	108160	108160	108160	108160

CAPITAL COSTS

LAND	20000									
IRRIGATION	20000									
MECHANICAL HARVESTER	30000									
TOTAL CAPITAL COSTS	70000									

ESTABLISHMENT COSTS

LAND PREPARATION	1270									
PLANTING MATERIAL	16800									
PLANTING LABOUR	9240									
SHELTER	2000									
FENCING	1400									
DRAINAGE	1000									
FERTILIZERS	5348									
WEED CONTROL	1300									
DISEASE CONTROL	1240									
TRACTOR OPERATING COSTS	1000									
R&M EQUIPMENT	2700									
IRRIGATION RUNNING COSTS	1000									
TOOLS AND CONSUMABLES	1000									
TOTAL ESTABLISHMENT COSTS	45298									

OPERATING COSTS

		2	3	4	5	6	7	8	9	10
FERTILIZERS		5348	5348	5348	5348	5348	5348	5348	5348	5348
WEED CONTROL		1880	1880	1880	1880	1880	1880	1880	1880	1880
DISEASE CONTROL		1240	1240	1240	1240	1240	1240	1240	1240	1240
TRACTOR OPERATING COSTS		1000	1000	1000	1000	1000	1000	1000	1000	1000
R&M EQUIPMENT		2700	2700	2700	2700	2700	2700	2700	2780	2780
IRRIGATION RUNNING COSTS		1000	1000	1000	1000	1000	1000	1000	1000	1000
TOOLS & CONSUMABLES		3000	3000	3000	3000	3000	3000	3000	3000	3000
TOTAL OPERATING COSTS		16168	16168	16168	16168	16168	16168	16168	16248	16248

HARVEST & EXTRACTION COSTS

	1	2	3	4	5	6	7	8	9	10
MACHINE HARVESTING COSTS	1448.761	1448.761	1448.761	1448.761	1448.761	1448.761	1448.761	1448.761	1448.761	1448.761
ROYALTY PAYMENT	278.3592	468.1496	847.7304	1265.269	1265.269	1265.269	1265.269	1265.269	1265.269	1265.269
TRANSPORT & STORAGE	371.8	625.3	1132.3	1690	1690	1690	1690	1690	1690	1690
EXTRACTION LABOUR	1070.784	1800.864	3261.024	4867.2	4867.2	4867.2	4867.2	4867.2	4867.2	4867.2
QUALITY CONTROL	237.952	400.192	724.672	1081.6	1081.6	1081.6	1081.6	1081.6	1081.6	1081.6
SOLVENT LOSS	118.976	200.096	362.336	540.8	540.8	540.8	540.8	540.8	540.8	540.8
HIRE OF EXTRACTOR	345.0304	580.2784	1050.774	1568.32	1568.32	1568.32	1568.32	1568.32	1568.32	1568.32
TOTAL EXTR/HARVEST COSTS	3871.662	5523.641	8827.597	12461.95	12461.95	12461.95	12461.95	12461.95	12461.95	12461.95

SALES OVERHEADS

	1	2	3	4	5	6	7	8	9	10
PACKAGING & FREIGHT	713.856	1200.576	2174.016	3244.8	3244.8	3244.8	3244.8	3244.8	3244.8	3244.8
ADVERTISING	475.904	800.384	1449.344	2163.2	2163.2	2163.2	2163.2	2163.2	2163.2	2163.2
ADMINISTRATION	237.952	400.192	724.672	1081.6	1081.6	1081.6	1081.6	1081.6	1081.6	1081.6
R & D	475.904	800.384	1449.344	2163.2	2163.2	2163.2	2163.2	2163.2	2163.2	2163.2
AGENTS COMMISSION	1189.76	2000.96	3623.36	5408	5408	5408	5408	5408	5408	5408
TOTAL SALES COSTS	3093.376	5202.496	9420.736	14060.8	14060.8	14060.8	14060.8	14060.8	14060.8	14060.8
TOTAL OPERATING COSTS	122263.0	26894.14	34416.33	42690.75	42690.75	42690.75	42690.75	42690.75	42770.75	42770.75
CASH FLOW	-98467.8	13125.06	38050.87	65469.25	65469.25	65469.25	65469.25	65469.25	65389.25	65389.25

IRR   .421
NET PRESENT VALUE   35.38138

TABLE 10.1.3 Sensitivity analysis for planting density

Planting Density (plants/m^2)	Bud Yield (kg/ha)	Internal Rate of Return of Cash Flows[*] Manual Harvest	Machine Harvest
1.0	206	3.6	27.3
2.0	298	20.1	39.9
2.8	338	24.2	42.1
4.0	336	20.4	35.1
4.8	343	19.4	32.5
5.6	384	22.8	34.9
6.7	391	21.1	31.4
7.7	461	26.7	36.4
9.1	335	10.5	16.3
11.1	383	12.8	17.0

[*] Price = $1000/kg

TABLE 10.1.4 Sensitivity analysis for price

Price ($/kg)	Internal Rate of Return of Cash Flows[*] Manual Harvest	Machine Harvest
500	-	2.2
600	-	12.1
700	-16.8	20.5
800	2.0	28.1
900	14.1	35.2
1000	24.2	42.1
1100	33.3	48.8
1200	42.0	55.5
1300	50.5	62.2

[*] at a planting density of 2.8 plants/m^2

FIGURE 10.1.1

THE RELATIONSHIP OF PLANTING DENSITY AND INTERNAL RATE OF RETURN
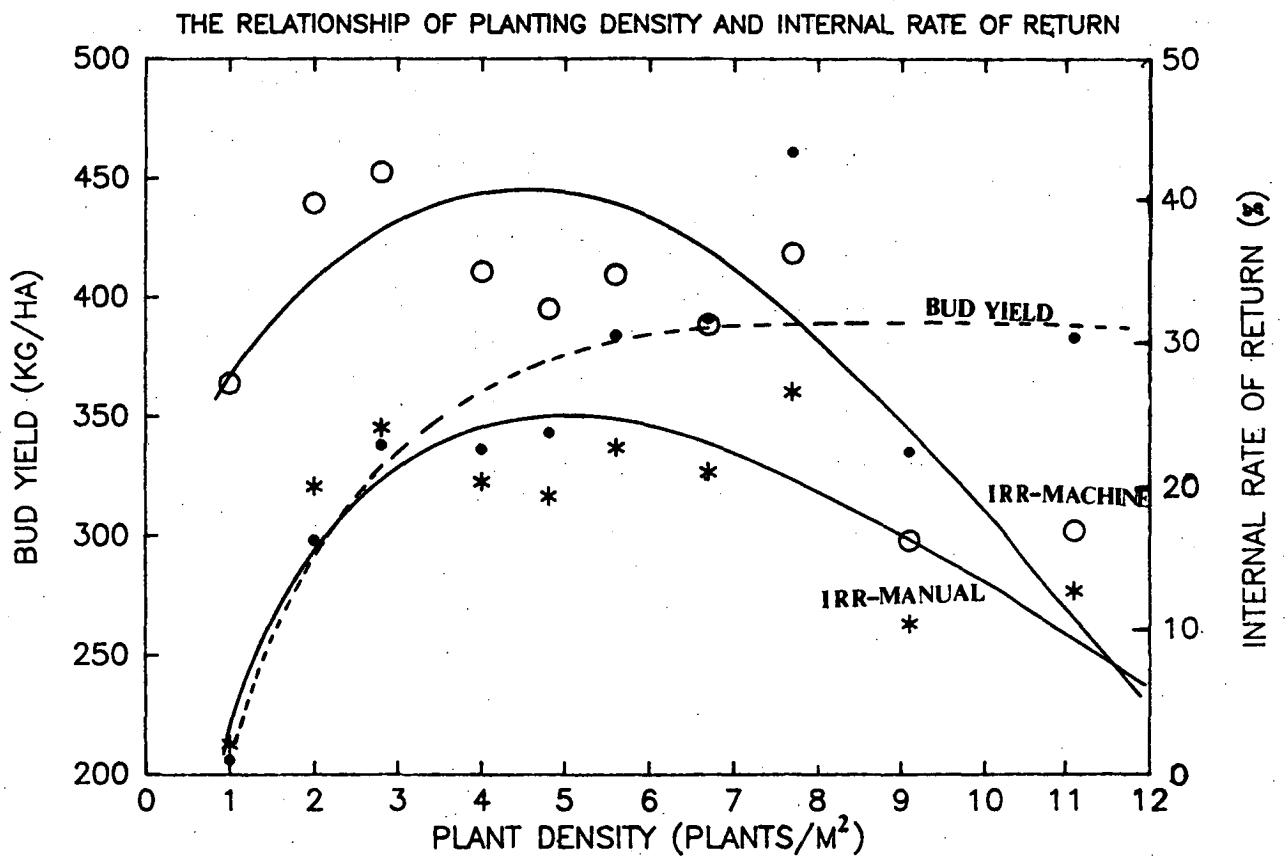
CHAPTER V
GENERAL DISCUSSION

GENERAL DISCUSSION

The following discussion relates the various aspects of the research programme in a logical sequence from production techniques through to product extraction and evaluation.

Plant Density and Other Factors Influencing Growth

In obtaining the data describing the form of the yield-density response, a systematic fan design was employed (Nelder 1962, Bleasdale 1967b). The advantage of these designs over conventional randomised complete block designs, are that they require fewer plants and a smaller area to adequately cover the wide range of planting densities examined, while retaining statistically valid comparisons.

Since the form of the yield-density response is known to vary (Frappell 1979), it is important to define this accurately; to relate field plantings to the yield of the economically important variable (in this case bud yield). Other variables important in this present study, for various cultural reasons, are those which give a measure of plant vigour - basal cane girth and number of shoots per plant. It is important to maintain a healthy balance between plant vigour and bud yield under the proposed cultural regimen of annually harvesting all the available cane material. Since such methods could potentially subject the blackcurrant plant to physiological stress and reduce bud yields in subsequent years.

With these considerations in mind, the statistical analysis was applied to determine the order of the polynomial which best described the relationship of each variable with plant density. Further, it was necessary to determine the linearity of the relationship between the reciprocal of bud yield and plant density. Since, as described by Frappell (1979), asymptotic relationships are based on a linear relation between these two variables.

As this relation proved to be linear, the final step of analysis was to select an appropriate yield-density model to define the response form. Ratkowsky (1983) had undertaken an extensive study on the statistical properties of various models proposed by Holliday (1960), Bleasdale-Nelder (1960) and Farazdaghi-Harris (1968); the former being the preferred equation. On the basis of his study, the Holliday model was fitted to the data set.

The asymptotic relationship demonstrated between bud yield and plant density is consistent with many other reports in the literature describing similar relationships for plant parts of economic interest (Bleasdale 1967a, Nichols et al. 1973, Frappell 1973 and Nes 1979). Some authors, for example Frappell (1979), often consider that the parameters of the yield-density model, α and β, as a measure of the genetic and environmental potential of the crop, respectively. The magnitudes of the parameter estimates and their standard errors presented here demonstrate that both α and β varied from year to year. Obviously, other factors are operating in this perennial crop which prevented α, in particular, from remaining constant; as it often does for annual crops of the same variety (Ratkowsky 1983). Hence to accept α and β as straightforward measures of genetic and environmental potential is an over-simplification and suggests a need to consider the genetic potential of a crop in terms of a community of plants and not

as individuals spaced apart.

Quadratic relationships with planting density were demonstrated to exist for both total cane fresh weight and shoot numbers per plant. These relationships indicate that the size of individual plants was decreased at higher planting densities due to increased competition between plants for available light and nutritional resources. However, the length of canes produced by these plants was reduced at both high and low planting densities. At high densities this growth depression can be readily attributed to the competition between neighbouring plants for a share of scarce resources. Other authors (Hughes 1971, Nes 1979) have demonstrated similar responses for blackcurrant plants grown at high density.

The reduction in cane length at low planting densities is confusing, unless one accepts the premise that the available light is excess to requirements for production of the leaf canopy. At low planting densities a discontinuous canopy exists and light penetration, as supported by the percent transmittance measurements, is intensified within each bush. Hence, with more available light closer to the ground there is less competition for light so a reduced phototropic response is observed. The canes produced were therefore shorter and probably thicker (since resources are not yield limiting as is the case at higher plant densities).

This premise was confirmed by measurements of basal cane girth in 1983, which demonstrated thicker shoots at low densities. The proof of a linear relationship between girth and both yield factors measured (bud weight and total fresh weight) confirms other work on apples (Moore 1978) which had shown that girth was a good estimator of plant productivity.

Although the canopy is very efficient in converting intercepted radiation into a productive yield, there was a decline in yield per

intercepted solar unit at the highest planting densities. This

decrease in conversion efficiency at high planting densities suggests

an hypothesis, that the conversion of solar energy inputs to productive

yield is dependent upon both the efficiency of photosynthesis and

the size of the leaf canopy. At high planting densities low light

conditions are more prevalent beneath the canopy surface. Now shade

leaves have lower photosynthetic efficiency rates than sun leaves,

due to the intensity of light to which they are exposed. Hence, shade

leaves at high planting densities will contribute less net photosynthate

than those at low densities, since light penetration is greater at the

low densities and therefore the net photosynthetic rate will be higher.

Further to this argument, the actual size of the leaf canopy (both sun

and shade leaves) would have an obvious effect on the efficiency of solar

energy conversion to yield.

In this current study, light resources have been shown to have a

major influence on shoot quality in blackcurrants. Nes (1979) con-

firmed that a reduction in blackcurrant shoot quality occurs at high

planting densities. In addition, other workers (Kranatz (1971) cited

in Nes (1979); Jackson and Palmer (1977)) have similarly proven that

light intensity is the causative factor in determining shoot quality for

blackcurrants and apples, respectively.

For blackcurrants this study established that all planting densities

suffer from inefficient light interception early in the season. This

conclusion is confirmed by other work carried out on annuals (Sceicz

1974, Sibma 1977) and orchard crops (Jackson 1980a). The development

of the canopy is initially slow due to dependence on carbohydrate

reserves (Wilson[*] pers. comm.), but once leaves are established they

rapidly export photosynthate for further growth. Two canopy types are

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[*] S.J. Wilson, Department of Agriculture, New Town, Tasmania

distinguishable over the range of planting densities examined. At high densities a continuous canopy exists consisting of erect bushes with a shallow layer of leaves at the cane tips; while at low densities there is a discontinuous canopy with clumped bushes covered in leaves.

The high density plantings are more efficient in terms of light interception, since long before they reach maturity, the plants have established a complete ground cover. The canopy is very uniform and intercepts light efficiently regardless of sun angles without any shading effects. The latter two points are significant factors influencing the efficiency of available light usage at low densities. The bimodal distribution of the relationship between percent ground cover and fractional transmitted light confirms the presence of the two canopy types. The difference between the two canopies is borne out by the percent transmittance data, which demonstrates an increased amount of variation observed for readings in the discontinuous, as opposed to the continuous canopy.

A comparison of the efficiency of solar energy conversion by the two canopies is possible from examination of the yields expressed as per solar energy input. At low planting densities both bud weight and cane fresh weight yields are depressed, which is consistent with a discontinuous canopy; since associated shading effects and less effective light interception will reduce conversion efficiency. At high planting densities yields per solar input rise to a slight peak at 7.7 plants/m^2, before declining. Overall there are higher yields per solar input, which is consistent with the more effective utilization of intercepted light by a continuous canopy. The decrease observed at the highest densities would be due to the increased competition for scarce resources between neighbouring plants; indicating that light in particular has become a limiting resource. The peak observed indicates the planting density (7.7 plants/m^2) enabling the most efficient

conversion of intercepted solar energy to yield. The continuous
canopy at this density meets the criteria laid down by Jackson (1980b)
for the ideal canopy. In particular, it reaches maximum size quickly,
before the incident radiation reaches its summer peak, as well as
being easy to maintain at maturity.

The caution concerning this peak previously outlined does not
now appear to be warranted. The fit of the asymptotic model suggested
this peak was anomalous. However, the evidence presented for the two
canopy hypothesis gives a sound physiological basis to this peak yield.
Further investigation is therefore required to examine more densities
surrounding this peak to ascertain its validity.

Regarding cultural management of a commercial venture the experi-
ment data establishes that a planting density of 7 to 8 plants/m² is
appropriate. At this density the highest yield of bud material is
obtainable on a per hectare basis, under a continuous canopy. This
canopy type is undeniably the most efficient, and has the added advantage
of being weed-free during the growing season; as well as meeting Jackson's
criteria for the ideal orchard canopy.

Observation of Oil Accumulation

The most rapid increase in gland size was determined to occur
during late November and early December, just prior to the normal fruit
harvesting period. This increase occurs at a time of rapid leaf growth,
corresponding to the period when the blackcurrant bushes reach maximum
canopy cover. In addition, analysis of total oil concentrations per
bud revealed that the period of most rapid oil accumulation was early
to mid January. These two observations are linked, gland size increas-
ing as photosynthate is made available from the expanding photosynthetic
surface. However, although the structural features are present before

January, the rate of oil synthesis does not increase rapidly until

photosynthate can be redirected from leaf growth into secondary

metabolism, particularly oil synthesis.

Air temperature was shown to be an important factor controlling

the rate of photosynthetic activity in blackcurrants; and effect which

had been previously demonstrated for other essential oil crops, e.g.

peppermint (Clark and Menary 1980a). Using these findings, the

plots of mean monthly maximum and minimum temperatures indicate that

the net photosynthetic rate would be reduced in both spring and autumn

months. Further, the plot of average daily incident solar energy

reveals a decline in available solar energy for photosynthesis during

the autumn period.

These conclusions concerning the amount of available photosynthate

within the plant, are in agreement with observations on the rate of oil

accumulation. At times of lower net photosynthetic gains (spring and

autumn), the rate of oil accumulation is slow. During the spring other

sink demands, particularly for leaf and shoot growth, have a marked

affect on reducing the amount of photosynthate available for oil syn-

thesis. However, once the canopy has reached its maximum, then photo-

synthate is readily available for oil synthesis. Particularly, since

environmental conditions at this time are very favourable for maintenance

of high photosynthate levels.

An attempt was made to identify the period of oil synthesis, using

a Carbon-14 tracing technique. This experiment produced highly variable

data, which did not enable any interpretation about oil synthesis. The

major difficulty with the experiment was exposing the whole plant to

sufficient C^{14} labelled carbon dioxide to obtain reproducible results

in bud samples taken from the plant. Any attempt to use similar

methodology would require the use of larger amounts of C^{14} labelled

carbon dioxide. Further, five to ten buds per cane should be taken

as a sample, pooled, and used as a replicate against the other canes
on the bush.

Effect of Bud Burst on Oil Quality

Under both glasshouse and field conditions the strength of the
catty note was proven to increase as the buds break from dormancy.
This fact, previously unreported in the literature, indicates that a
number of important oil compositional changes are occurring at this
time.   Although, the particular compounds responsible for the complex
blackcurrant bud aroma were not identified, there is a considerable
amount of evidence in the data for oil biosynthetic activity during
this period.

For example, the monoterpenes delta-3-carene and alpha terpinolene
decreased and increased, respectively, over the harvest period suggest-
ing that since both compounds are thought to derive from the same
precursor (Charlewood and Banthorpe 1978), delta-3-carene is an inter-
mediate for other products.   Gamma terpinene and alpha thujene, which
also have a common precursor (Loomis and Croteau 1980), rise to a peak
level and then decline; indicating further interconversions are taking
place.   Finally alpha terpineol, which is considered to be a precursor
of limonene (Manitto 1981), was observed to decrease as the levels of
limonene rose, thereby supporting this proposal.   Limonene levels then
fall indicating further conversion takes place, while alpha terpineol
levels rose indicating an increased availability of photosynthate for
oil synthesis.

The levels of alpha and beta pinene both decline until about
sixty percent of the buds have burst.   This suggests that, since these
components are formed by interconversion from the three alcohols,
geraniol, nerol and linalool (Manitto 1981); the availability of photo-

synthate for oil synthesis is limited until the buds burst. After bud burst all the plant resources are being channelled into preparatory spring growth, but as the leaves expand they rapidly commence photosynthesis (Wilson[*] pers. comm.) and there is an increase in photosynthate available for biosynthetic changes in oil composition.

Wright (1975) has shown that total abscisic acid levels, which had remained fairly steady after an autumn peak, decline sharply at bud burst.   His study also revealed that the inner bud is the major site of abscisic acid accumulation during dormancy.   Hence, it seems possible that the decline in abscisic acid levels is related to the increase in oil synthesis noted at bud burst.   In addition, although the substitution pattern of the cycohexyl ring of abscisic acid suggests an origin from carotenes, recent experimental results show that abscisic acid is biosynthesised from farnesyl pyrophosphate and exclude the degradation of an intermediate carotenoid (Manitto 1981).   Importantly, farnesyl pyrophosphate is now considered to be the precursor of the sesquiterpenes (Loomis and Croteau 1980, Manitto 1981).   This biosynthetic linkage between abscisic acid and the sesquiterpenes, raises the question as to the effect of changes in abscisic acid levels on sesquiterpene biosynthesis and vice versa.   While no data has been presented in this study to support this assertion, the control abscisic acid exercises over the buds' release from dormancy indicates that this relationship warrants investigation.

Varietal Differences

A close association between oils extracted from selections belonging firstly to the Goliath and Baldwin groups, and secondly to the Boskoop and French groups, has been revealed.   Importantly, this analysis

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[*] S.J. Wilson, Department of Agriculture, New Town, Tasmania.

of varietal oils confirms the relationships Todd (1962) proposed in

his key for identification, based solely on the phenotypic features

of each selection.

The French workers, Latrasse and Lantin (1974) in their first

study also grouped Goliath and Baldwin together. However, later

these authors (1976 and 1977) proposed that a number of monoterpene

and sesquiterpene phenotypes could be distinguished. This present

study provides evidence from a number of closely related varietal

selections disagreeing with the basis on which these phenotypes were

declared distinct. For example, Baldwin under the French workers'

scheme was classified as a BIV phenotype, yet the relative proportions

of sabinene, delta-3-carene and terpinolene are very different in this

present study. Further, alpha humulene, which is the second most

important sesquiterpene constituting Baldwin oils in the current study,

is not considered important by the French workers for this phenotype.

Several factors could contribute to these differences, for example,

the climate and/or extraction method employed. However, the major

contributing factor is probably the advances in combined gas chromato-

graphy/mass spectrometry instrumentation, particularly, the improved

resolving powers of capillary versus packed columns. These advances

have allowed for more accurate separation and identification of component

peaks. Differences resulting from climatic influence or differences

in varietal strains examined cannot be ruled out; as Latrasse et al.

(1982) in recent work suggested that terpinen-4-ol was a discrimatory

feature of each cultivar. A conclusion which is not supported by the

present work. These workers noted that terpinen-4-ol levels were

higher for Baldwin than Boskoop Giant, and that these high levels could

be traced in offspring from Baldwin crosses. This current work however

revealed that terpinen-4-ol levels were of the same magnitude in all

the selections examined.

Importantly, this study showed that most of the varieties grown widely in Tasmania are closely related to the main English variety Baldwin. Any suspicion that the main local variety, White Bud, was not of Baldwin parentage is removed supporting Wilson's claim (Wilson and Jones 1980) that White Bud is a local selection of Baldwin. From a commercial viewpoint, organoleptic assessment of extracts from White Bud selections revealed a preference for these over all other varieties. A comparison between the White Bud and the two French varieties preferred to Baldwin - Noir de Bourgogne and Royal de Naples (Latrasse 1974; Latrasse et al. 1982), has not been possible in this laboratory. However, commercial sources consider the Tasmanian product to be equal to the best French extracts.

Harvesting Methods

Manual harvesting of bud material from prunings was time-consuming and yielded only a low monetary return per kilogram of harvested buds, as previously reported in the literature (Thomas 1979). If first year wood is used, as a source of bud material, higher bud picking efficiencies are obtained if the buds are picked directly from the bushes, than by cutting and collecting the canes before hand picking the buds. Even though at present the economic analysis indicates this is a profitable situation, higher labour costs and the difficulty of obtaining sufficient labour in the future will increase the pressure for new harvesting techniques. Of the alternative harvesting methods, one chemical and the other mechanical, available, the latter has considerable potential.

The chemically based technique involved application of the growth regulator, Ethephon, which is widely used to aid harvesting of fruit crops (Edgerton and Hatch 1972, Martin et al. 1972, Lavee and Martin

1974).   This growth regulator proved successful, with factors such as storage temperature, length of storage and applied Ethrel concentration determined to be important in controlling the effectiveness of bud removal from the treated canes.

Storage temperatures of 10°C and above were determined to increase bud removal at all Ethrel concentrations examined, which is in agreement with the results of Olien and Bukovac (1978). These workers had been able to demonstrate the temperature dependence of ethylene evolution from Ethephon *in vitro*.   High temperatures have subsequently been reported (Klien *et al.* 1979) to cause more rapid breakdown of Ethephon.   This trend is not clear from the present study, although there is some evidence from the first harvest period to support this proposal.

Ethephon has been successfully used to aid the harvest of black-currant berries (Zandke 1977, Pankova *et al.* 1979, Sande 1980); however, at concentrations tenfold lower than was necessary to remove buds in the current study.   These differences are most likely due to restricted uptake of Ethephon due to cuticular waxes on mature tissues (as reported by Nir and Lavee (1981) for grapes) and the larger surface area of the bud pedicel as compared to that of the berry.   In order to improve the rate of Ethephon uptake further investigation of chemicals which could damage or remove the cuticular waxes needs to be carried out.

All Ethephon sprays contained added urea because of its reported biological effect (De Wilde 1971) in increasing the rapidity of leaf abcission in deciduous trees. The application of only urea, in the current study, was no more effective in aiding bud removal than the unsprayed control; proving that the reported effect is not due to a direct urea effect on abcission.   In addition, other workers have reported that this effect is not due to an increase in ethylene production per se (Biddle *et al.* 1978), suggesting that urea has an effect

on the rate of uptake of Ethephon.   This point has been clarified by

two studies (Poovaiah and Leopold 1976, Poovaiah 1979) which demonstrated

that NH_4^+ increases the permeability of root and leaf tissues and thus

urea acts to increase the rate of Ethephon uptake.

Despite the demonstrated effectiveness of Ethephon in aiding bud

removal, any operation involving chemical removal of buds still requires

high labour inputs.   For example, labour is required to spray the canes,

cut and collect them, and then to harvest the buds from these canes.

Although mechanisation can improve the efficiency of the operation,

chemical harvesting certainly requires more labour inputs than was

initially envisaged.   It was this requirement to reduce the labour

content of harvesting that led to the evolutionary development of a

once-over, mechanical bud harvester.

The design of the picking rollers required much attention, especially

in relation to feeding the canes tip or base first.   Tip first would be

preferable, since it is relatively easy with a standard cutter bar and

header comb to gather canes in this manner.   However, it is not possible

to feed canes tip first and still produce good quality bud material.

It is envisaged to use a chain arrangement which would put the tips

into a pair of rollers and cut the canes at the base.   Next the canes

are reversed on a belt arrangement before they enter the picking rollers

base first.   The buds would be collected in a hopper underneath the

two sets of picking rollers or picked up by a cyclone and passed to a

storage vat.   The stripped canes would pass to a shredder at the rear

of the harvester and be returned to the paddock as mulch.

The picking rollers are based on the simple premise that the

direction of the force required to remove buds is the most important

factor controlling the design of these rollers.   The transition from

rubber protruberances to nylon brushes was necessary to discover the

most effective way of applying such a force. These rollers need some minor modifications to improve efficiency, i.e. slightly shorter and stiffer nylon bristles with two less brushes per roller. The former to provide a stronger picking force and the latter to enable more effective intermeshing, which would also include a timing gear arrangement. In addition, more durable materials are required for the feeding rollers. Setting up a transverse movement on one of the infeed rollers relative to the other would turn the shoot through 180° and achieve complete exposure of all buds on the cane to the picking rollers.

Regarding the organoleptic quality of machine harvested materials, assessment by two commercial users and this laboratory showed that a handpicked product was preferable. However, the improvements discussed in harvester design will result in less damage to bud material, which should improve the product. Likewise, the development of a single-pass field harvester will mean that buds are picked on site, without the time now involved in cutting, transporting and picking. This improvement will decrease the storage time of buds at undesirable temperatures, resulting in less oxidation and reduced loss of important volatiles.

Component Separation and Identification

In this present study a variety of liquid chromatography techniques were examined in order to isolate and hopefully, identify the catty note of blackcurrants. Despite that silica gel was confirmed to be effective in separating hydrocarbons from oxygenated compounds, as shown earlier (Scheffer et al. 1975, 1976b and 1981), the catty note was not eluted. Since none of the fractions possessed this catty aroma, it appears that the precautions taken to deactivate and neutralize the silica gel were not sufficient to ensure this compound's stability.

The compound responsible for this catty aroma, it is argued is therefore very labile and readily undergoes chemical rearrangement.

An alternative hypothesis exists, that the catty note is the result of two or more compounds which have separated into different fractions, hence the loss of aroma is readily explained. There is a lack of confirmational evidence for this proposal, and indeed the study provides circumstantial evidence suggesting the involvement of only a single compound.

Florisil which has been preferred to silica gel (Ayling 1976) for difficult separations of terpene constituents, was also unable to elute the catty note, thereby supporting the contention that this aroma compound is extremely labile. Although other workers (Ayling 1976 and Scheffer et al. 1976a) suggest that isomerization processes can be avoided by using purification and deactivation procedures, this current work demonstrates such is not the case when the catty is in contact with polar absorbants.

The failure to achieve elution of the catty note from a polar absorbant suggested the need to attempt a reversed phase separation. Such separations are frequently employed using High Pressure Liquid Chromatography (HPLC). These techniques are considered (Jones et al. 1979) to considerably speed up any necessary prefractionation required for optimal separation of complex mixtures, having varied functionality, prior to gas chromatography.

An effective HPLC method for prefractionation of monoterpene and sesquiterpene hydrocarbons from the oxygenated compounds was developed confirming the results of Kubeczka (1981). This method enabled the catty aroma to pass through the column unchanged in one small fraction; suggesting that the catty note is a single component that undergoes some chemical change on polar absorbants. It is important to realize that the polarity system was reversed with a non-polar absorbant

(Bondpak C18) and a highly polar solvent (methanol/water), as opposed to the previous polar absorbant (silica gel) and non-polar solvent (hexane).

Most communications in the past have reported on HPLC methods for compounds which are ultra violet (UV) detectable above 240 nm, e.g. Komae and Hayashi (1975), Ross (1976, 1978). This study and other recent work, e.g. Strack *et al*. (1980), Schwanbeck and Kubeczka (1979), demonstrate the applicability of HPLC in the lower wavelengths 200-220 nm for terpene separations. Indeed the UV absorption trace for the vacuum distilled oil illustrates the pattern of absorption for the terpene fraction at these low wavelengths, and their suitability detection. To some extent the availability of HPLC solvents with improved transparency to UV has ensured the wider application of lower detection wavelengths.

Gas chromatography proved to be an efficient, reliable tool for analysis of compositional changes in various oil samples. The siliceous glass capillary columns provided good resolution, and were able to pass the catty note without alteration, whereas packed columns have been noted (e.g. Jennings 1981b) for their failure to pass nitrogen or sulphur containing compounds unchanged. The fused silica columns which provide increased resolving power were considered so successful that they became the column of choice for any gas chromatography associated with component identification, in this current work.

Effluent trapping of gas chromatographic samples was not found to be useful, other than as a confirmatory technique for components identified by other separatory procedures. This was due to two factors; firstly, the resolving power of the glass capillary column and secondly, the nature of the peaks of real interest. The complexity of the black-currant aroma, determined that minor peaks in the chromatogram were of

greatest interest. The resolving power of the glass column meant that some of these peaks were not separated adequately from major components. Alternatively, the inability to load the column with samples large enough to enhance the peaks of interest sufficiently, restricted the usefulness of trapping procedures. Prefractionation procedures, particularly by HPLC, improved this situation markedly; however, at this stage the combination of a fused silica column and the fast scan capabilities of the mass spectrometer made the trapping requirement redundant.

Headspace analysis was a useful technique in separating and identifying a number of early eluting components. These peaks were never seen in routine gas chromatographic analysis of blackcurrant concretes due to the presence of residual solvent peaks. There is an extensive literature, some of which was reviewed earlier (Section II 4.6), which supports the results obtained in this study confirming the ability of headspace analysis to reliably reproduce the natural aroma. Importantly, the presence of these early components was confirmed by combined gas chromatography/mass spectrometry analysis of the liquid carbon dioxide extract; demonstrating the superiority of this extract in retaining the true natural aroma, free from solvent contamination.

Utilizing all these techniques, most of the compounds previously detected (refer to Section II 2.3) in blackcurrant bud oil were identified in the current work. However, the following components - delta-cadinene, citronellol, ethyl oleate, methyl palmitate - reported by Williams (1972), and, sabinol and geraniol, reported by Glichitch and Igolen (1937) have not been identified in Tasmanian extracts.

Wide differences are reported in the literature concerning the relative percentages of components in blackcurrant buds. For example, Fridman et al. (1971) reported limonene (23.91%) as the most abundant component, whereas Latrasse (1968, 1969) noted that myrcene (34%) and

caryophyllene (21.2%) were present in larger amounts than limonene (10.9%). Likewise, Williams (1972), in extracts from mixed cultivars, recorded that limonene (0.8%) was only of secondary importance to the major compounds, delta-3-carene (15%), beta-pinene (24%) and terpinolene (9%). Whereas, in this current study, sabinene (15.44%), delta-3-carene (12.65%), alpha terpinolene (11.63%) and beta caryophyllene (12.39%) were recognized as major components. Further, limonene (3.25%), beta pinene (0.71%) and myrcene (2.81%) were of lesser importance in the extracts studied.

Part of this present work, which has already been discussed in detail, suggests that the major reason for such conflicting results, is of genetic origin. This hypothesis has been supported by Williams (1972), Latrasse and Lantin (1974, 1976 and 1977), as well as Latrasse, Rigaud and Sarris (1982). In addition, the amount of oxidation that takes place during extraction or storage may also account for some of the reported compositional differences. While no evidence has been presented here to support this premise, it is known that monoterpenes in blackcurrant bud oils readily oxidise on exposure to air (Latrasse and Demaizieres 1971). Likewise, Williams (1972) determined that estimates of limonene were found to vary with the degree of oxidation that occurred during the extraction process.

This attempt to relate odours to compounds eluting from the gas chromatography column revealed that the blackcurrant bud aroma is complex, with five regions of major interest. Aroma regions 3 to 5 possess blackcurrant fruit aromas and are, most likely, the cause of Andersson and von Sydow's claim (1966b) that the characteristic blackcurrant note was localized in the high boiling point fraction. Similarly, Williams (1972) associated the heavy sweet smell of commercial blackcurrant flavours with the high boiling point region.

The catty aroma was not identified by Williams (1972), but he suggested that peaks with green or cucumber aromas could contribute to this catty note. In addition, he reported difficulty in eluting the catty note from a packed Carbowax 20 M column. No such difficulty was encountered using a capillary OV 101 column in this study. Improvements in column resolution and the use of a non-polar phase are the most likely reasons for this result. Likewise, Latrasse, Rigaud and Sarris (1982) reported that the catty note passed through three columns of differing polarity; SF96, Carbowax 20 M and Pluronic L64; supporting the contention concerning improvements in column technology.

Various sulphur-containing compounds with similar odours have been suggested as possibilities for the 'catty' constituent. For example, both von Sundt *et al.* (1971) and Kaiser *et al.* (1975) associated a catty note with (+) menthon-8-thiol in Buchu oil, while the former also presented a synthesis based on pulegone. This compound was not detected in the current work although pulegone and related components menthone and cis-p-menth-2-ene 1,8 diol were detected. Indeed, despite the use of prefractionation techniques and a sulphur-specific gas chromatographic detector, no sulphur-containing compound was elucidated.

Lewis *et al.* (1980) confirmed the presence of pulegone and a compound of molecular weight 186 with similar mass spectral and gas chromatographic characteristics as p-menthon-8-thiol; but made no mention of the aroma associated with the latter compound. Many components utilized for a synthetic catty note have the same structural elements, $-C(CH_3)_2-SH$, as the keto thiol in Buchu (e.g. Pickenhagen and Demole (1983), Stoffelsma and Pijpker (1973) cited in Latrasse *et al.* (1982)). It is evident that the responsible component is present only in a very small amount, has a very low odour threshold, and while the probability that it contains sulphur remains, its identity

is still unknown.

In the most recent paper on blackcurrant fruit aromas, Latrasse et al. (1982) report that methyl and ethyl butyrates, 1,8-cineole, diacetyl and a catty unknown are important aroma constituents. None of these four named components were identified in this study of blackcurrant bud oils. Although it is possible some may be among the fifty-seven unknowns recognized in this study, since identification was hampered by deficiencies in our data base. Of course there are also distinct differences between the fruit and bud aromas, which might also account for the failure to identify these components in the bud oil.

Extraction Procedures

The examination of various extraction solvents was aimed at achieving the traditional quality associated with the French products, without using benzene, because of its carcinogenic properties. A wide polarity range of solvent mixtures was used to obtain extracts, with different aroma characteristics. Organoleptic comparison of these extracts revealed those extracted with petroleum ether were considered most like the French products. Petroleum ether extracts were considered superior to those extracted with pentane or hexane alone. This work suggests that the relative percentages of pentane, hexane and heptane in the petroleum ether fraction have a determining effect on extract quality; a factor that requires further examination in this non-polar solvent system.

Some samples were first extracted with the polar solvent methanol, followed by a non-polar solvent (e.g. n-hexane, petroleum ether). All these products were considered to be inferior to that produced when the extraction was carried out using only the particular non-polar solvent. These inferior products are enriched in the sesquiterpene

fraction when compared to the other samples. The unacceptable quality of such extracts may be due to a reduced proportion of important lower boiling point aroma volatiles (identified as regions 1-3). Further, the vacuum distillate produced from the petroleum ether extract, was not enriched in sesquiterpenes, but contains a very powerful catty aroma. This finding was opposite to that reported by Tucknott and Williams (1971), who considered methanol/pentane extracts were superior to those produced with pentane alone. This conflict strongly suggests that the criteria used for selection of acceptable extracts in the two studies were different; since the sensory assessor in this study was calibrated to test his ability to determine appropriate aroma differences (Appendix 14).

The fact that phellandrenes and cymenes can be easily formed by rearrangement but delta-3-carene and beta-caryophyllene could not, suggests that the extraction methods used in this present work (Section IV 2.1) cause few unwanted chemical changes since the latter components are present in greater abundance.

Although the petroleum ether extract was most like the French product, there still are noticeable differences between the two products, some of which are due to factors, other than extraction method, such as climate and variety. However, the liquid carbon dioxide extracts produced were considered superior to all other products, exciting the marketplace as they retain a freshness and strength unmatched by any other sample. The quality of this product supports claims (Reineccius and Anandaraman 1981, Clarke 1983) of the superiority of liquid carbon dioxide to retain the true nature of any aroma. In addition, the fact that such extracts are free of solvent contamination, adds much to their universal appeal to perfumers and flavourists alike.

Economic Aspects

Blackcurrant (Cassis) absolute has been marketed for about
twenty years, and despite the high price demand is growing strongly
(Thomas 1979). Therefore, provided the price can be maintained in
real terms, Tasmanian extracts should be competitive on the world
market; as the quality of Tasmanian extracts has already been proven
(Section IV 5). Presently, buds are obtained from prunings of the
fruit plantations of France and England. This method was demonstrated
to be uneconomic in this study due to the amount of labour required
to pick the buds (with a financial return of only $40 per kilogram of
buds, it cost $36 (6 hrs labour) just to pick the bud material).

This fact encouraged a proposal to grow blackcurrants for bud
production alone, in a stool bed plantation. This situation appeared
ideal, and was demonstrated so, since it encouraged maximum production
of first year canes, which carry most of the bud material. Manual
harvesting was practical in this case, provided buds were picked directly
from the bushes. Despite this success, chemical and mechanical harvest-
ing methods were examined in further efforts to reduce labour inputs.
Both methods were effective in harvesting bud material, but the chemical
method was more labour intensive; therefore consideration of its effect-
iveness was discontinued.

A sensitivity analysis was then undertaken to compare both manual
and machine harvesting situations; examining the effect of price and
planting density on profitability of a commercial operation. Despite
the high yields attained at high planting densities, favoured for
agronomic reasons, low planting densities were shown to be more profit-
able. Mostly due to the high costs associated with establishment,
particularly the cost of planting material and the labour required for
planting. This conclusion, which is the reverse of that proposed for

agronomic reasons earlier, strongly supports Saville's (1983) arguments that the realistic approach to experimental data is to consider it in terms of economic parameters.

The effect of price fluctuation on the internal rate of return, demonstrates effectively the handicap labour intensive harvesting presents, particularly in a low return situation. Despite having to write off the purchase price of a harvester against the operation, machine harvesting was proven to yield a higher rate of return for capital invested, than manual harvesting, at all price levels.

CHAPTER VI

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CHAPTER VII

PUBLICATIONS

PUBLICATIONS

1. KERSLAKE, M.F. and MENARY, R.C. (1983). Analysis of the
    essential oil extracted from the buds of the black-
    currant (*Ribes nigrum* L.). Proceedings 9th Inter-
    national essential oils congress, Singapore (in press).

MANUSCRIPTS IN PREPARATION

KERSLAKE, M.F. and MENARY, R.C. (1984).

1. Aroma constituents of blackcurrant buds (*Ribes nigrum* L.).

2. Varietal differences in extracts from blackcurrant buds (*Ribes
    nigrum* L.).

3. The influence of plant density and light interception on yield
    factors in blackcurrants (*Ribes nigrum* L.).

CHAPTER VIII

APPENDICES

VIII APPENDICES

1. Appendix Section III 10.1

Calculation of parameters for systematic fan design. For a full explanation refer to Nelder (1962), Bleasdale (1967b) and Section 3, Figure 10.1.1.

Let the number of densities (arcs) be N = 15 and the areas per plant range from A_1 = 0.09 m^2 to A_{15} = 1 m^2. Firstly the value of α, a constant governing the rate of change of spacing, was calculated using the equation:

$$(2N - 2) \log \alpha = \log A_N - \log A_1 \qquad (1)$$

$$(30 - 2) \log \alpha = \log 1 - \log 0.09$$

$$\text{therefore} \quad \log \alpha = \frac{\log 1 - \log 0.09}{28}$$

$$= \frac{0 - (-2.4079)}{28}$$

$$= 0.0861$$

$$\text{hence} \quad \alpha = e^{0.0861} = 1.0899$$

Nelder (1962) has shown that for the deviation from regularity, caused by any one plant not occupying the midpoint between its immediate neighbours on the same radius, not to exceed 5%, α must be less than 1.11. As α is <1.11, the number of steps (N = 15) is adequate for the range of densities required in the design.

The angle between the radii (θ) was determined from the following equation:

$$\theta = \tau(\alpha - 1)/ \; \alpha$$

where τ is the rectangularity of plant arrangement. For a 'square' plant arrangement (within the 5% limit) which was used in this design, $\tau = 1$.

Thus, $\theta = 1(1.0899 - 1)/ \; 1.0899$

$= 0.0861$ radians

$= 4°56'$ this angle was approximated to 5° for actual field layout.

As it was required to fit this arrangement into a rectangular plot, with approximately twice as many plants at a geometrical mean area of the range as at the extremes, the half angle of the fan was 30° (Bleas-dale 1967b). This had the advantage of improving the accuracy of the results over the range of densities most likely to be of commercial interest. Thirteen full radii were accommodated in the plot with a half angle of 30°(6 × 5°). The spacings of the plants along a radius, measured from the centre, were obtained as follows. The distance (r_0) of the first plant from the centre was given by

$$r_0 = \frac{2 \; A_1}{\theta \; \alpha^3 - \alpha)}$$

$$= \frac{2 \times 0.09}{0.0861(1.2947 - 1.0899)}$$

$$= 3.195 \text{ m}$$

$r_1, r_2 \ldots \ldots r_{N+1}$ are obtained from the relation $r_{n+1} = 2r_n$, thus $r_1 = r_0 \times 1.0899 = 3.483$ m and so on. A full list is contained in the table below.

The length of the rectangular plot (L) was calculated from the equation

$$L = 2\ r_{N+1}\ \sin \chi \quad \text{where } \chi \text{ is the half angle of the fan } (30°)$$

thus $\quad L = 2 \times 12.669 \times 0.5$

$$= 12.669\ m.$$

In order to mark out the plot in the field the distance 'a' (Section III, Figure 10.1.1) needs to be calculated. 'a' is shortest distance from the centre to the base line of the rectangular plot and was given by the equation:

$$a = \cos \chi\ r_0$$

$$= 0.8660 \times 3.195$$

$$= 2.767\ m$$

The breadth (B) of the plot is then given by:

$$B = r_{N+1} - a$$

$$= 12.669 - 2.767$$

$$= 9.902\ m.$$

Now, let the point at which the central radius crosses the base line (at right angles) be M and let the next radius cross at M_1, the next at M_2 and so on. The value 'a' was used to calculate the distances along the base line from the point M at which it is intersected by each of the radii. These needed to be calculated for one half of the plot, as it was symmetrical about the central radius. The distance M to $M_1 = \alpha \tan \theta$, M to $M_2 = \alpha \tan 2\theta$ and so on.

Hence, $\quad MM_1 = 1.0899 \times \tan 5° = 0.095\ m$

$$MM_2 = 1.0899 \times \tan 10° = 0.192\ m \text{ etc.}$$

The plant density at each arc is calculated from the equation:

$$A_n = r_n^2\ \theta\ (\alpha^2 - 1)/2\alpha$$

where A_n is the area per plant on the arc which is distance r_n from

the centre.   The number of plants per unit area is therefore $1/A_n$.

Each arc of the fan was harvested separately with the outer arcs

(r_0 and r_{15+1}) and end plants acting as guards.

For arc 2 $A_2 = (3.483)^2 \times 0.0861 \times [(1.0899)^2 - 1]/2 \times 1.0899$

$= 0.09 \text{ m}^2/\text{plant}$

i.e. 11.1 plants/m²

A full list is contained in the table below.

Arc	Radius, r (m)	Plant density, plants/m²	
r_0	3.195	guard row	MM_1 = 0.095 m
r_1	3.483	11.1	MM_2 = 0.192 m
r_2	3.796	9.1	MM_3 = 0.292 m
r_3	4.137	7.7	MM_4 = 0.397 m
r_4	4.509	6.7	MM_5 = 0.508 m
r_5	4.915	5.6	MM_6 = 0.629 m
r_6	5.357	4.8	
r_7	5.838	4.0	
r_8	6.363	3.3	
r_9	6.935	2.8	
r_{10}	7.559	2.4	
r_{11}	8.238	2.0	
r_{12}	8.979	1.7	
r_{13}	9.789	1.4	
r_{14}	10.666	1.2	
r_{15}	11.624	1.0	
r_{15+1}	12.669	guard row	

2. Appendix   Section IV 1.2   Mechanical harvester design

265 mm

38mm

72mm

7mm

picking roller

rubber hose

2 x 2½″

25mm

feeding roller

10″

1

chain

2½″ sprocket

10″

2 x 2½″

1″

2″

pulley sizes (inches)
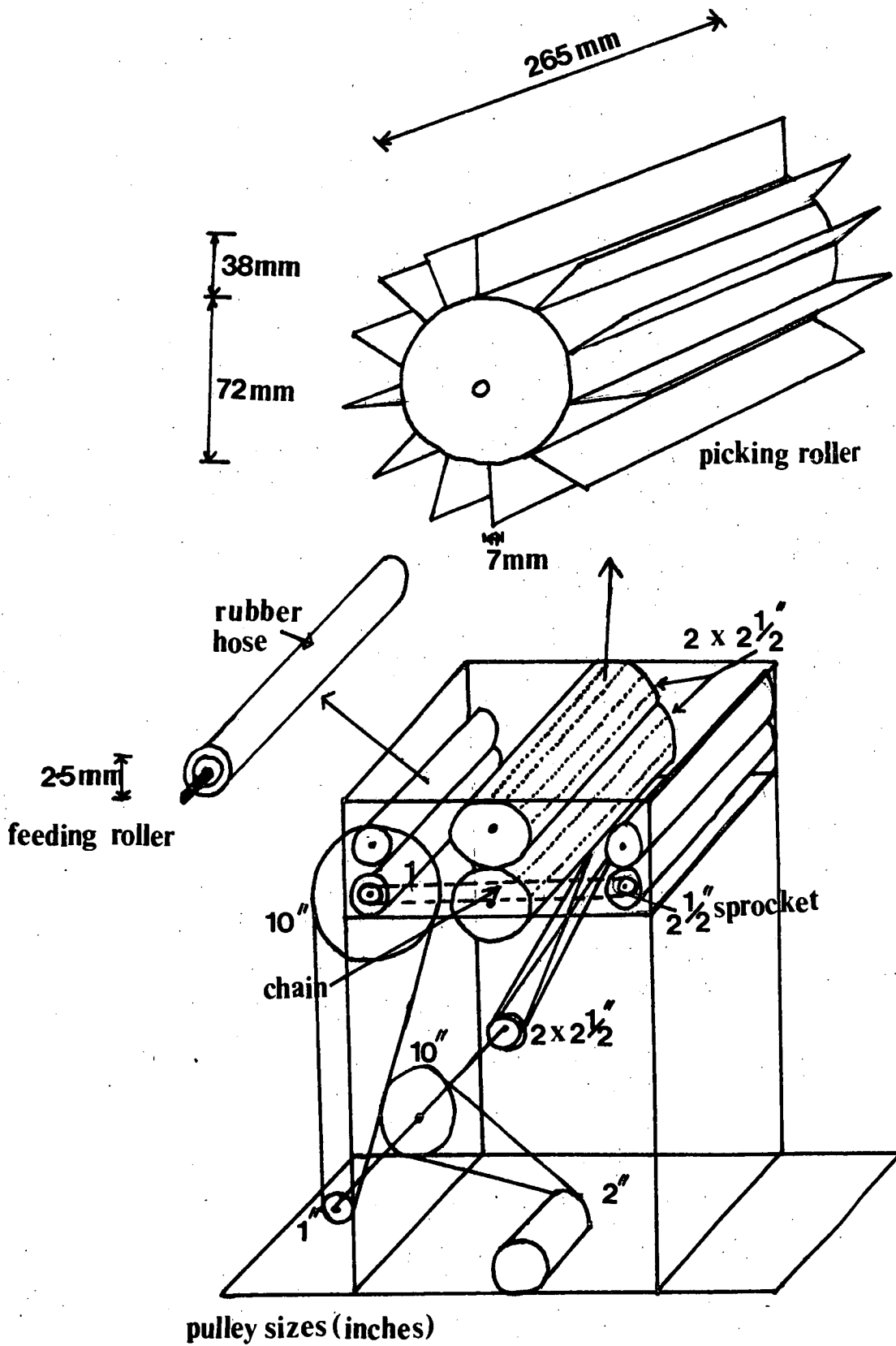
3.   Appendix III 8.1   Calibration curve - Infrared gas analyser

CALIBRATION CURVE—INFRA RED GAS ANALYZER

$Y = 0.6403X - 0.5665$

$r = 0.98$

CHART RECORDER RESPONSE (DIVS)

CO2 RESPONSE (PPM)
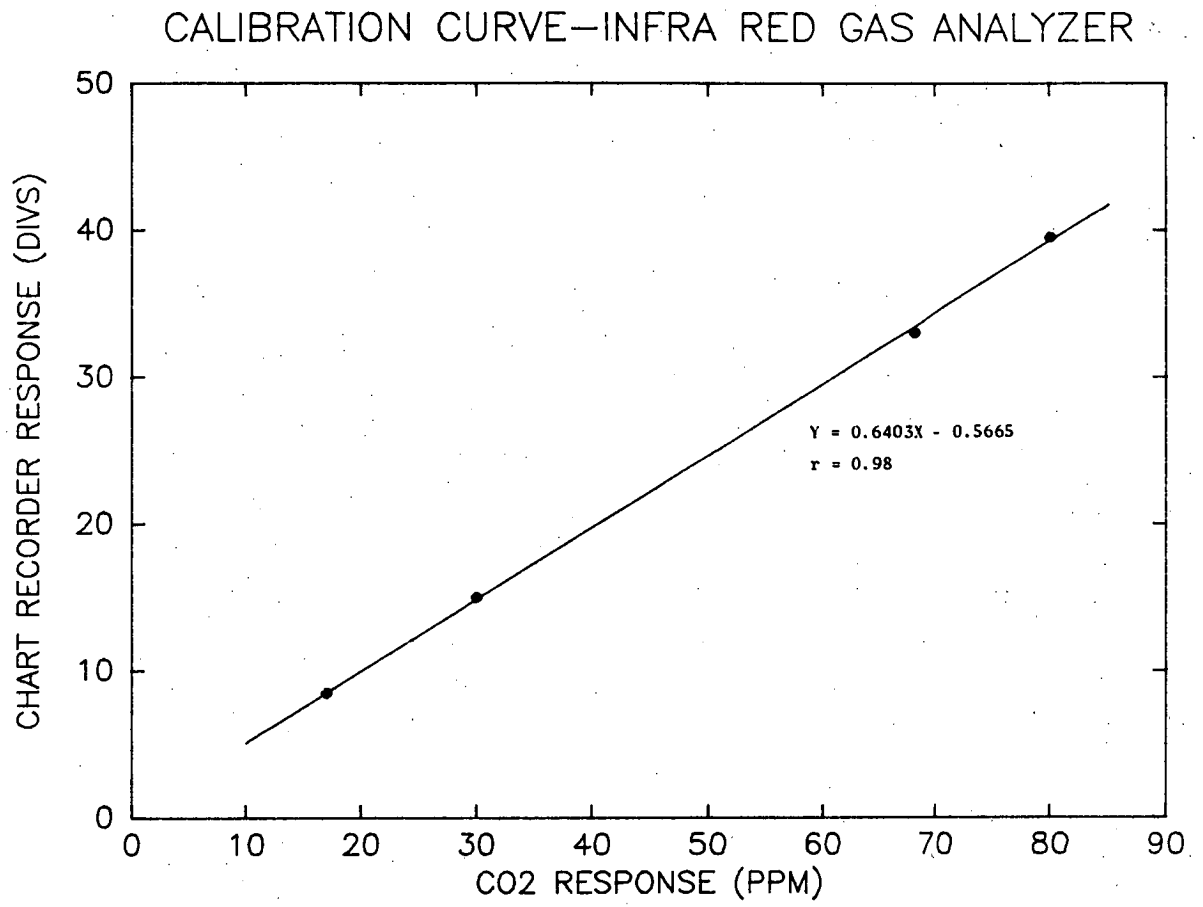
3. APPENDIX SECTION IV 7

Effect of light intensity on apparent photosynthetic activity (at 24° ambient temperature)

Light intensity ($\mu\Sigma m^{-2}s^{-1}$)	Replicate	Net carbon dioxide exchange ($mgCO_2dm^{-2}hr^{-1}$)
8	1	2.4
	2	1.8
	3	1.8
	mean	2.0
55	1	2.9
	2	3.5
	3	4.1
	mean	3.5
100	1	5.9
	2	6.5
	3	7.1
	mean	6.5
300	1	14.7
	2	14.1
	3	15.3
	mean	14.7
400	1	15.9
	2	16.5
	3	17.7
	mean	16.7
650	1	16.5
	2	16.5
	3	15.3
	mean	16.1
700	1	15.3
	2	16.5
	3	16.5
	mean	16.1
1100	1	18.3
	2	17.7
	3	17.7
	mean	17.9

3. APPENDIX SECTION IV 7

Effect of temperature on net photosynthetic activity $(mgCO_2dm^{-2}hr^{-1})$

Temperature (°C)	Replicate	Apparent Photo-synthesis 1	Dark Respir-ation 2	Enhanced net CO_2 exchange 3	Photo Respir-ation (1-3)	Time Photo-synethesis (3-2)
10	1	13.0	-4.1	17.7		
	2	12.4	-3.5	17.7		
	3	13.0	-4.7	17.1		
	mean	12.8	-4.1	17.5	-4.7	21.6
17	1	10.0	-3.5	16.5		
	2	10.6	-4.1	16.5		
	3	11.2	-4.1	17.7		
	mean	10.6	-3.9	16.9	-6.3	20.8
20	1	17.7	-5.9	26.5		
	2	16.5	-5.3	25.9		
	3	17.7	-4.7	27.1		
	mean	17.3	-5.3	26.5	-9.2	31.8
24	1	15.3	-3.8	28.1		
	2	15.9	-4.7	28.9		
	3	15.9	-3.5	27.7		
	mean	15.7	-4.0	28.2	-12.5	32.2
26	1	21.8	-4.7	30.9		
	2	21.2	-5.3	30.3		
	3	22.4	-5.9	30.9		
	mean	21.8	-5.3	30.7	-8.9	36.0
29	1	22.4	-8.3	35.9		
	2	21.2	-7.1	35.3		
	3	20.6	-8.3	37.1		
	mean	21.4	-7.9	36.1	-14.7	42.0
35	1	17.1	-7.1	34.2		
	2	15.9	-6.5	33.0		
	3	17.7	-6.5	33.6		
	mean	16.9	-6.7	33.6	-16.7	40.3

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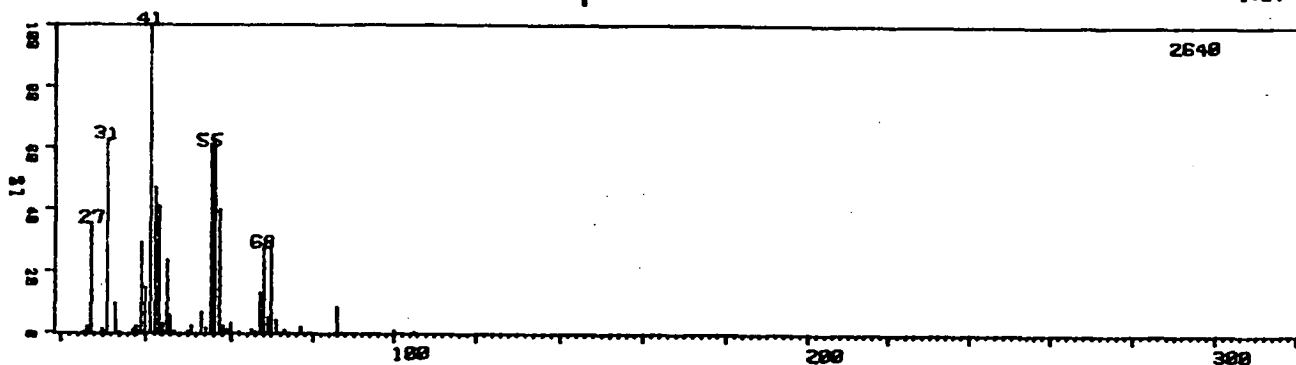
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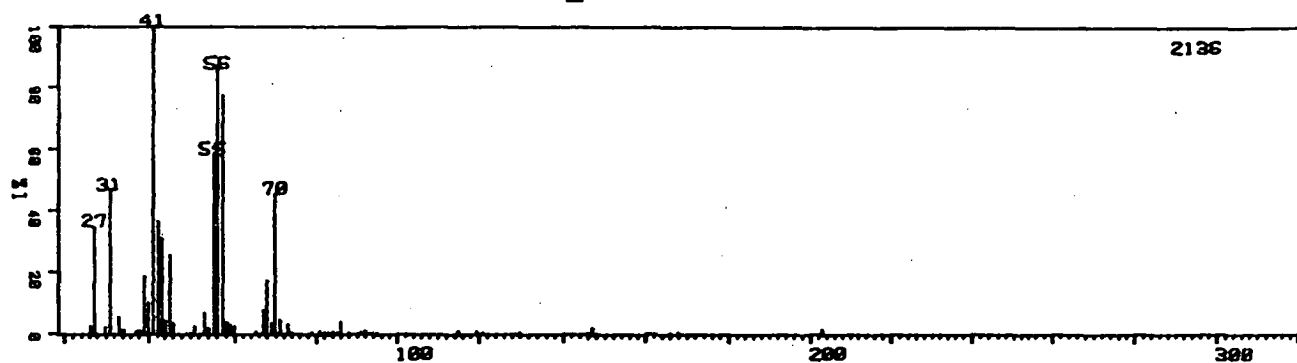
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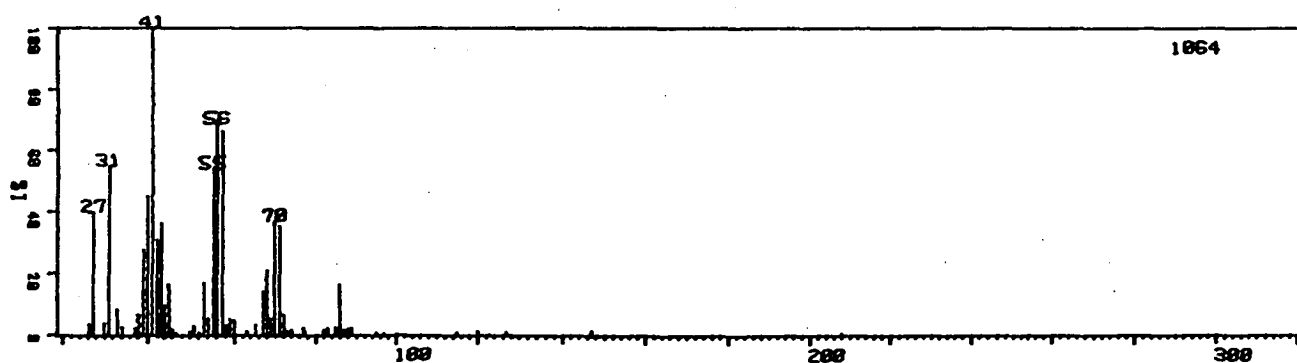
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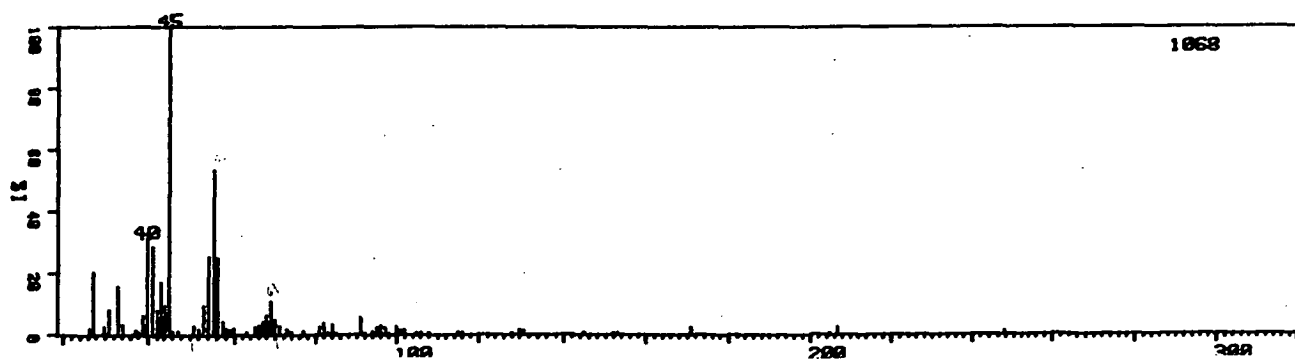
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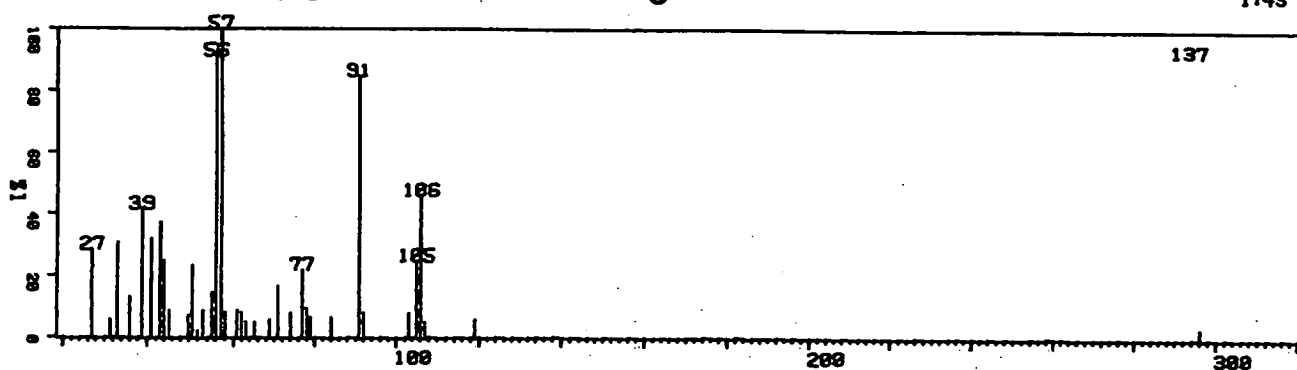
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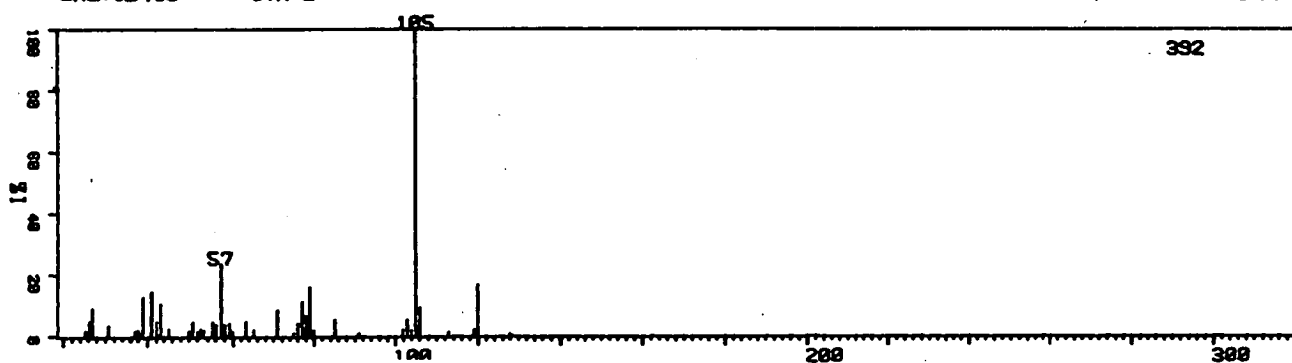
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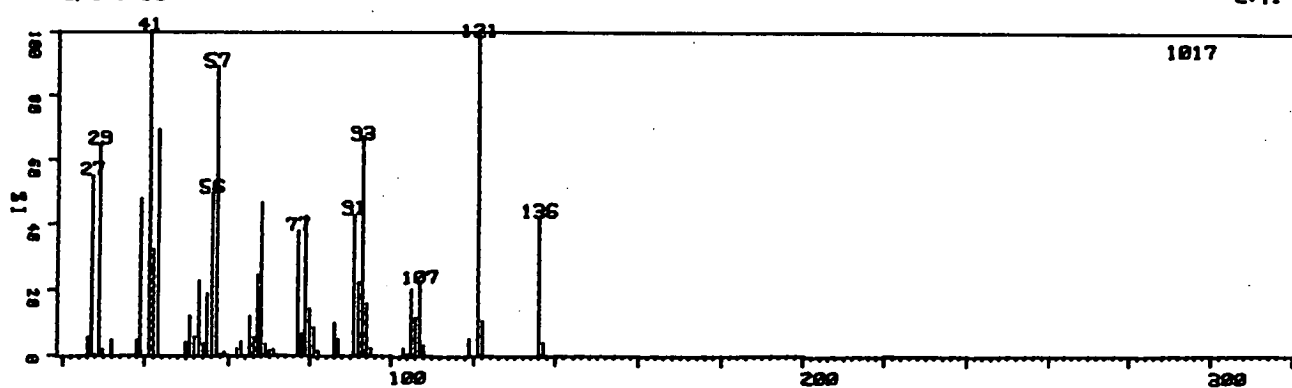
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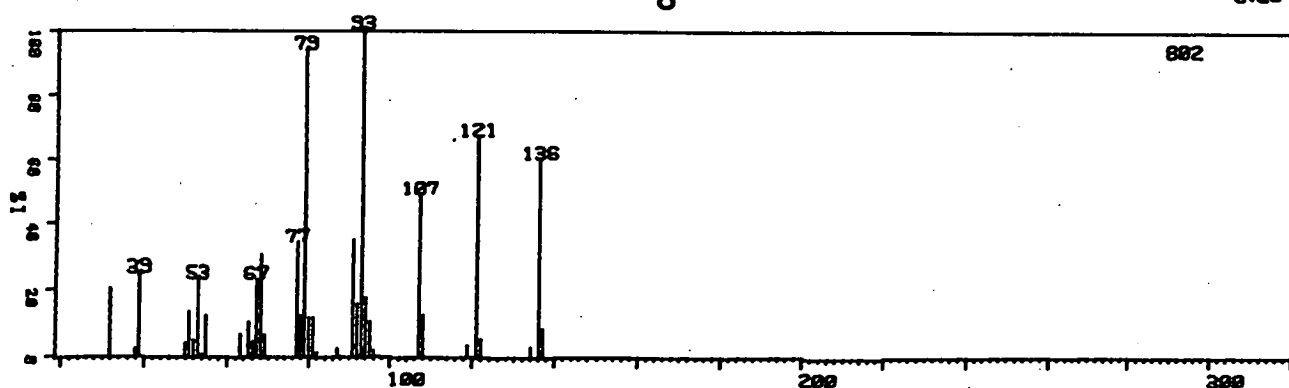
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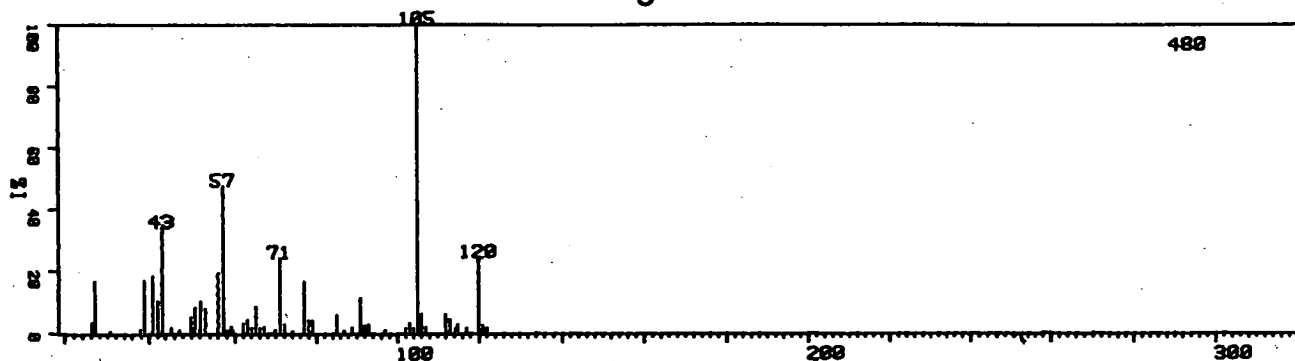
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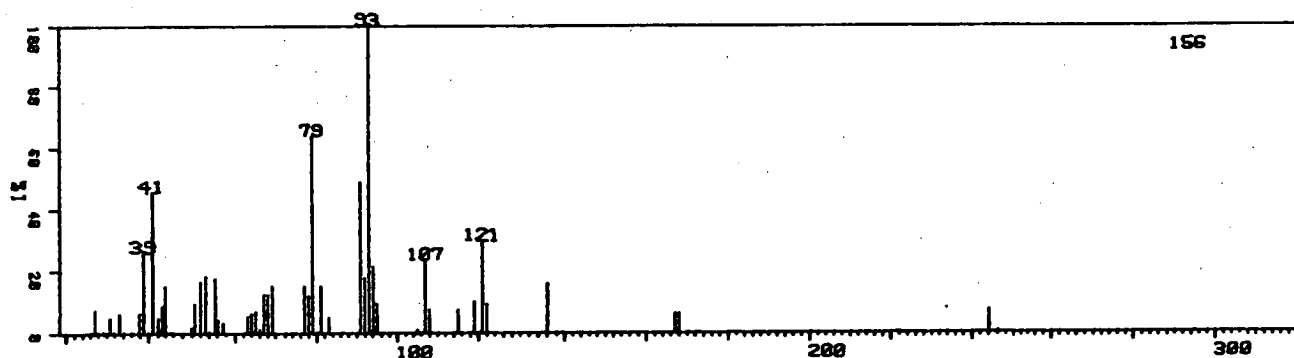
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11
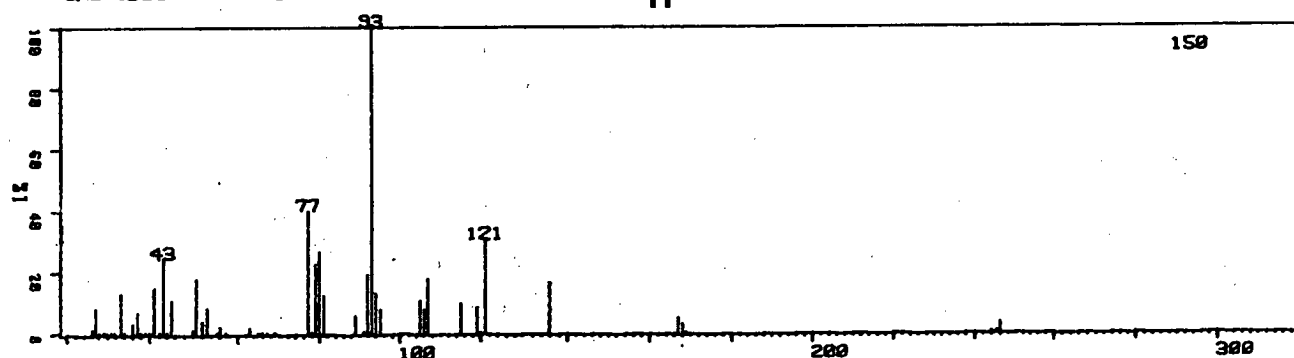
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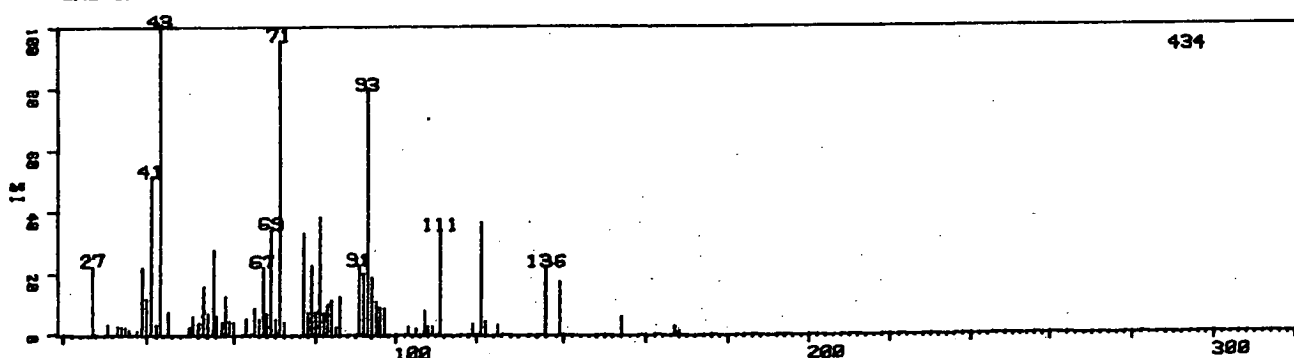
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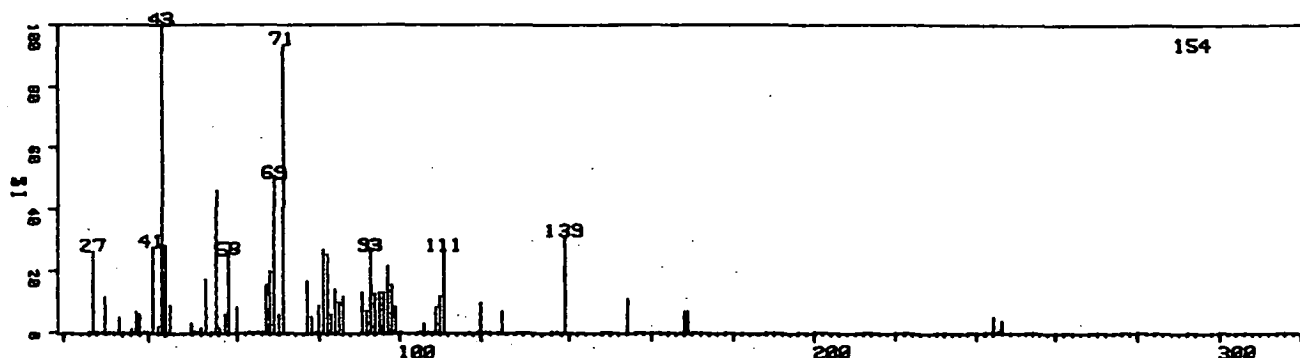
43
71
69
154
27 41 59 33 111 139

100   200   300

14

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43
29
27
71 93
483

100   200   300

AG1000 356 FRN 17 EX FLORISIL. BLACKCURRANT. OV101 80-200 4   13-MAY-82
CAL:1D400   STA:E.   11:9

15

32
67
601

100   200   300

16

AG1000 362 FRN 17 EX FLORISIL. BLACKCURRANT. OV101 80-200 4   13-MAY-82
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29
43
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244
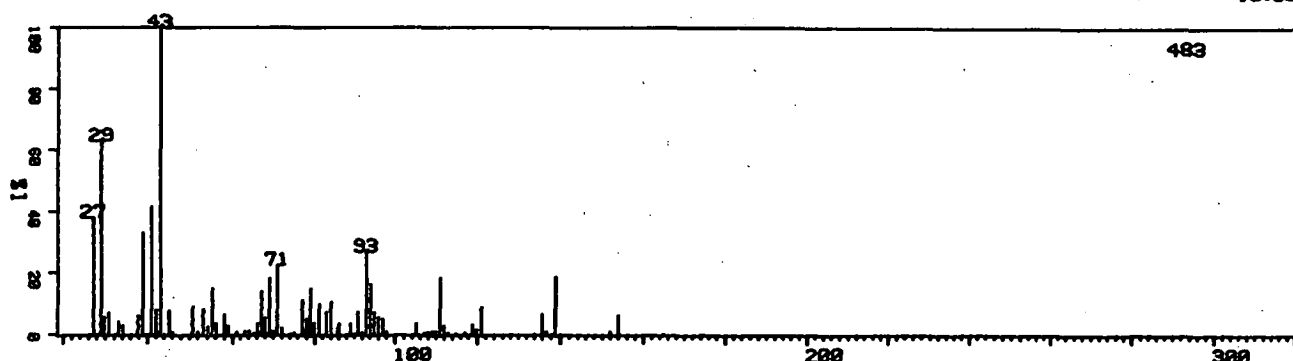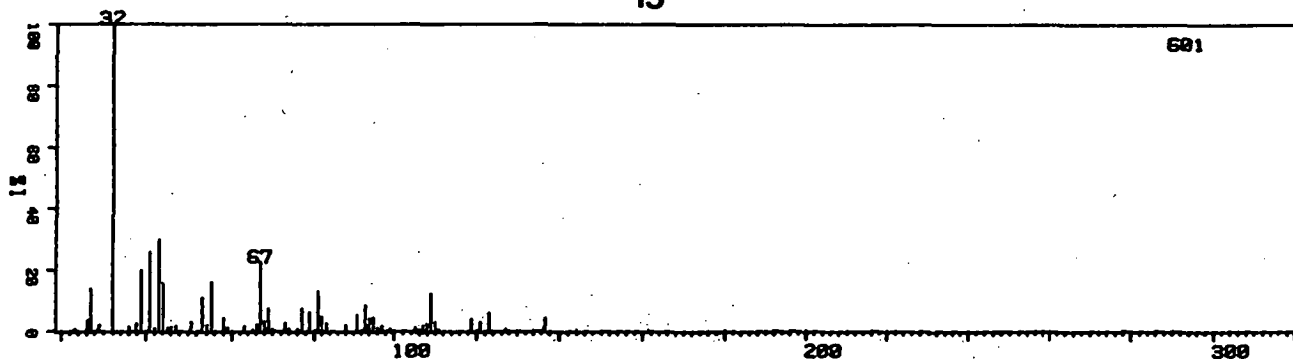
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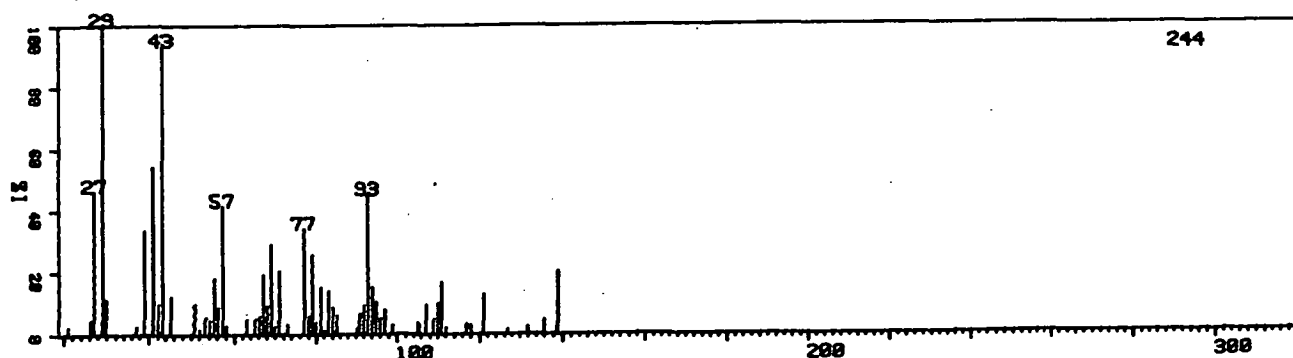
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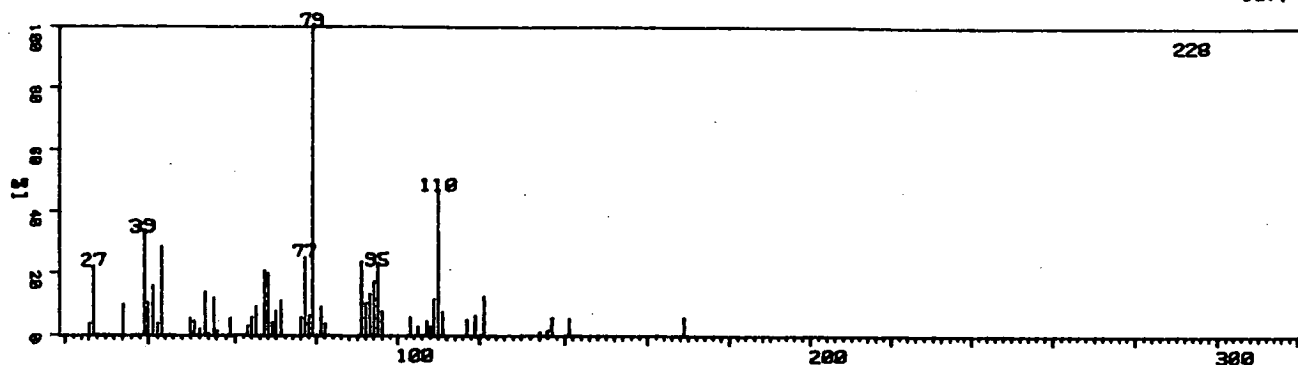
18

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CO2REP 197   **19**   04-MAR-83
CAL:1DSS0   STA:E.   S:19

20

CO2REP 209   04-MAR-83
CAL:1DSS0   STA:E.   S:38

21
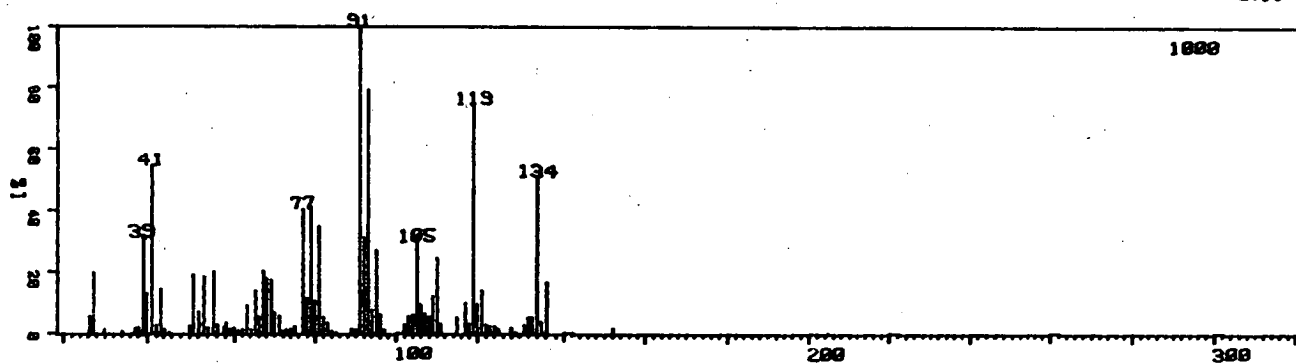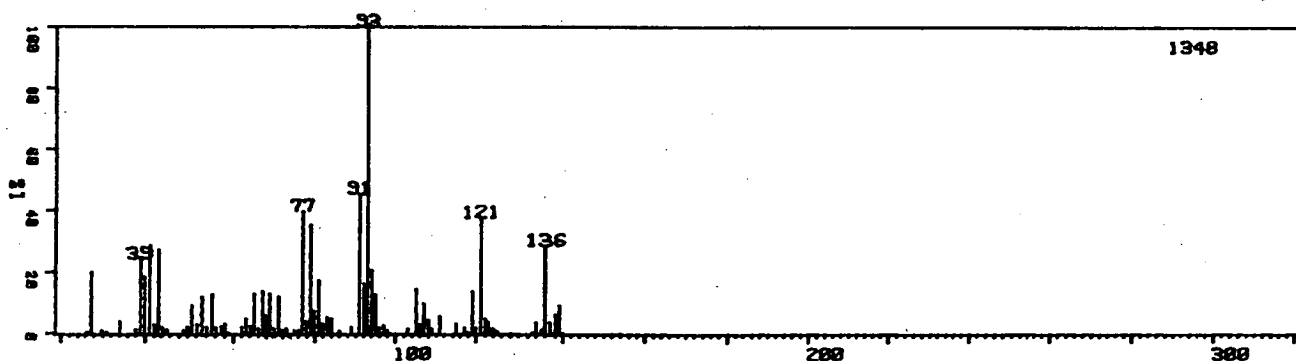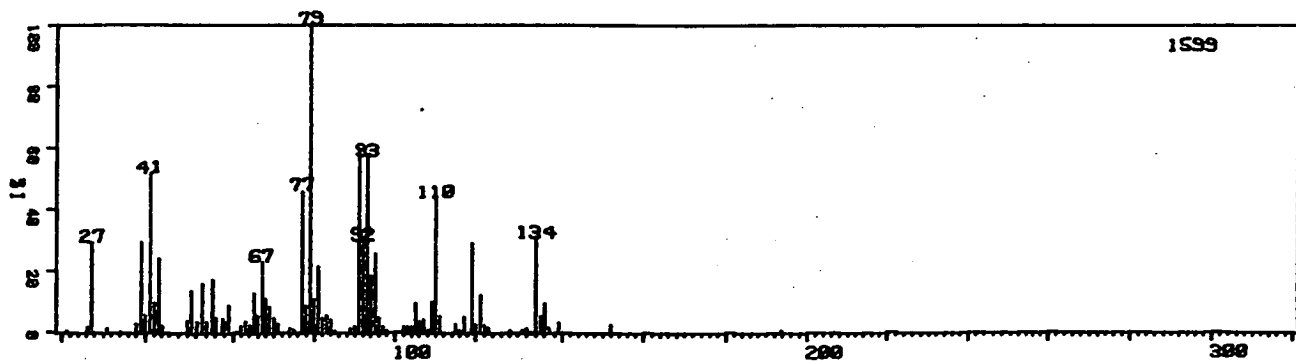
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04-MAR-83
5:49

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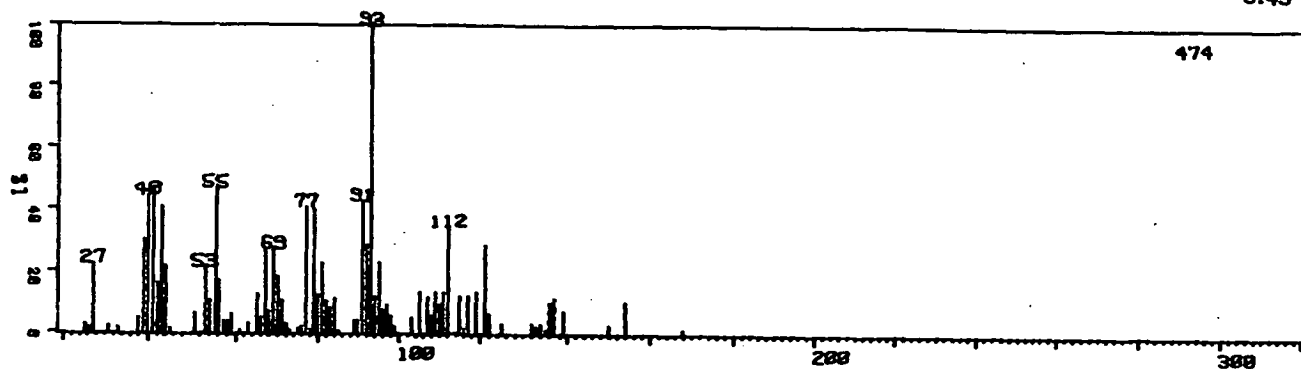
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CAL:1C350

21-JAN-81

23
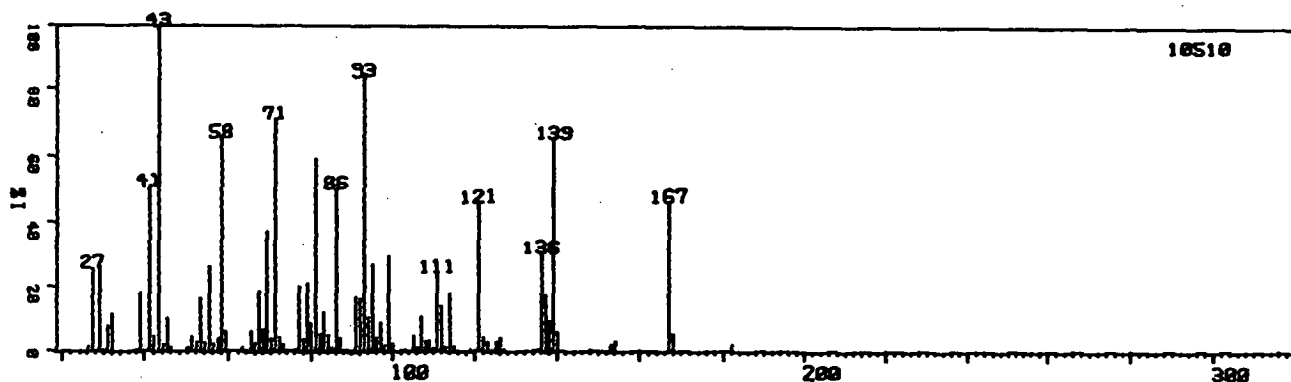
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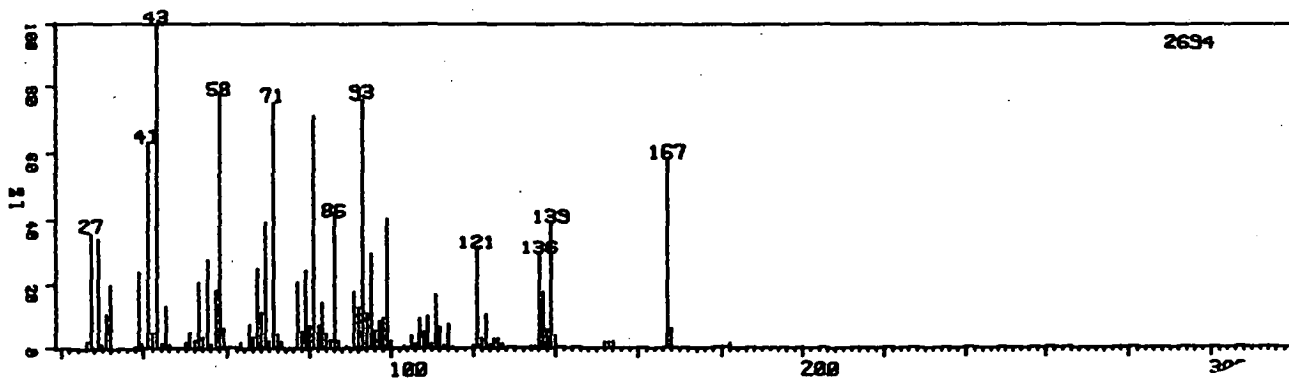
21-JAN-81

24

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04-MAR-83
6:40
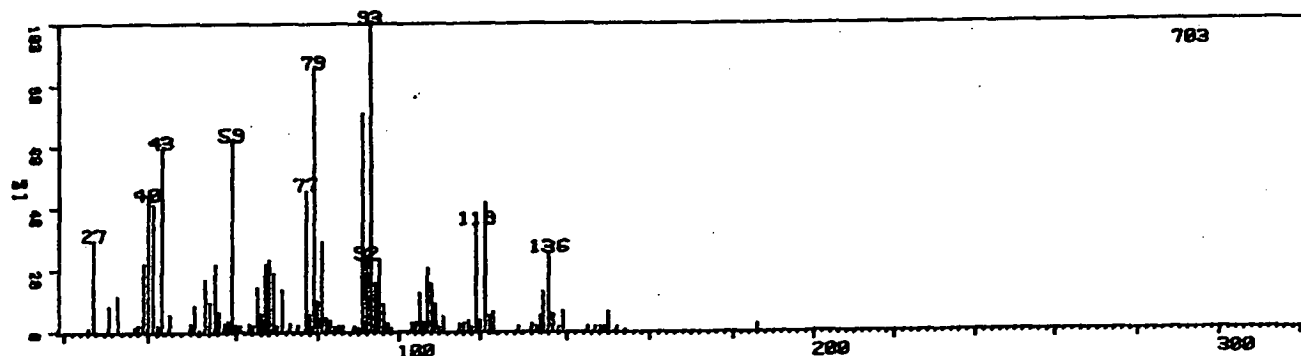
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26

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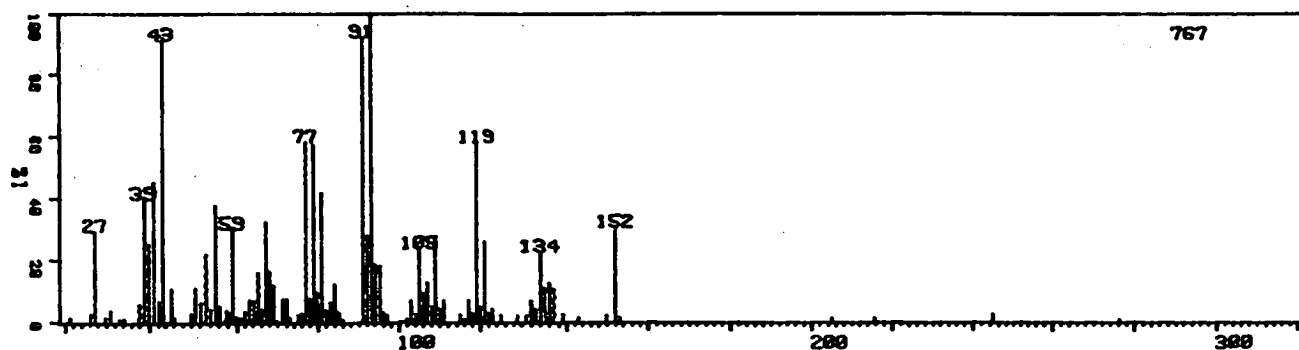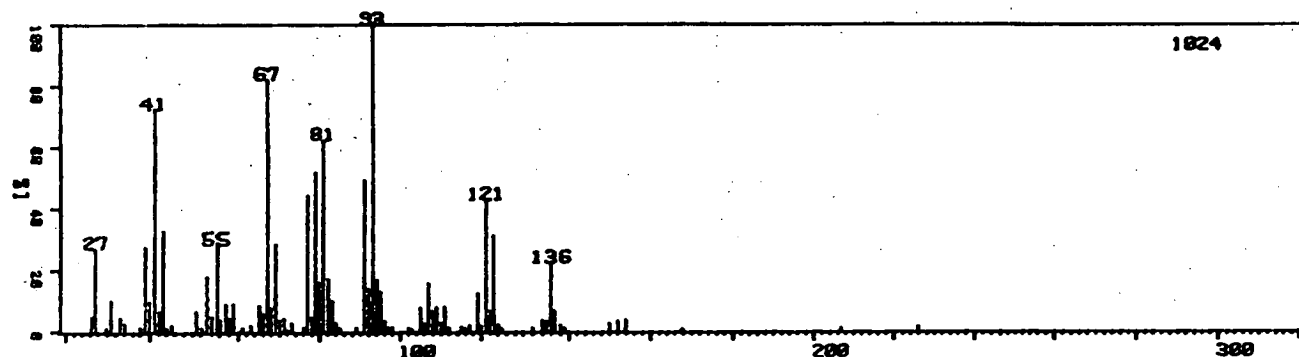
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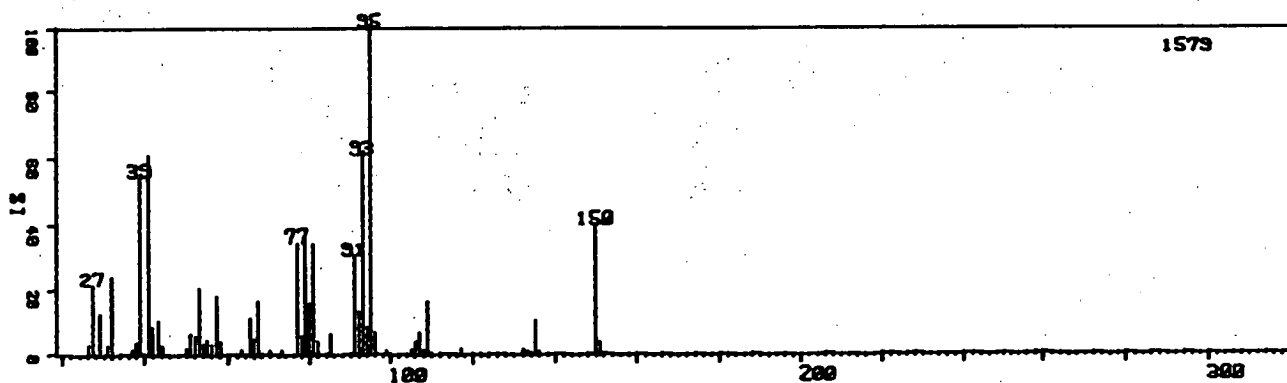
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29
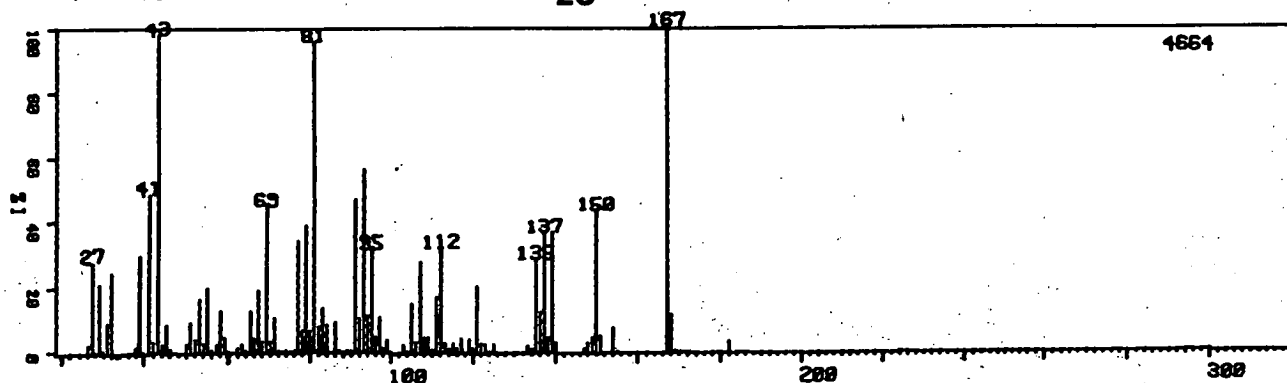
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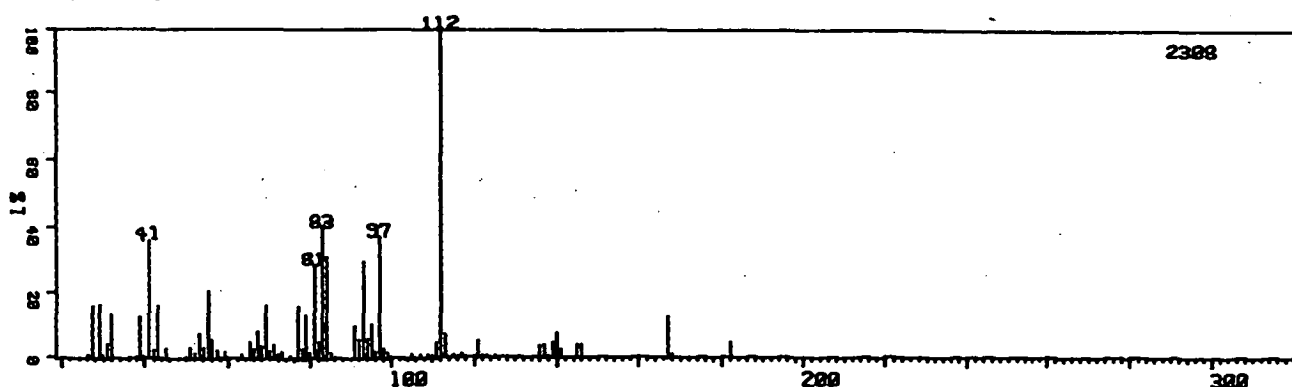
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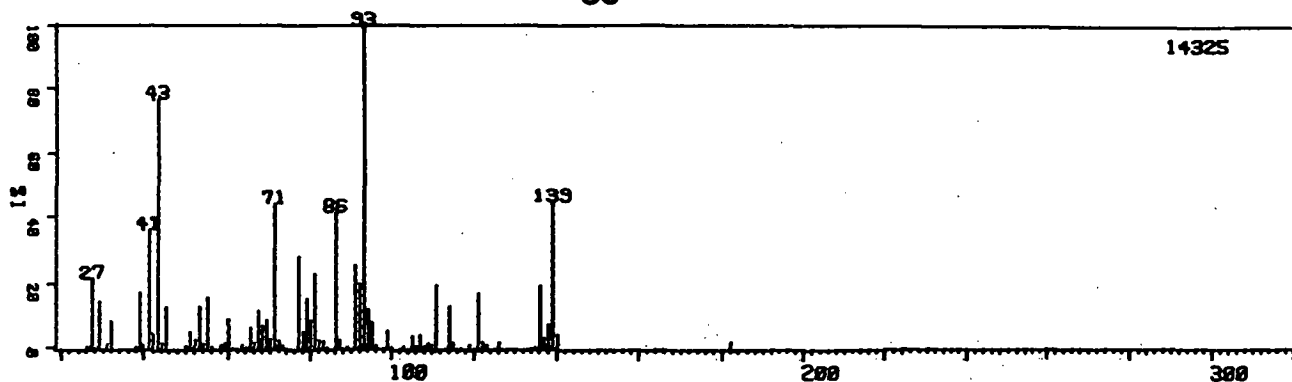
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AGS001 304 MIXTURE   21-JAN-81
CAL:1C3S0

32

AGS001 307 MIXTURE   21-JAN-81
CAL:1C3S0

33
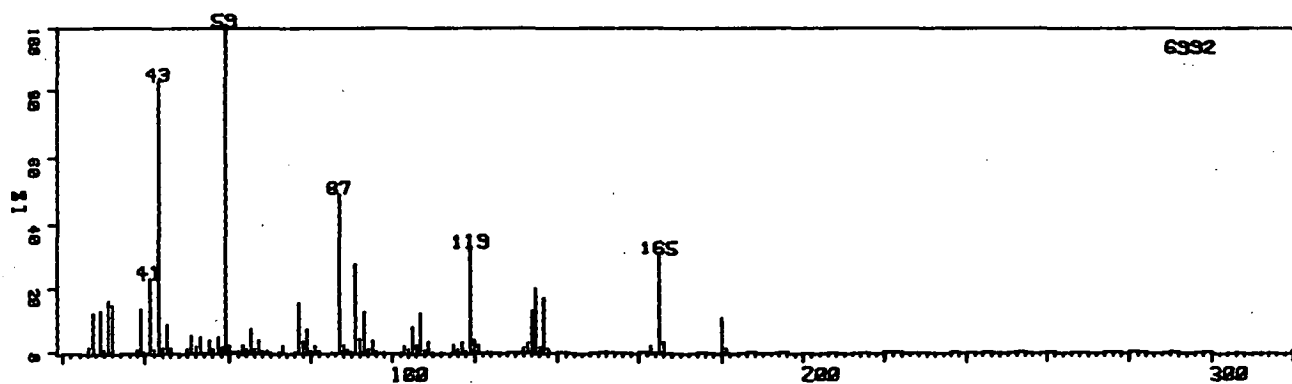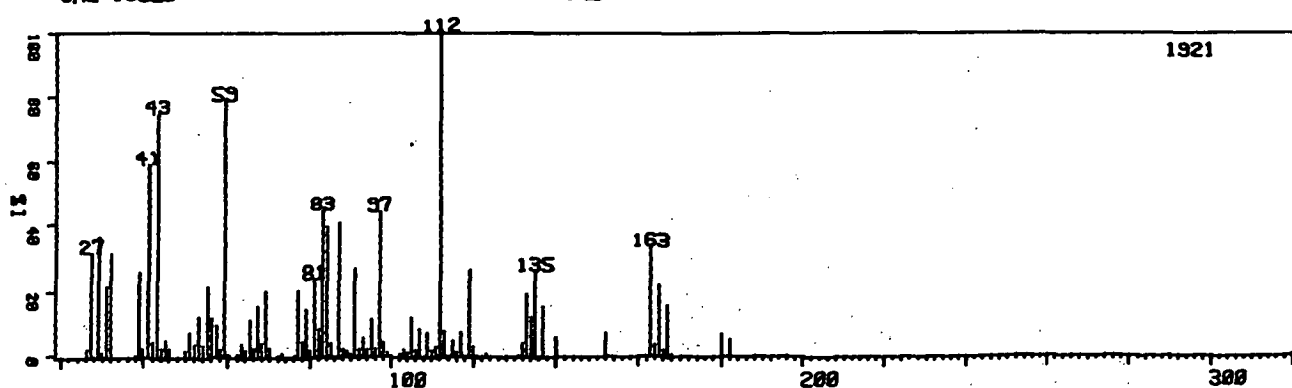
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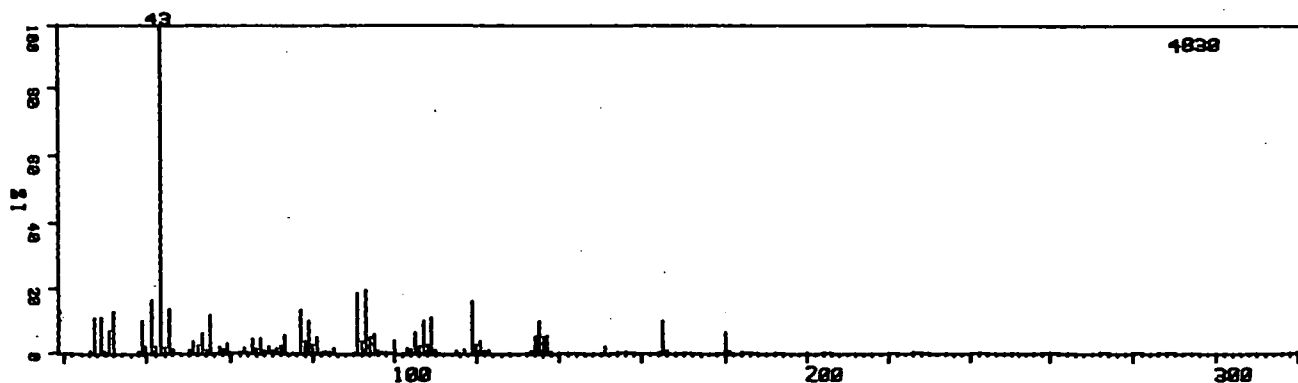
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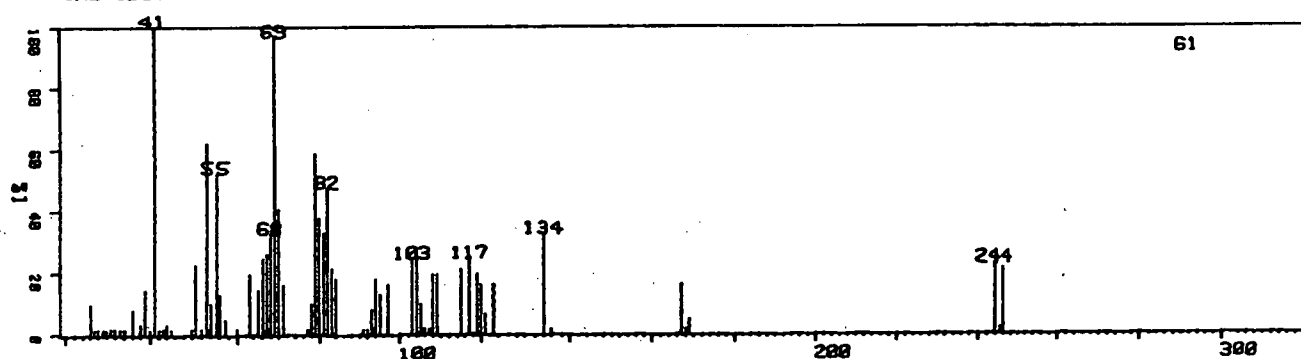
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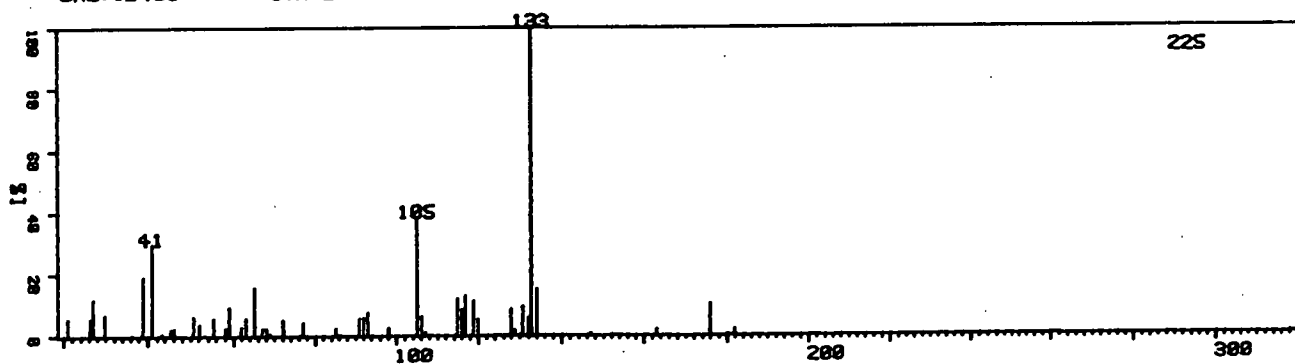
36

CO2REP 420   04-MHK-83
CAL:1D350   STA:E.   11:15

37

CO2REP 450   04-MAR-83
CAL:1D3S0   STA:E.   12:3

38

CO2REP 506   04-MAR-83
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39
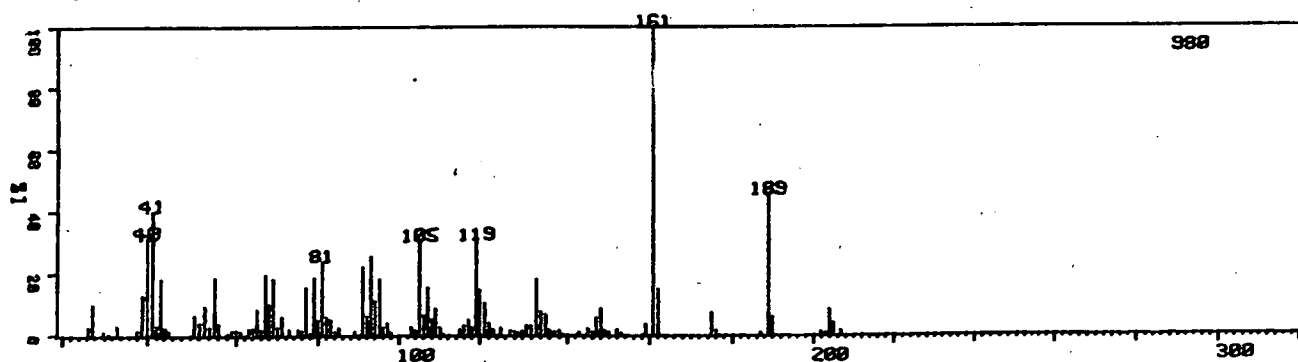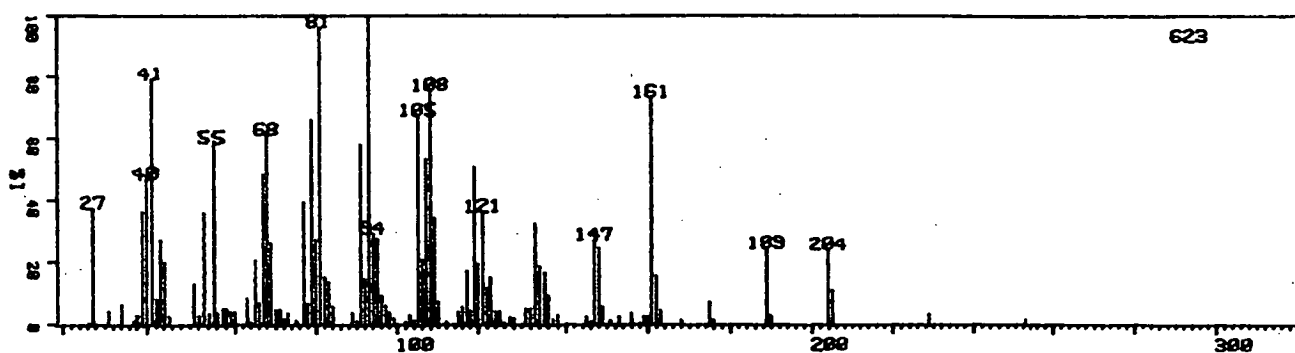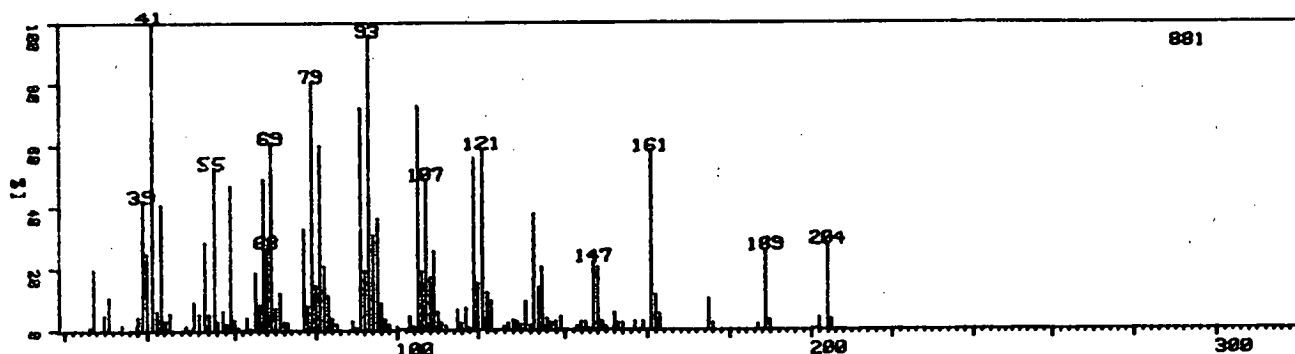
AG1S07 6S3 BLACKCURANT VAC DIST SILICA CW20M   11-NOV-81
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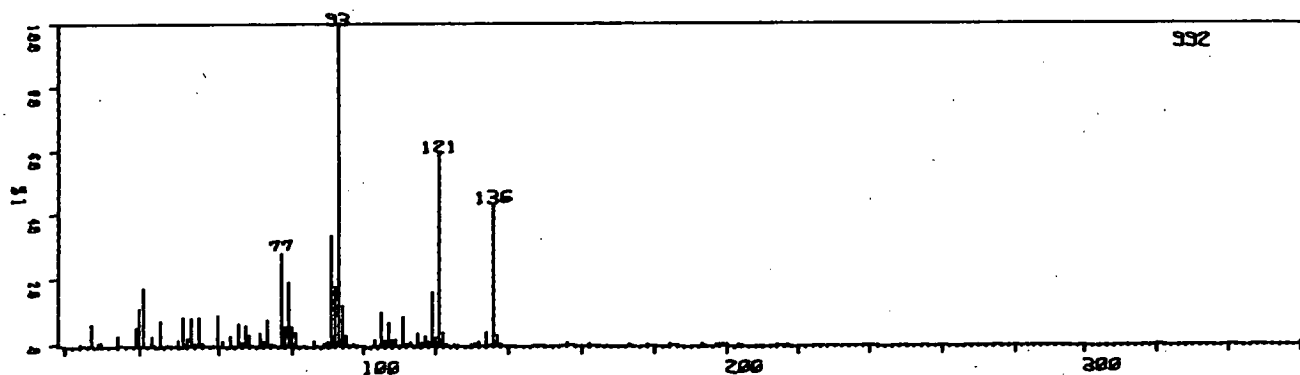
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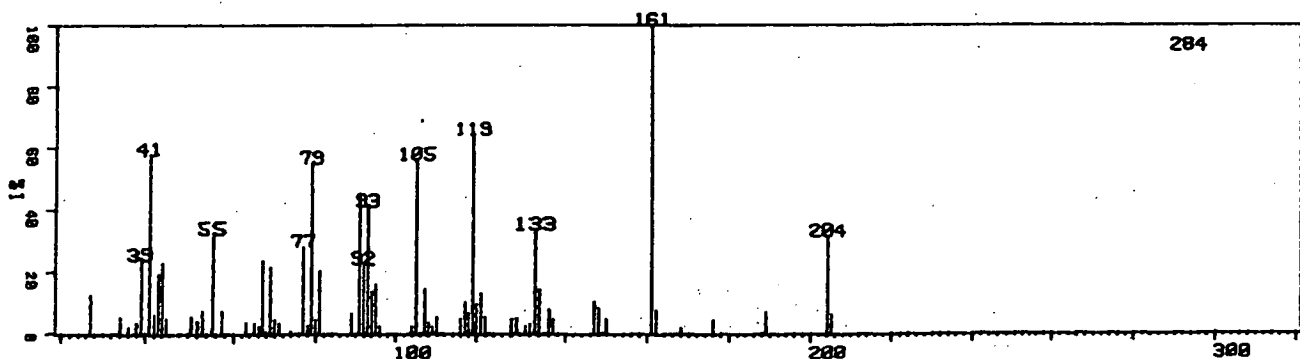
AGS005 364 LSC RUN 6.FRACTION 8

03-DEC-80
12:17

CAL:1C350

42
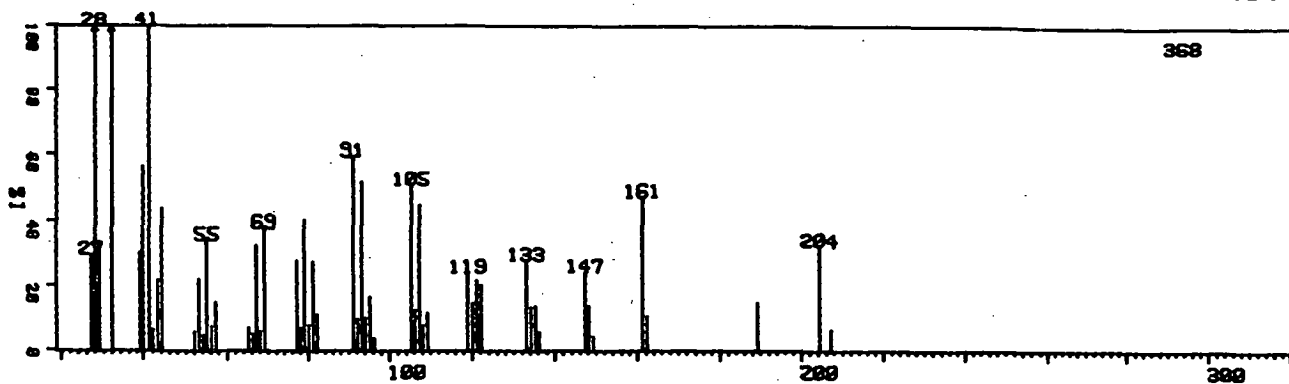
AGS007 413 LSC RUN 6. FRACTION 12

03-DEC-80
13:50
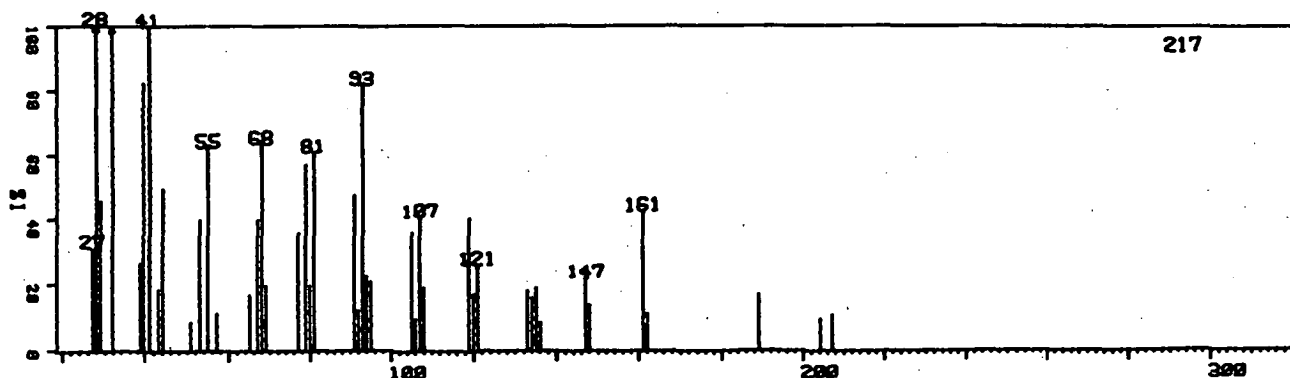
CAL:1C350

43

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06-JUN-83
29:29

CAL:1DS50   STA:E.

44
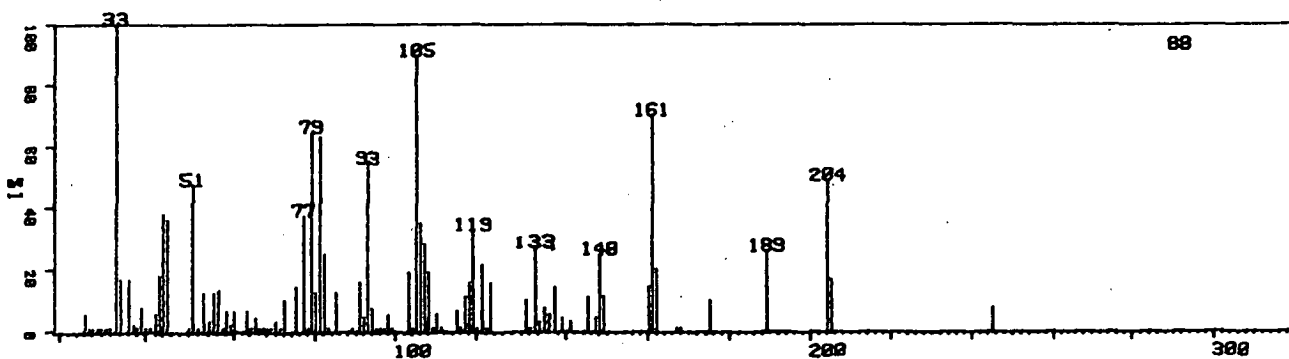
AGS001 621 CASSIS. LSC 9. FR.19
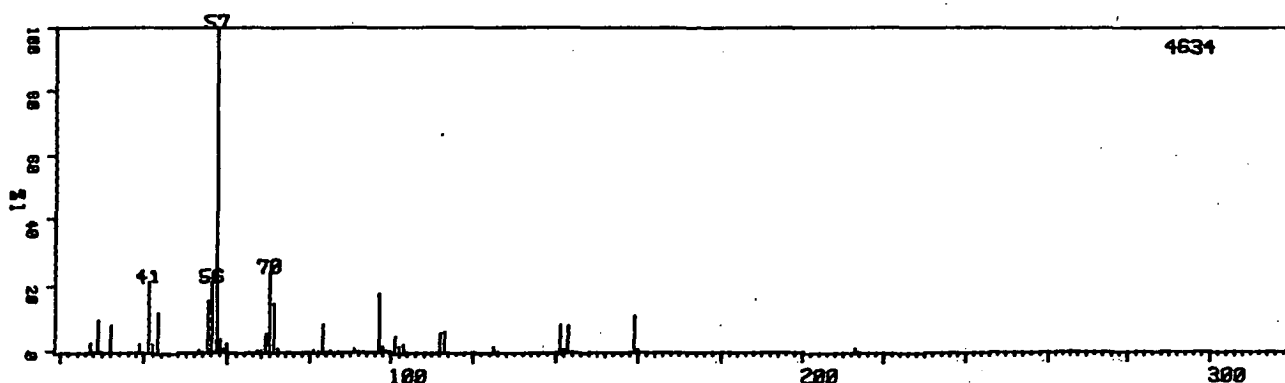
21-JAN-81

CAL:1C350

45

AGS008 478 LSC RUN 6, FRACTION 13
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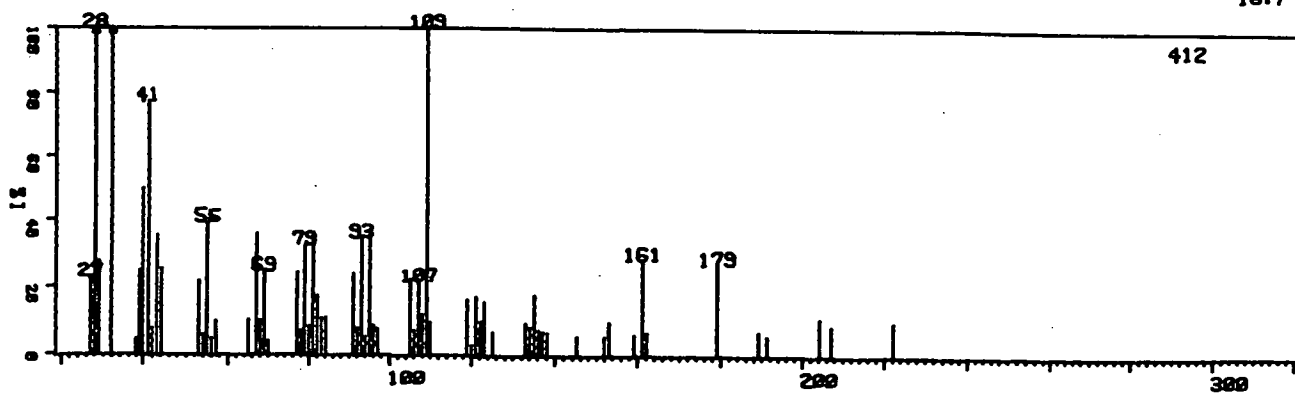
46

AGS008 482 LSC RUN 6, FRACTION 13
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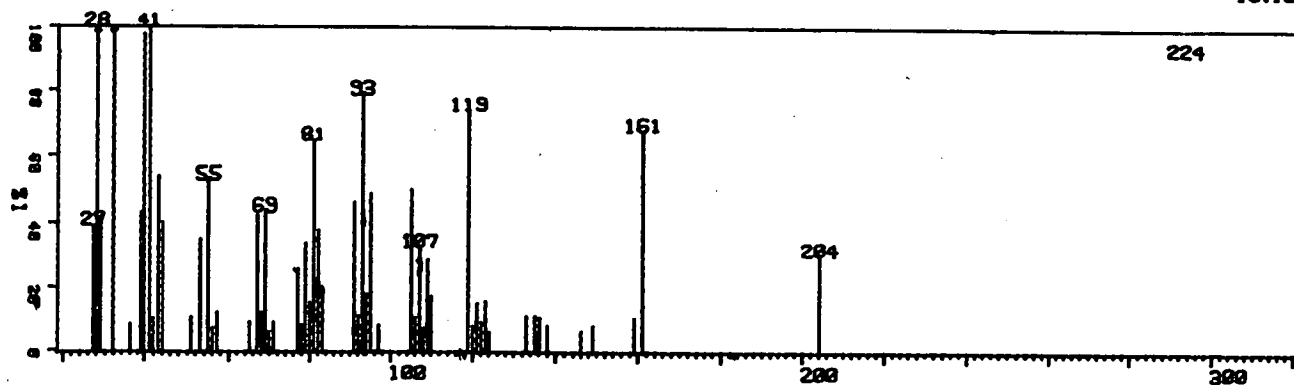
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47   16:29
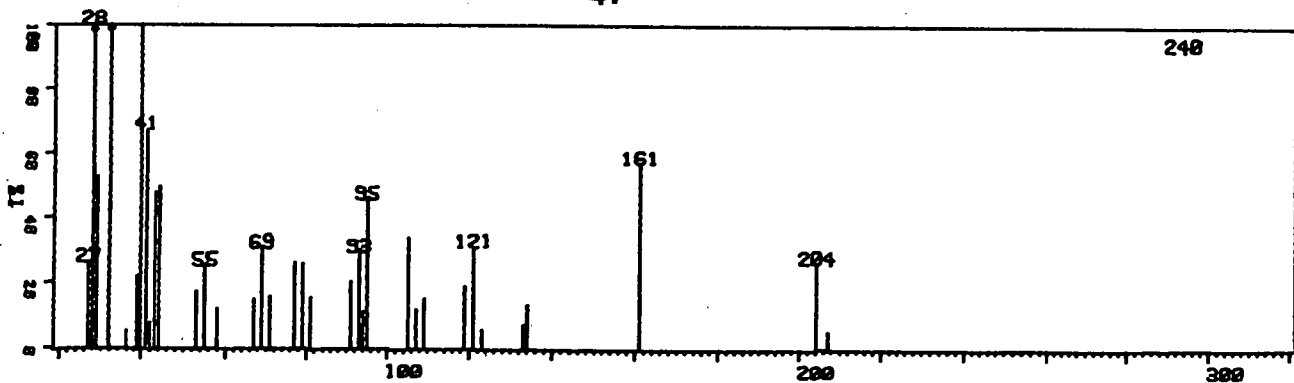
48

AGS001 642 CASSIS, LSC 9, FR.19   21-JAN-81
CAL:1C350

49

CO2REP 670
CAL:1D350   STA:E.   04-MAR-83
                                                                17:54

1439

50
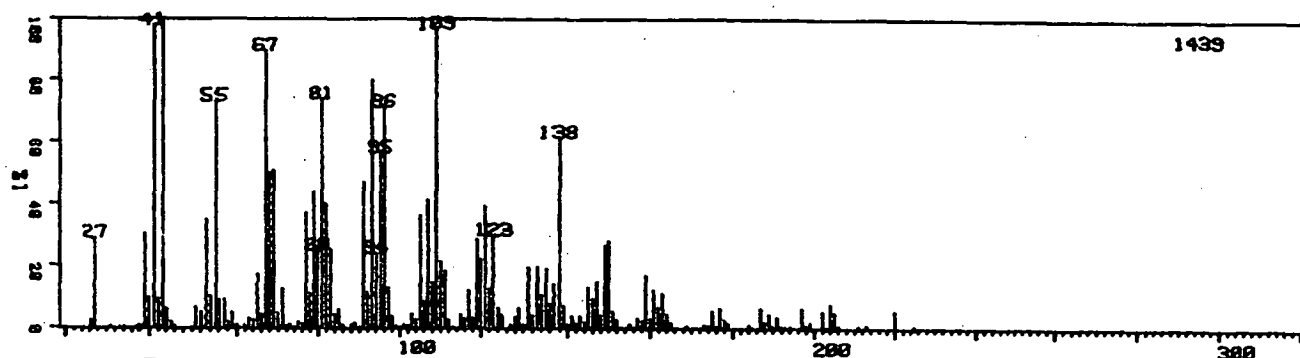
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1040

51

CO2REP 696
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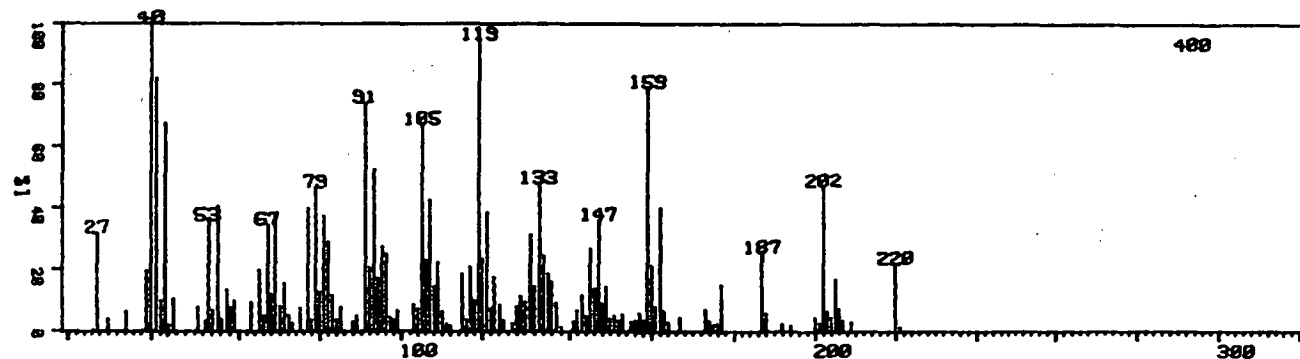
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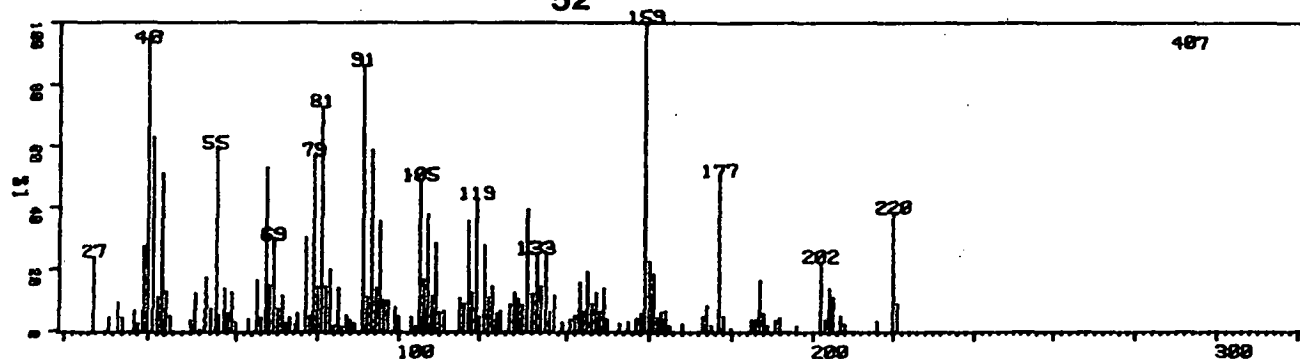
52

407

53

CO2REP 730
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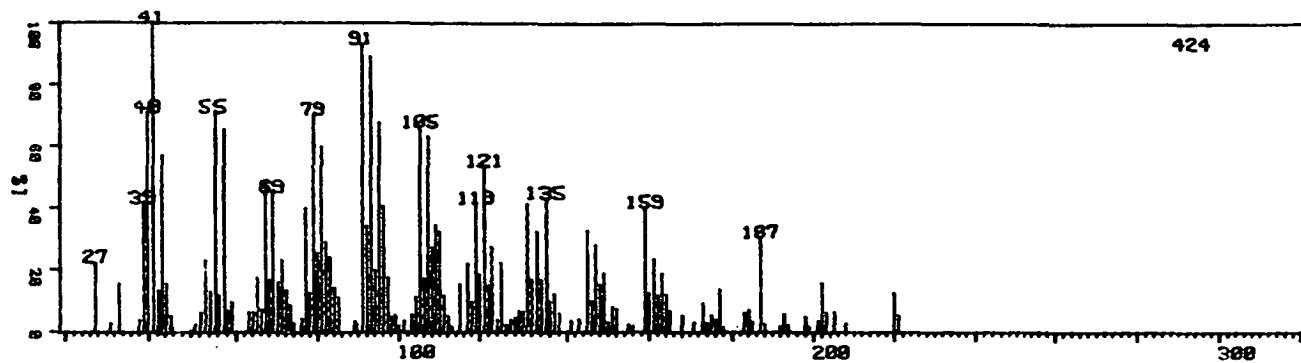
424

27 41 48 35 55 69 79 91 105 119 121 135 159 187

100   200   300

CO2REP 743
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54   19:50

159

366

27 41 48 55 69 68 79 93 107 121 135 177 220

100   200   300

55

AGS001 686 CASSIS. LSC 9. FR.19   21-JAN-81
CAL:1C350

99

2048

29 41 43 58 71 86 93 135

100   200   300

56
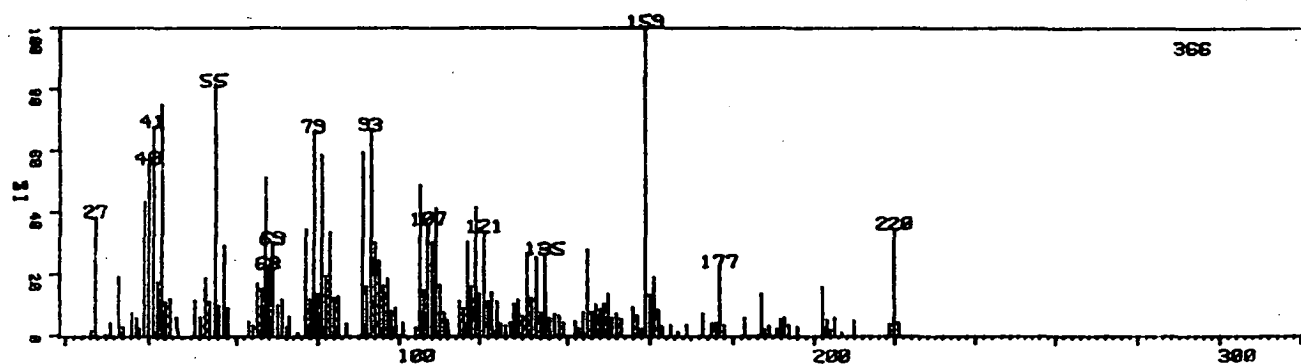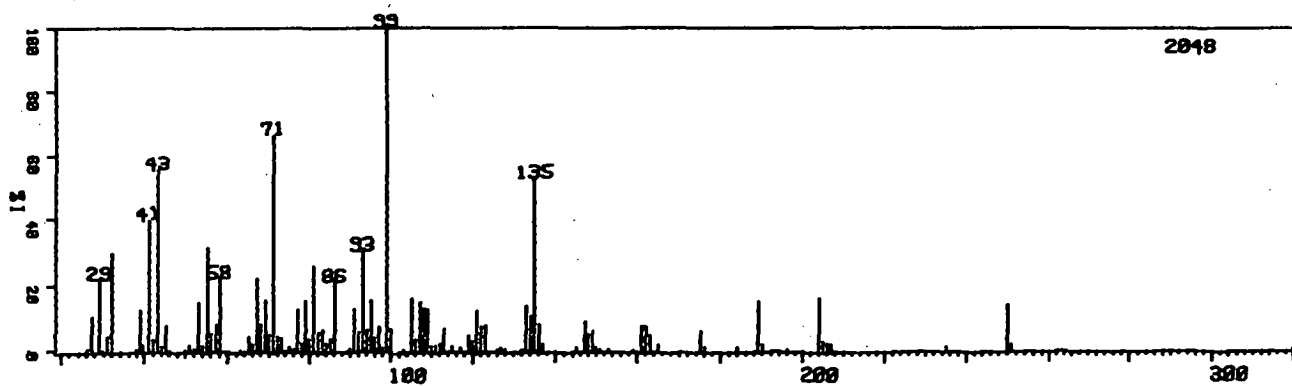
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CAL:1C350

99

1649

32 43 71 91 93 121 161 204

100   200   300
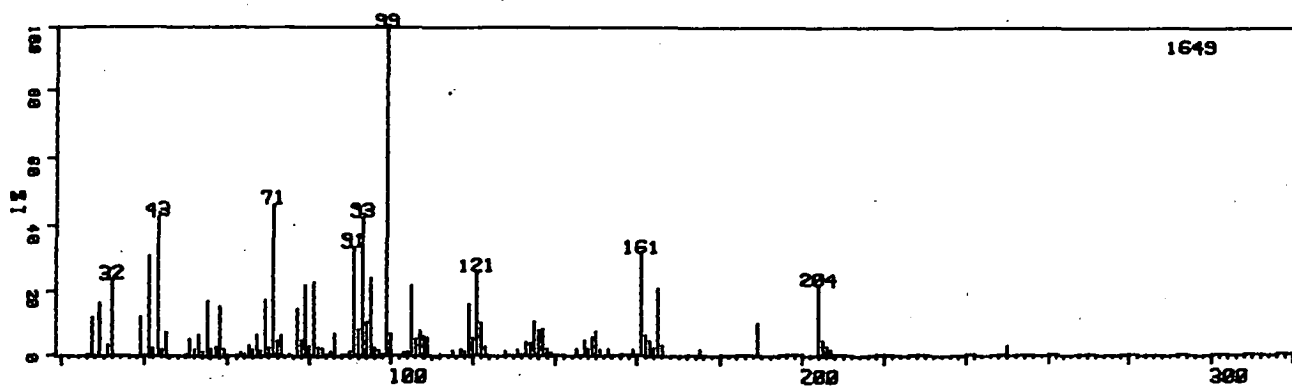
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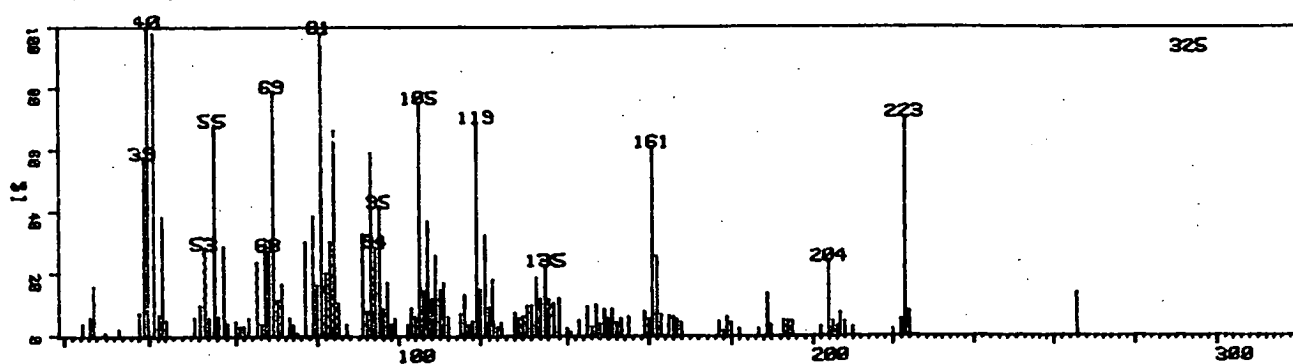
5. Appendix Section IV 10.1   Mean Bud Yield 1981

Density Plants/ m²	BLOCK 1 Ass. Ct.	1 Mean Yld. (g)	2 Ass. Ct.	2 Mean Yld. (g)	3 Ass. Ct.	3 Mean Yld. (g)	TOTAL Ass. Ct.	Mean Yld. (g) per plant	Mean Yld. (g) per m²
1.0	11	10.33	10	7.05	11	10.85	32	9.48	9.48
1.2	12	7.51	14	8.68	11	8.92	37	8.38	10.06
1.4	14	8.66	15	9.08	16	8.07	45	8.59	12.03
1.7	17	9.36	17	5.66	16	7.94	50	7.65	13.01
2.0	22	8.99	20	6.47	19	6.68	61	7.44	14.88
2.4	21	7.85	20	7.04	21	5.90	62	6.93	16.63
2.8	19	8.80	15	6.09	18	4.60	52	8.56	18.37
3.3	23	7.90	18	4.31	18	4.62	59	5.84	19.27
4.0	21	6.30	15	3.72	14	3.80	50	4.83	19.32
4.8	17	6.11	13	4.05	15	3.13	45	4.52	21.70
5.6	17	5.21	11	3.71	13	2.86	41	4.06	22.74
6.7	16	5.35	7	3.81	12	2.93	35	4.21	28.21
7.7	16	5.39	7	4.69	9	2.47	32	4.42	34.03
9.1	12	3.11	5	2.81	7	2.68	24	2.92	26.57
11.1	12	3.23	5	2.76	5	1.71	22	2.78	30.86
TOTAL	250	7.08	192	5.73	205	5.43	647	6.16	19.81

Mean Bud Yield 1982

Density Plants/ m^2	BLOCK 1		BLOCK 2		BLOCK 3		TOTAL		
	Ass. Ct.	Mean Yld. (g)	Ass. Ct.	Mean Yld. (g)	Ass. Ct.	Mean Yld. (g)	Ass. Ct.	Mean Yld. (g) per plant	per m^2
1.0	13	16.84	13	12.20	13	13.37	39	14.14	14.14
1.2	15	12.64	15	14.80	15	11.85	45	13.12	15.74
1.4	17	14.41	16	15.71	16	12.28	49	14.14	19.80
1.7	20	14.80	19	10.49	18	10.62	57	12.04	20.47
2.0	23	13.09	21	10.40	21	9.58	65	11.09	22.18
2.4	23	10.54	23	11.47	22	9.07	68	10.38	24.91
2.8	11	9.38	12	9.90	23	6.63	68	8.62	24.14
3.3	23	8.91	22	7.75	22	7.97	67	8.22	27.13
4.0	21	6.30	22	6.61	20	6.38	63	6.43	25.72
4.8	21	5.00	21	5.57	20	4.79	62	5.13	24.62
5.6	20	5.14	19	5.66	17	4.21	56	5.03	28.17
6.7	19	3.82	18	4.25	11	4.08	48	4.04	27.07
7.7	16	4.24	13	4.83	12	4.49	41	4.50	34.65
9.1	14	2.28	12	2.80	11	3.30	37	2.75	25.03
11.1	10	2.42	10	2.40	8	2.45	28	2.42	26.86
TOTAL	277	8.81	267	8.53	249	7.70	793	8.37	

Mean Bud Yield 1983

Density Plants/ m^2	BLOCK 1 Ass. Ct.	Mean Yld. (g)	BLOCK 2 Ass. Ct.	Mean Yld. (g)	BLOCK 3 Ass. Ct.	Mean Yld. (g)	TOTAL Ass. Ct.	TOTAL Mean Yld. (g) per plant	TOTAL Mean Yld. (g) per m^2
1.0	15	22.85	14	19.01	13	19.88	42	20.65	20.65
1.2	15	19.44	15	20.93	15	16.94	45	19.10	22.92
1.4	16	22.48	17	18.65	17	16.12	50	19.02	26.72
1.7	19	19.88	19	14.14	19	15.39	57	16.47	28.00
2.0	23	16.26	23	15.00	22	13.52	68	14.95	29.85
2.4	23	14.15	23	14.95	23	13.17	69	14.09	33.82
2.8	21	12.31	23	13.02	23	10.91	67	12.07	33.82
3.3	23	11.29	21	9.91	22	11.00	66	10.76	35.45
4.0	21	8.71	20	8.17	21	8.35	62	8.41	33.64
4.8	20	7.89	21	7.34	20	6.24	61	7.16	34.35
5.6	18	6.81	18	7.22	19	6.58	55	6.87	38.47
6.7	15	5.53	17	6.52	15	5.47	47	5.87	39.13
7.7	14	5.64	14	6.32	10	6.00	38	5.98	46.10
9.1	11	3.17	11	3.66	10	4.21	32	3.66	33.49
11.1	11	3.62	7	3.13	6	3.60	24	3.47	38.30
TOTAL	265	12.41	263	11.68	255	10.99	783	11.70	

Mean Cane Fresh Weight 1981

Density Plants/ m²	BLOCK 1 Ass. Ct.	Mean Fwt (g)	BLOCK 2 Ass. Ct.	Mean Fwt (g)	BLOCK 3 Ass. Ct.	Mean Fwt (g)	TOTAL Ass. Ct.	Mean Fwt (g)
1.0	11	161.29	10	81.21	11	148.06	32	131.71
1.2	12	105.19	14	108.59	11	111.57	37	108.37
1.4	15	129.05	15	110.57	16	98.72	46	112.47
1.7	17	135.63	17	66.79	16	101.99	50	101.46
2.0	22	147.26	20	79.75	19	77.16	61	103.29
2.4	21	118.26	21	90.37	21	69.91	63	92.85
2.8	19	139.87	15	84.30	18	52.98	52	93.76
3.3	23	128.28	18	54.55	18	49.43	59	81.73
4.0	21	116.86	15	46.29	14	43.82	50	75.24
4.8	17	107.89	13	47.57	15	36.90	45	66.80
5.6	17	91.79	11	46.84	13	35.11	41	61.76
6.7	16	101.32	7	48.73	12	35.97	35	68.40
7.7	16	93.93	7	70.78	9	38.59	32	73.30
9.1	12	52.72	5	44.65	7	32.75	24	45.22
11.1	12	60.84	5	33.19	5	21.46	22	45.61
TOTAL	251	115.32	193	72.12	205	66.26	649	86.98

Mean Cane Fresh Weight 1982

Density Plants/ m²	BLOCK 1		2		3		TOTAL	
	Ass. Ct.	Mean Fwt (g)	Ass. Ct.	Mean Fwt (g)	Ass. Ct.	Mean Fwt (g)	Ass. Ct.	Mean Fwt (g)
1.0	13	513.44	13	287.14	13	333.80	39	378.12
1.2	15	415.49	15	395.29	15	265.03	45	358.60
1.4	17	490.59	16	417.05	16	306.27	49	406.39
1.7	20	505.08	19	283.16	18	279.00	57	359.71
2.0	23	519.33	22	305.12	21	224.84	66	354.22
2.4	23	399.76	23	383.57	22	241.67	68	343.14
2.8	22	442.41	23	325.69	23	174.37	68	312.27
3.3	23	374.26	22	264.48	21	210.49	66	285.56
4.0	21	317.26	22	235.31	19	174.36	62	244.39
4.8	21	229.57	21	197.19	20	118.88	62	182.95
5.6	20	206.39	19	223.47	17	97.27	56	179.06
6.7	19	169.27	18	164.52	11	92.96	48	150.00
7.7	16	192.26	13	187.46	12	107.05	41	165.80
9.1	14	104.32	12	132.90	11	76.80	37	105.41
11.1	10	122.52	10	110.48	8	53.10	28	98.39
TOTAL	277	344.47	268	269.48	247	192.82	792	271.80

Mean Cane Fresh Weight 1983

Density Plants/ m²	BLOCK 1		2		3		TOTAL	
	Ass. Ct.	Mean Fwt (g)	Ass. Ct.	Mean Fwt (g)	Ass. Ct.	Mean Fwt (g)	Ass. Ct.	Mean Fwt (g)
1.0	15	792.48	14	576.89	13	654.97	42	678.05
1.2	15	705.96	15	757.43	15	550.37	45	671.25
1.4	16	820.46	17	684.58	17	572.82	50	690.06
1.7	19	777.48	19	524.68	19	585.77	57	629.31
2.0	23	736.93	23	609.16	22	499.42	68	616.87
2.4	23	569.78	23	610.77	23	508.16	69	562.90
2.8	21	537.34	23	536.04	23	420.72	67	496.86
3.3	23	474.81	21	415.83	22	447.38	66	446.90
4.0	21	362.51	20	337.23	21	372.76	62	357.83
4.8	20	292.42	21	307.07	20	280.14	61	293.44
5.6	18	235.21	18	304.58	19	322.48	55	288.06
6.7	15	191.45	17	248.64	15	247.28	47	229.95
7.7	14	185.21	14	250.50	10	277.79	38	233.63
9.1	11	88.84	11	150.33	10	195.37	32	143.27
11.1	11	107.54	6	139.78	6	160.56	23	129.78
TOTAL	265	428.85	262	454.45	255	426.64	782	455.01

Mean Shoot Number 1981

Density Plants/ m^2	BLOCK 1		2		3		TOTAL	
	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.
1.0	11	9.55	10	5.80	11	7.18	32	7.56
1.2	12	6.92	14	6.57	11	7.09	37	6.84
1.4	15	7.20	15	7.07	16	5.81	46	6.67
1.7	17	7.65	17	5.18	16	6.25	50	6.36
2.0	22	7.68	20	6.40	19	5.68	61	6.64
2.4	21	6.71	21	6.71	21	4.71	63	6.05
2.8	19	7.63	16	7.25	18	5.78	53	6.89
3.3	23	7.17	18	5.06	18	4.83	59	5.81
4.0	21	6.71	15	4.73	14	5.50	50	5.78
4.8	17	7.24	13	5.85	15	4.07	45	5.78
5.6	17	6.35	11	5.73	13	4.85	41	5.71
6.7	16	7.38	7	5.57	12	5.17	35	6.26
7.7	16	6.63	7	6.43	9	4.33	32	5.94
9.1	12	5.00	5	8.00	7	4.71	24	5.54
11.1	12	6.25	5	4.40	5	3.60	22	5.23
TOTAL	251	7.08	194	6.06	205	5.37	650	6.24

Mean Shoot Number 1982

Density Plants/ m^2	BLOCK 1		2		3		TOTAL	
	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.
1.0	13	18.00	13	12.23	13	13.46	39	14.56
1.2	15	13.60	15	14.47	15	11.80	45	13.29
1.4	17	15.76	16	13.56	16	12.31	49	13.92
1.7	20	14.35	19	9.42	18	11.94	57	11.95
2.0	23	13.83	22	11.00	21	10.00	66	11.67
2.4	23	12.09	23	12.39	22	10.00	68	11.51
2.8	22	13.00	23	11.61	23	7.52	68	10.68
3.3	23	12.09	22	9.09	22	8.59	67	9.96
4.0	21	10.76	22	9.05	20	7.95	63	9.27
4.8	21	9.71	21	8.71	20	6.45	62	8.32
5.6	20	9.45	19	8.26	17	6.24	56	8.07
6.7	19	7.74	18	8.39	11	4.91	48	7.33
7.7	16	8.25	13	8.92	12	6.58	41	7.98
9.1	14	6.50	12	6.67	11	5.27	37	6.19
11.1	10	8.30	10	6.80	8	5.25	28	6.89
TOTAL	277	11.64	268	10.15	249	8.77	794	10.24

Mean Shoot Number 1983

Density Plants/ m^2	BLOCK 1		2		3		TOTAL	
	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.	Ass. Ct.	Mean No.
1.0	15	33.00	14	28.14	13	31.38	42	30.88
1.2	15	29.67	15	32.80	15	25.80	45	29.42
1.4	16	32.88	17	30.47	17	25.47	50	29.54
1.7	19	32.11	19	22.47	19	25.79	57	26.79
2.0	23	27.13	23	25.13	22	21.82	68	24.74
2.4	23	25.13	23	24.65	23	20.87	69	23.55
2.8	21	23.10	23	23.13	23	16.96	67	21.00
3.3	23	21.22	21	19.19	22	17.45	66	19.32
4.0	21	17.38	20	15.80	21	14.76	62	15.98
4.8	20	15.70	21	15.52	20	11.65	61	14.31
5.6	18	13.00	18	14.61	19	12.00	55	13.18
6.7	15	12.80	17	14.29	15	9.53	47	12.30
7.7	14	11.00	14	13.14	10	10.60	38	11.68
9.1	11	6.09	11	10.91	10	8.60	32	8.53
11.1	11	9.55	7	8.57	6	7.17	24	8.67
TOTAL	265	21.44	263	20.62	255	18.04	783	20.06

Mean Cane Length 1981

Density Plants/ m²	BLOCK 1		BLOCK 2		BLOCK 3		TOTAL	
	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)
1.0	11	39.63	10	34.63	11	44.82	32	39.85
1.2	12	36.84	14	37.99	11	35.35	37	36.83
11.4	15	44.83	15	37.51	16	36.57	46	39.57
1.7	17	42.46	17	32.80	16	36.26	50	37.19
2.0	22	48.25	20	35.53	19	36.94	61	40.56
2.4	21	46.51	20	38.22	21	40.31	62	41.74
2.8	19	41.82	15	31.75	18	26.92	52	33.76
3.3	23	44.67	18	34.92	18	35.06	59	38.76
4.0	21	48.30	14	32.11	14	25.84	49	37.26
4.8	17	41.79	10	28.43	15	30.54	42	34.59
5.6	17	40.94	11	26.34	13	25.02	41	31.97
6.7	16	39.75	7	28.17	12	22.95	35	31.67
7.7	16	39.40	7	28.64	9	26.38	32	33.38
9.1	12	39.04	5	18.54	7	25.44	24	30.80
11.1	12	31.21	5	25.20	5	23.10	22	28.00
TOTAL	251	42.48	188	33.08	205	32.50	644	36.56

Mean Cane Length 1982

Density Plants/ m²	BLOCK						TOTAL	
	1		2		3			
	Ass. Ct.	Mean Lgth. (cm)	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth. (cm)
1.0	13	50.07	13	43.15	13	43.83	39	45.68
1.2	15	52.73	15	48.07	15	41.11	45	47.31
1.4	17	54.54	16	52.24	16	44.04	49	50.36
1.7	20	56.49	19	49.42	18	44.48	57	50.34
2.0	23	60.44	21	50.09	21	46.26	65	52.52
2.4	23	56.33	23	53.38	22	47.28	68	52.41
2.8	22	61.04	23	54.59	23	45.50	68	53.60
3.3	23	62.04	21	56.86	22	48.08	66	55.74
4.0	21	59.23	22	55.48	20	42.53	63	52.62
4.8	21	67.36	21	52.68	20	43.82	62	51.41
5.6	20	54.17	19	53.60	17	42.11	56	50.31
6.7	19	54.58	18	52.75	11	43.41	48	51.34
7.7	16	59.67	13	54.56	12	39.76	41	52.22
9.1	14	52.77	12	57.36	11	38.92	37	50.14
11.1	10	50.23	10	53.44	8	32.04	28	46.18
TOTAL	277	56.75	266	52.68	249	43.73	792	51.29

Mean Cane Length 1983

Density Plants/ m²	BLOCK 1		BLOCK 2		BLOCK 3		TOTAL	
	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)	Ass. Ct.	Mean Lgth.(cm)
1.0	15	48.54	14	44.99	13	45.78	42	46.50
1.2	15	48.76	15	49.94	15	46.60	45	48.44
1.4	16	50.30	17	49.86	16	48.14	49	49.44
1.7	19	50.94	19	49.79	19	48.46	57	49.73
2.0	23	55.08	23	51.83	22	49.22	68	52.08
2.4	23	51.37	23	53.41	23	50.39	69	51.72
2.8	21	52.58	23	53.26	23	52.27	67	52.71
3.3	23	52.73	21	52.05	22	54.15	66	52.99
4.0	21	49.96	20	52.99	21	54.64	62	52.52
4.8	20	47.10	21	50.93	20	54.42	61	50.82
5.6	18	44.99	18	51.73	19	59.77	55	52.30
6.7	15	43.54	17	46.10	15	56.68	47	48.66
7.7	14	44.18	14	47.51	10	57.15	38	48.82
9.1	11	43.31	11	45.57	10	56.04	32	48.06
11.1	11	39.01	7	49.96	6	57.58	24	46.84
TOTAL	265	48.96	263	50.46	254	52.44	782	50.60

Mean Basal Cane Girth 1983

Density Plants/ m^2	BLOCK 1		2		3		TOTAL	
	Ass. Ct.	Mean Girth	Ass. Ct.	Mean Girth	Ass. Ct.	Mean Girth	Ass. Ct.	Mean Girth
1.0	15	1.09	14	1.00	13	1.01	42	1.03
1.2	15	1.08	15	1.01	15	0.97	45	1.02
1.4	16	1.11	17	0.98	16	1.04	49	1.04
1.7	19	1.05	19	1.02	19	1.00	57	1.02
2.0	23	1.08	23	1.03	22	1.03	68	1.05
2.4	23	1.03	23	1.02	23	1.05	69	1.03
2.8	21	0.98	23	0.94	23	1.05	67	0.99
3.3	23	0.96	21	0.95	22	1.01	66	0.98
4.0	21	0.90	20	0.92	21	0.98	62	0.93
4.8	20	0.93	21	0.93	20	0.97	61	0.94
5.6	18	0.86	18	0.92	19	1.03	55	0.94
6.7	15	0.79	17	0.91	15	1.07	47	0.92
7.7	14	0.84	14	0.86	10	0.97	38	0.88
9.1	11	0.83	11	0.81	10	0.97	32	0.87
11.1	11	0.76	7	0.90	6	0.96	24	0.85
TOTAL	265	0.96	263	0.95	254	1.01	782	0.98

6.   **Appendix IV 9.1   GENSTAT program for fit polynomial**

```
100    'REFE' POLY83A
200    'UNITS' $45
300    'FACT' BLOCK $3
400       :    DEN $15
500    'GENE' BLOCK,DEN
600    'VARI' DENSITY=1,1.2,1.4,1.7,2,2.4,2.8,3.3,4,4.8,5.6,6.7,7.7,9.1,11.1
700    'BLOCK' BLOCK
800    'TREAT' POL(DEN,3,DENSITY)
900    'FOR' I=1...5
1000   'READ' Y
1100   'CALC' LOGY = LOG(Y)
1200   'PRIN/P' DEN,BLOCK,Y,LOGY $10.2,10.4
1300   'ANOVA' Y
1400      :    LOGY
1500   'REPE'
1600   'RUN'
```

Analysis of Variance
Variate: Bud Weight 1981

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	29.499	11.28	14.749	13.084
Block. Units Stratum					
Den	14	200.418	76.65	14.316	12.699
Lin	1	182.732	69.88	182.732	162.103
Quad	1	12.337	4.72	12.337	10.944
Cub	1	0.715	0.27	0.715	0.535
Deviations	11	4.633	1.77	0.421	0.374
Residual	28	31.563	12.07	1.127	
Total	42	231.981	88.72	5.523	
GRAND TOTAL	44	261.480	100.00		

Variate: Bud Weight 1982

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	12.567	1.64	6.283	4.332
Block. Units Stratum					
Den	14	712.774	93.06	50.912	35.098
Lin	1	641.952	83.81	641.952	442.547
Quad	1	59.417	7.76	59.417	40.961
Cub	1	0.028	0.00	0.028	0.019
Deviations	11	11.376	1.49	1.034	0.713
Residual	28	40.616	5.30	1.451	
Total	42	753.391	98.36	17.938	
GRAND TOTAL	44	765.957	100.00		

Variate: Bud Weight 1983

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	16.977	1.13	8.489	4.749
Block. Units Stratum					
Den	14	1436.095	95.54	102.578	57.387
Lin	1	1194.671	79.48	1194.671	668.350
Quad	1	197.553	13.14	197.553	110.520
Cub	1	33.166	2.21	33.166	18.554
Deviations	11	10.705	0.71	0.973	0.544
Residual	28	50.050	3.33	1.787	
Total	42	1486.145	98.87	35.384	
GRAND TOTAL	44	1503.122	100.00		

Variate: Fresh Weight 1981

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	22264.2	36.01	11132.1	32.783
Block. Units Stratum					
Den	14	30057.7	48.61	2147.0	6.323
Lin	1	25212.7	40.78	25212.7	74.250
Quad	1	2313.4	3.74	2313.4	6.813
Cub	1	1187.6	1.92	1187.6	3.497
Deviations	11	1343.9	2.17	122.2	0.360
Residual	28	9507.9	15.38	339.6	
Total	42	39565.6	63.99	942.0	
GRAND TOTAL	44	61829.8	100.00		

Variate: Fresh Weight 1982

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	167914	23.21	83957	31.435
Block. Units Stratum					
Den	14	480828	66.46	34345	12.859
Lin	1	436286	60.30	436286	163.353
Quad	1	29153	4.03	29153	10.916
Cub	1	104	0.01	104	0.039
Deviations	11	15284	2.11	1389	0.520
Residual	28	74783	10.34	2671	
Total	42	555611	76.79	13229	
GRAND TOTAL	44	723526	100.00		

Variate: Fresh Weight 1983

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	20457	1.08	10229	1.841
Block. Units Stratum					
Den	14	1718857	90.71	122776	22.099
Lin	1	1539652	81.25	1539652	277.128
Quad	1	149074	7.87	149074	26.832
Cub	1	3506	0.19	3506	0.631
Deviations	11	26626	1.41	2421	0.436
Residual	28	155561	8.21	0556	
Total	42	1874418	98.92	44629	
GRAND TOTAL	44	1894875	100.00		

Variate: Shoot Number 1981

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	23.6155	36.01	11.8078	14.726
Block. Units Stratum					
Den	14	19.5125	29.75	1.3937	1.738
Lin	1	11.7768	17.96	11.7768	14.587
Quad	1	0.5788	0.88	0.5788	0.722
Cub	1	3.8291	5.84	3.8291	4.775
Deviations	11	3.3277	5.07	0.3025	0.377
Residual	28	22.4519	34.24	0.8019	
Total	42	41.9643	63.99	0.9992	
GRAND TOTAL	44	65.5799	100.00		

Variate: Shoot Number 1982

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	67.984	16.57	33.992	25.341
Block. Units Stratum					
Den	14	304.792	74.28	21.771	16.230
Lin	1	278.938	67.98	278.938	207.950
Quad	1	15.729	3.83	15.729	11.726
Cub	1	0.953	0.23	0.953	0.711
Deviations	11	9.172	2.24	0.834	0.622
Residual	28	37.558	9.15	1.341	
Total	42	342.351	83.43	8.151	
GRAND TOTAL	44	410.335	100.00		

Variate: Shoot Number 1983

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	91.763	3.26	45.882	10.135
Block. Units Stratum					
Den	14	2596.574	92.24	185.470	40.971
Lin	1	2254.869	80.10	2254.869	498.105
Quad	1	297.908	10.58	297.908	65.809
Cub	1	27.413	0.97	27.413	6.056
Deviations	11	16.384	0.58	1.489	0.329
Residual	28	126.753	4.50	4.527	
Total	42	2723.327	96.74	64.841	
GRAND TOTAL	44	2815.090	100.00		

Variate: Cane Length 1981

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	1058.17	43.53	529.09	34.927
Block. Units Stratum					
Den	14	948.34	39.02	67.74	4.472
Lin	1	519.01	21.35	519.01	34.262
Quad	1	219.69	9.04	219.69	14.503
Cub	1	72.10	2.97	72.10	4.760
Deviations	11	137.53	5.66	12.50	0.825
Residual	28	424.15	17.45	15.15	
Total	42	1372.49	56.47	32.68	
GRAND TOTAL	44	2430.66	100.00		

Variate: Cane Length 1982

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	1404.636	69.81	702.318	71.394
Block. Units Stratum					
Den	14	332.067	16.50	23.719	2.411
Lin	1	31.154	1.55	31.154	3.167
Quad	1	174.998	8.70	174.998	17.789
Cub	1	60.035	2.98	60.035	6.103
Deviations	11	65.880	3.27	5.989	0.609
Residual	28	275.443	13.69	9.837	
Total	42	607.510	30.19	14.465	
GRAND TOTAL	44	2012.146	100.00		

Variate: Cane Length 1983

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	160.37	19.79	80.18	4.632
Block. Units Stratum					
Den	14	165.24	20.39	11.80	0.682
Lin	1	4.24	0.52	4.24	0.245
Quad	1	53.86	6.65	53.86	3.111
Cub	1	79.53	9.81	79.53	4.594
Deviations	11	27.62	3.41	2.51	0.145
Residual	28	484.71	59.82	17.31	
Total	42	649.95	80.21	15.48	
GRAND TOTAL	44	810.32	100.00		

Variate: Basal Cane Girth 1983

Source of Variation	DF	SS	SS%	MS	VR
Block stratum	2	0.033524	11.01	0.016762	4.574
Block. Units Stratum					
Den	14	0.168311	55.28	0.012022	3.281
Lin	1	0.150008	49.27	0.150008	40.934
Quad	1	0.006892	2.26	0.006892	1.881
Cub	1	0.000358	0.12	0.000358	0.098
Deviations	11	0.011054	3.63	0.001005	0.274
Residual	28	0.102609	33.70	0.003665	
Total	42	0.270920	88.99	0.006450	
GRAND TOTAL	44	0.304444	100.00		

7. **Appendix VI 9.1 GENSTAT program for correlation regression analysis**

```
100     'REFE' POLY83B
200     'UNITS' $45
300     'FACT' BLOCK $3
400       :    DEN $15
500     'GENE' BLOCK,DEN
600     'VARI' DENX=11.1, 9.1, 7.7, 6.7, 5.6, 4.8, 4.0, 3.3, 2.8, 2.4, 2.0, 1.7, 1.4, 1.2, 1.0,
700                   11.1, 9.1, 7.7, 6.7, 5.6, 4.8, 4.0, 3.3, 2.8, 2.4, 2.0, 1.7, 1.4, 1.2, 1.0,
800                   11.1, 9.1, 7.7, 6.7, 5.6, 4.8, 4.0, 3.3, 2.8, 2.4, 2.0, 1.7, 1.4, 1.2, 1.0
900     'FOR' I=1...5
1000    'READ' Y
1100    'CALC' DENXX=DENX*DENX
1200       :    DENXXX=DENXX*DENX
1300    'TERMS/PRIN=C' DENX,DENXX,DENXXX,Y
1400    'Y' Y
1500    'FIT/PRIN=CAU' DENX
1600    'ADD/PRIN=CAU' DENXX
1700          :      DENXXX
1800    'REPE'
1900    'RUN'
```

Correlation Matrices and analysis of variance tables for each variate

Bud Weight 1981   Correlation Matrix   DF = 43

DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	0.8369	0.7579	0.6775	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	2.91616	0.35129	8.30
DENX	0.66853	0.06668	10.03

Analysis of Variance

	DF	SS	MS
Regression	1	182.95	182.946
Residual	43	78.25	1.829
Total	44	261.20	5.936
Change	-1	-182.95	182.945

Percentage variance accounted for 69.3

Regression Coefficients Y-Variate: Y

	Estimate	S.E.	T
Constant	1.670185	0.552872	3.02
DENX	1.350029	0.251802	5.36
DENXX	-0.061173	0.021908	-2.79

Analysis of Variance

	DF	SS	MS
Regression	2	195.20	97.599
Residual	42	66.00	1.571
Total	44	261.20	5.936
Change	-1	-12.25	12.252

Percentage variance accounted for 73.5

Regression Coefficients Y-Variate: Y

	Estimate	S.E.	T
Constant	1.1361509	0.9821044	1.16
DENX	1.8118505	0.7442305	2.43
DENXX	-0.1570710	0.1469627	-1.07
DENXXX	0.0054280	0.0082241	0.65

Analysis of Variance

	DF	SS	MS
Regression	3	195.89	65.297
Residual	41	65.30	1.593
Total	44	261.20	5.936
Change	-1	-0.69	0.694

Percentage variance accounted for 73.2

Bud Weight 1982

		Correlation Matrix	DF = 43		
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.8883	-0.7786	-0.6800	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients - Y-Variate: Y

	Estimate	S.E.	T
Constant	13.3974	0.5037	26.56
DENX	-1.2158	0.0956	-12.72

Analysis of Variance

	DF	SS	MS
Regression	1	605.1	605.073
Residual	43	160.9	3.741
Total	44	756.0	17.408
Change	-1	-605.1	605.073

Percentage variance accounted for 78.5

Regression Coefficients Y-Variate: Y

	Estimate	S.E.	T
Constant	16.69643	0.58563	28.51
DENX	-3.03008	0.26672	-11.36
DENXX	0.16286	0.02321	7.02

Analysis of Variance

	DF	SS	MS
Regression	2	691.91	345.954
Residual	42	74.05	1.763
Total	44	765.96	17.408
Change	-1	-86.83	86.834

Percentage variance accounted for 98.9

Regression Coefficients Y-Variate: Y

	Estimate	S.E.	T
Constant	18.805259	0.966391	19.46
DENX	-4.853751	0.732323	-6.63
DENXX	0.541542	0.144611	3.74
DENXXX	-0.021434	0.008093	-2.65

Analysis of Variance

	DF	SS	MS
Regression	3	702.73	234.242
Residual	41	63.23	1.542
Total	44	765.96	17.408
Change	-1	-10.82	10.819

Percentage variance accounted for 91.1

Bud Weight 1983

		Correlation Matrix	DF = 43		
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	0.9460	0.8606	0.7690	1.0000
15		1	2	3	4

	Regression Analysis - Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	3.4016	0.4991	6.82
DENX	1.8128	0.0947	19.14

	Analysis of Variance		
	DF	SS	MS
Regression	1	1345.2	1345.159
Residual	43	158.0	3.674
Total	44	1503.1	34.162
Change	-1	-1345.2	1345.159

Percentage variance accounted for 89.2

	Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	0.24294	0.60574	0.40
DENX	3.54046	0.27588	12.83
DENXX	-0.15508	0.02400	-6.46

	Analysis of Variance		
	DF	SS	MS
Regression	2	1423.90	711.950
Residual	42	79.22	1.886
Total	44	1503.12	34.162
Change	-1	-78.74	78.741

Percentage variance accounted for 94.5

	Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	0.0968823	1.0813591	0.09
DENX	3.6667672	0.8194448	4.47
DENXX	-0.1813089	0.1618152	-1.12
DENXXX	0.0014846	0.0090553	0.16

	Analysis of Variance		
	DF	SS	MS
Regression	3	1423.95	474.651
Residual	41	79.17	1.931
Total	44	1503.12	34.162
Change	-1	-0.05	0.052

Percentage variance accounted for 94.3

Fresh Weight 1981

		Correlation Matrix		DF = 43	
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.6386	-0.5713	-0.5116	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	115.2106	7.5992	15.15
DENX	-7.8482	1.4423	-5.44

Analysis of Variance

	DF	SS	MS
Regression	1	25213	25212.7
Residual	43	36617	851.6
Total	44	61830	1405.2
Change	-1	-25213	25212.7

Percentage variance accounted for 39.4

Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	132.33163	12.60465	10.50
DENX	-17.21273	5.74070	-3.00
DENXX	0.84059	0.49946	1.68

Analysis of Variance

	DF	SS	MS
Regression	2	27526	13763.1
Residual	42	34304	816.8
Total	44	61830	1405.2
Change	-1	-2313	2313.4

Percentage variance accounted for 41.9

Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	154.42605	22.11608	6.98
DENX	-36.31952	16.75978	-2.17
DENXX	4.80813	3.30946	1.45
DENXXX	-0.22457	0.18520	-1.21

Analysis of Variance

	DF	SS	MS
Regression	3	28714	9571.3
Residual	41	33116	807.7
Total	44	61830	1405.2
Change	-1	-1188	1187.6

Percentage variance accounted for 42.5

Fresh Weight 1982

		Correlation Matrix		DF = 43	
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.7764	-0.7033	-0.6251	1.0000
		1	2	3	4
15					

	Regression Analysis :- Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	400.334	21.282	18.81
DENX	-32.636	4.039	-8.08

	Analysis of Variance		
	DF	SS	MS
Regression	1	435975	435975
Residual	43	287186	6679
Total	44	723161	16435

	Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	460.9959	34.5774	13.33
DENX	-65.8150	15.7480	-4.18
DENXX	2.9783	1.3701	2.17

	Analysis of Variance		
	DF	SS	MS
Regression	2	465017	232508
Residual	42	258145	6146
Total	44	723161	16435
Change	-1	-29042	29042

Percentage variance accounted for 62.6

	Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	454.615156	61.735818	7.36
DENX	-60.297092	46.782884	-1.29
DENXX	1.832483	9.238183	0.20
DENXX	0.064854	0.516974	0.13

	Analysis of Variance		
	DF	SS	MS
Regression	3	465116	155039
Residual	41	258046	6294
Total	44	723161	16435
Change	-1	-99	99

Percentage variance accounted for 61.7

Fresh Weight 1983

		Correlation Matrix	DF = 43		
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.9014	-0.8047	-0.7097	1.0000
		1	2	3	4

Regression Analysis - Regression Coefficients Y-Variate: Fresh Weight

	Estimate	S.E.	T
Constant	696.677	23.669	29.43
DENX	-61.330	4.492	-13.65

Analysis of Variance

	DF	SS	MS
Regression	1	1539652	1539652
Residual	43	355223	8261
Total	44	1894875	43065
Change	-1	-1539652	1539652

Percentage variance accounted for 80.8

Regression Coefficients Y-Variate: Fresh Weight

	Estimate	S.E.	T
Constant	834.1143	30.8995	26.99
DENX	-136.5024	14.0730	-9.70
DENXX	6.7477	1.2244	5.51

Analysis of Variance

	DF	SS	MS
Regression	2	1688726	844363
Residual	42	206149	4908
Total	44	1894375	43065
Change	-1	-149074	149074

Percentage variance accounted for 88.6

Regression Coefficients Y-Variate: Fresh Weight

	Estimate	S.E.	T
Constant	872.07460	54.70858	15.94
DENX	-169.32964	41.45770	-4.08
DENXX	13.56473	8.18662	1.66
DENXXX	-0.38583	0.45813	-0.84

Analysis of Variance

	DF	SS	MS
Regression	3	1692232	564077
Residual	41	202644	4943
Total	44	1893875	43065
Change	-1	-3506	3506

Percentage variance accounted for 88.5

Shoot Number 1981

		Correlation Matrix	DF = 43		
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.4238	-0.3876	-0.3628	1.0000
		1	2	3	4

15

Regression Analysis - Regression Coefficients Y-Variate: Y			
	Estimate	S.E.	T
Constant	6.87453	0.29129	23.60
DENX	-0.16962	0.05529	-3.07

Analysis of Variance			
	DF	SS	MS
Regression	1	11.78	11.777
Residual	43	53.80	1.251
Total	44	65.58	1.490
Change	-1	-11.78	11.777

Percentage variance accounted for 16.0

Regression Coefficients Y-Variate: Y			
	Estimate	S.E.	T
Constant	7.145355	0.496485	14.39
DENX	-0.317748	0.226125	-1.41
DENXX	0.013296	0.019674	0.68

Analysis of Variance			
	DF	SS	MS
Regression	2	12.36	6.178
Residual	42	53.22	1.267
Total	44	65.58	1.490
Change	-1	-1.58	0.579

Percentage variance accounted for 15.0

Regression Coefficients Y-Variate: Y			
	Estimate	S.E.	T
Constant	8.399917	0.854144	9.83
DENX	-1.402666	0.647263	-2.17
DENXX	0.238581	0.127815	1.87
DENXXX	-0.012751	0.007153	-1.78

Analysis of Variance			
	DF	SS	MS
Regression	3	16.18	5.395
Residual	41	49.40	1.205
Total	44	65.58	1.490
Change	-1	-3.83	3.829

Percentage variance accounted for 19.2

Shoot Number 1982

		Correlation Matrix	DF = 43		
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.7823	-0.6792	-0.5870	1.0000
		1	2	3	4
15					

	Regression Analysis - Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	13.43397	0.50113	26.81
DENX	-0.78322	0.09511	-8.23

	Analysis of Variance		
	DF	SS	MS
Regression	1	251.1	251.100
Residual	43	159.2	7.703
Total	44	410.3	9.316
Change	-1	-251.1	251.100

Percentage variance accounted for 60.3

	Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	15.75007	0.73581	21.41
DENX	-2.05003	0.33512	-6.12
DENXX	0.11371	0.02916	3.90

	Analysis of Variance		
	DF	SS	MS
Regression	2	293.4	146.718
Residual	42	116.9	2.783
Total	44	410.3	0.326
Change	-1	-42.3	42.336

Percentage variance accounted for 70.2

	Regression Coefficients Y-Variate: Y		
	Estimate	S.E.	T
Constant	16.823239	1.298154	12.96
DENX	-2.978090	0.983730	-3.03
DENXX	0.306425	0.194256	1.58
DENXXX	-0.010908	0.010871	-1.00

	Analysis of Variance		
	DF	SS	MS
Regression	3	296.2	98.746
Residual	41	114.1	2.763
Total	44	410.3	9.325
Change	-1	-2.8	2.802

Percentage variance accounted for 70.2

Shoot Number 1983

		Correlation Matrix	DF = 43		
DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	0.9147	0.8175	0.7207	1.0000
		1	2	3	4

15

Regression Analysis - Regression Coefficients Y-Variate: Y			
	Estimate	S.E.	T
Constant	8.9356	0.8514	10.49
DENX	2.3988	0.1616	14.84

Analysis of Variance			
	DF	SS	MS
Regression	1	2355.4	2355.42
Residual	43	459.7	10.69
Total	44	2815.1	63.98
Change	-1	-2355.4	2355.42

Percentage variance accounted for 83.3

Regression Coefficients Y-Variate: Y			
	Estimate	S.E.	T
Constant	3.62901	1.04865	3.46
DENX	5.30129	0.47760	11.10
DENXX	-0.26054	0.04155	-6.27

Analysis of Variance			
	DF	SS	MS
Regression	2	2577.7	1288.829
Residual	42	237.4	5.653
Total	44	2815.1	63.979
Change	-1	-222.2	222.241

Percentage variance accounted for 91.2

Regression Coefficients Y-Variate: Y			
	Estimate	S.E.	T
Constant	2.6485564	1.8634112	1.42
DENX	6.1491608	1.4120773	4.35
DENXX	-0.4365975	0.2788419	-1.57
DENXXX	0.0099654	0.0156042	0.64

Analysis of Variance			
	DF	SS	MS
Regression	3	2580.0	859.999
Residual	41	235.1	5.734
Total	44	2815.1	63.979
Change	-1	-2.3	2.339

Percentage variance accounted for 91.0

Cane Length 1981   Correlation Matrix   DF = 43

DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	0.4621	0.3739	0.3092	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	29.9738	1.7363	17.26
DENX	1.1260	0.3296	3.42

Analysis of Variance

	DF	SS	MS
Regression	1	519	519.01
Residual	43	1912	44.46
Total	44	2431	55.24
Change	-1	-519	519.01

Percentage variance accounted for 19.5

Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	24.69770	2.79974	8.82
DENX	4.01182	1.27494	3.15
DENXX	-0.25904	0.110092	-2.34

Analysis of Variance

	DF	SS	MS
Regression	2	739	369.35
Residual	42	1692	40.28
Total	44	2431	55.24
Change	-1	-220	219.69

Percentage variance accounted for   27.1

Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	19.253785	4.891330	3.94
DENX	8.719605	3.706609	2.35
DENXX	-1.236614	0.731941	-1.69
DENXXX	0.055332	0.040960	1.35

Analysis of Variance

	DF	SS	MS
Regression	3	811	270.27
Residual	41	1620	79.51
Total	44	2431	-5.24
Change	-1	-72	-2.10

Percentage variance accounted for 28.5

Cane Length 1982   Correlation Matrix   DF = 43

DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.0941	-0.1607	-0.1918	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	51.40185	1.77348	28.98
DENX	-0.20861	0.33661	-0.62

Analysis of Variance

	DF	SS	MS
Regression	1	18	17.81
Residual	43	1994	46.38
Total	44	2012	45.73
Change	-1	-18	17.81

Residual variance exceeds variance of Y-variate

Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	46.89200	2.91433	16.09
DENX	2.25809	1.32731	1.70
DENXX	-0.22142	0.11548	-1.92

Analysis of Variance

	DF	SS	MS
Regression	2	178	89.16
Residual	42	1834	43.66
Total	44	2012	45.73
Change	-1	-161	160.52

Percentage variance accounted for 4.5

Regression Coefficients   Y-Variate: y

	Estimate	S.E.	T
Constant	42.709879	5.143622	8.30
DENX	5.874705	3.897793	1.51
DENXX	-0.972413	0.769695	-1.26
DENXXX	0.042508	0.043073	0.99

Analysis of Variance

	DF	SS	MS
Regression	3	221	-3.63
Residual	41	1791	43.69
Total	44	2012	45.73
Change	-1	-43	42.55

Percentage variance accounted for 4.5

Cane Length 1983   Correlation Matrix   DF = 43

DENX	1	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	-0.1649	-0.2427	-0.2769	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	51.30440	1.11499	46.01
DENX	-0.23199	0.21163	-1.10

Analysis of Variance

	DF	SS	MS
Regression	1	22.0	22.03
Residual	43	788.3	18.33
Total	44	810.3	18.42
Change	-1	-22.0	22.03

Percentage variation accounted for 0.5

Regression Coefficients Y-Variate: Y

	Estimate	S.E.	T
Constant	47.89004	1.79679	26.67
DENX	1.63553	0.81788	2.00
DENXX	-0.16763	0.07116	-2.36

Analysis of Variance

	DF	SS	MS
Regression	2	114.0	57.02
Residual	42	696.3	16.58
Total	44	810.3	18.42
Change	-1	-92.0	92.0

Percentage variance accounted for 10.0

Regression Coefficients Y-Variate: Y

	Estimate	S.E.	T
Constant	44.447743	3.139800	14.16
DENX	4.612354	2.379314	1.94
DENXX	-0.785775	0.469841	-1.67
DENXXX	0.034988	0.026293	1.33

Analysis of Variance

	DF	SS	MS
Regression	3	142.9	47.62
Residual	41	667.5	16.28
Total	44	810.3	18.42
Change	-1	-28.8	28.83

Percentage variance accounted for 11.6

Basal Cane Girth 1983   Correlation Matrix   DF = 43

DENX	1.	1.0000			
DENXX	2	0.9693	1.0000		
DENXXX	3	0.9111	0.9834	1.0000	
Y	4	0.6275	0.5205	0.4322	1.0000
15		1	2	3	4

Regression Analysis - Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	0.894960	0.017061	52.46
DENX	0.017113	0.003238	5.28

Analysis of Variance

	DF	SS	MS
Regression	1	0.1199	0.119878
Residual	43	0.1846	0.004292
Total	44	0.3044	0.006919
Change	-1	-0.1199	0.119878

Percentage variance accounted for 38.0

Regression Coefficients   Y-Variate: Y

	Estimate	S.E.	T
Constant	0.8249491	0.0259934	31.74
DENX	0.0554061	0.0118385	4.68
DENXX	-0.0034373	0.0010300	-3.34

Analysis of Variance

	DF	SS	MS
Regression	2	0.1586	0.079281
Residual	42	0.1459	0.003473
Total	44	0.3044	0.006919
Change	-1	-0.0387	0.038683

Percentage variance accounted for 49.8

Regression Coefficients   Y-Variate:Y

	Estimate	S.E.	T
Constant	0.79266242	0.04601328	17.23
DENX	0.08332699	0.03486847	2.39
DENXX	-0.00923509	0.00688545	-1.34
DENXXX	0.00032817	0.00038531	0.85

Analysis of Variance

	DF	SS	MS
Regression	3	0.1611	0.053699
Residual	41	0.1433	0.003496
Total	44	0.3044	0.006919
Change	-1	-0.0025	0.002536

Percentage variance accounted for 49.5

8. <u>Appendix IV 9.1</u> GENSTAT computer program to test form of yield-density relationship

```
100   'REFE' ANOVA
200   'UNITS' $819
300   'INPUT' 2
400   'READ/NUN=Q' DENSITY,YIELD $S ,1,3x,1,/
500   'INPUT' 1
600   'FACT' BLOCK $3= 273(1),273(2),273(3)
700     :   DEN $15
800   'VARI' DENS=1,1.2,1.4,1.7,2,2.4,2.8,3.3,4,4.8,5.6,6.7,7.7,9.1,11.1
900     :   LIMS=1,1.2,1.4,1.7,2,2.4,2.8,3.3,4,4.8,5.6,6.7,7.7,9.1
1000  'GROUP' DEN=LIMITS (DENSITY;LIMS)
1100  'CALC' RECIP=1/YIELD
1200  'BLOCK' BLOCK
1300  'TREAT' POL(DEN,3,DENS)
1400  'ANOVA' RECIP ;FVAL=F ; RES=R
1500  'GRAPH' R;FVAL
1600  'RUN'
1700  'CLOSE'
1800  'STOP'
```

Analysis of Variance tables for the Reciprocal of Bud Yield
Variate: Reciprocal Bud Yield 1981

Source of Variation	DF	(MV)	SS	SS%	MS	VR
Block stratum	2		2.63382	3.99	1.31691	14.840*
Block. Units Stratum						
Den	14		11.52985	17.47	0.82356	9.281*
Lin	1		10.29730	15.60	10.29730	116.038***
Quad	1		0.32046	0.49	0.32046	3.611 NS
Cub	1		0.15118	0.23	0.15118	1.704 NS
Deviations	11		0.76091	1.15	0.06917	0.780
Residual	630	(172)	55.90644	64.69	0.08874	
Total	644		67.43629	102.15	0.10471	
GRAND TOTAL	646		70.07011	106.14		

Variate: Reciprocal Bud Yield 1982

Source of Variation	DF	(MV)	SS	SS%	MS	VR
Block stratum	2		10.4569	1.54	5.2285	6.443*
Block. Units Stratum						
Den	14		52.7030	7.78	3.7645	4.639*
Lin	1		39.5118	5.83	39.5118	48.693**
Quad	1		2.2616	0.33	2.2616	2.787 NS
Cub	1		1.7359	0.26	1.7359	2.139 NS
Deviations	11		9.1937	1.36	0.8358	1.030
Residual	765	(37)	620.7579	91.59	0.8114	
Total	779		673.4609	99.37	0.8645	
GRAND TOTAL	781		683.9178	100.91		

Variate: Reciprocal Bud Yield 1983

Source of Variation	DF	(MV)	SS	SS%	MS	VR
Block stratum	2		0.23418	0.74	0.11709	3.518*
Block. Units Stratum						
Den	14		6.85241	21.67	0.48946	14.705*
Lin	1		6.17171	19.52	6.17171	185.418***
Quad	1		0.00609	0.02	0.00609	0.183 NS
Cub	1		0.00599	0.02	0.00599	0.180 NS
Deviations	11		0.66863	2.11	0.06078	1.826
Residual	763	(39)	25.39669	80.31	0.03129	
Total	777		32.24911	101.98	0.04150	
GRAND TOTAL	779		32.48328	102.72		

9. Appendix IV 9.3 Yield of concrete at budburst

Harvest Date	Replicate	Total Bud Nos./10 g	Open Bud Nos./10 g	Percentage Open Buds	Percentage Concrete Yield (Frest wt Basis)	Adjusted Yield
12/8/83	I	291	0	0	2.38	
	II	239	0	0	3.71	
	III	192	0	0	4.69	
	Mean	241	0	0	3.59	3.59
19/8/83	I	191	0	0	2.41	
	II	181	27	14.9	2.17	$2.25 \times \frac{241}{189}$
	III	195	14	7.2	2.17	
	Mean	189	13.7	7.3	2.25	2.87
26/8/83	I	88	41	46.6	1.30	
	II	89	36	40.5	1.92	$1.72 \times \frac{241}{92}$
	III	99	39	39.4	1.93	
	Mean	92	38.7	42.1	1.72	4.51
29/8/83	I	91	47	51.7	1.91	
	II	86	47	54.7	1.55	$1.72 \times \frac{241}{82.7}$
	III	71	41	57.8	1.70	
	Mean	82.7	45	54.7	1.72	5.06
30/8/83	I	83	43	51.8	1.40	
	II	76	54	71.0	1.33	$1.48 \times \frac{241}{81.7}$
	III	86	55	64.0	1.72	
	Mean	81.7	50.7	62.3	1.48	4.35
2/9/83	I	45	44	97.8	1.10	
	II	46	41	89.1	1.26	$1.12 \times \frac{241}{45.3}$
	III	45	43	95.6	1.00	
	Mean	45.3	42.7	94.2	1.12	6.00

9. Appendix IV 9.3

	Harvest Date					
	12/8	19/8	26/8	29/8	30/8	2/9
Alpha-thujene	0.65	1.58	0.71	2.86	0.64	0.74
Alpha-pinene	1.51	1.24	0.43	0.15	0.51	0.71
Sabinene	44.3	36.5	20.5	19.1	24.2	27.4
Beta-pinene	1.20	1.80	1.26	0.67	1.62	1.60
Myrcene	0.60	0.25	0.43	0.80	0.55	0.39
Alpha-phellandrene	0.24	0.67	0.17	0.71	0.58	0.25
Delta-3-carene	22.8	16.9	10.9	12.5	12.7	13.2
Beta-phellandrene	0.71	0.68	2.03	1.18	1.80	2.49
Limonene	1.84	4.73	3.54	3.71	3.55	3.54
Cis beta-ocimene	0.20	0.43	1.90	1.81	1.92	1.94
Trans beta-ocimene	0.30	1.11	1.34	2.44	1.17	1.20
Gamma-terpinene	0.64	0.91	1.92	1.21	1.41	1.18
Alpha-terpinolene	3.29	7.51	12.8	11.4	10.9	8.64
Non-an-2-one	0.25	0.30	0.68	0.36	0.50	0.50
Unknown MW152 (17)	1.24	0.33	0.38	0.46	0.61	0.42
Terpinen-4-ol	0.10	0.19	0.48	0.38	0.61	0.42
Alpha-terpineol	2.93	1.26	2.55	2.36	2.41	3.34
Trans-piperitol	0.08	0.59	1.26	0.96	1.02	0.20
Carvone	0.32	0.31	0.59	0.42	0.65	0.37
Beta terpinyl acetate	0.08	0.15	0.16	0.71	0.13	0.13
Beta-elemene	0.08	0.17	0.18	0.11	0.19	0.14
Beta-caryophyllene	6.84	7.35	14.9	14.0	11.9	11.0
Unknown MW204 (39)	0.08	0.20	0.12	0.11	0.13	0.11
Humulene	2.71	3.08	4.52	4.42	4.13	3.79
Alloaromadrene	0.08	0.08	0.10	0.21	0.08	0.12
Germacrene-D	1.38	2.61	5.95	5.27	5.22	5.26
Gamma-elemene	0.08	0.77	1.42	1.39	1.00	0.88
Gamma-cadinene	0.15	0.21	0.38	0.71	0.28	0.26
Caryophyllene epoxide	1.20	1.20	1.73	1.48	1.29	1.45
Humulene epoxide	1.43	0.73	1.01	0.87	0.85	0.83
Unknown (45)	0.60	0.34	0.36	0.40	0.38	0.33
Unknown (46)	0.25	0.18	0.22	0.30	0.19	0.18

10. <u>Appendix IV 9.4</u> Solarimeter Measurements (I/I_o)

Quadrat		1	2	3	4	5	6	7	8
Plant Density (plants/m²)		10.1	7.2	5.6	4.4	3.3	2.0	1.4	1.0
Date	Replicate	(14/9/82 Budburst)							
22/10/82	I	0.32	0.63	0.58	0.56	0.53	0.65	0.61	0.63
	II	0.93	0.61	0.54	0.64	0.52	0.68	0.55	0.66
	Mean	0.63	0.62	0.56	0.60	0.53	0.67	0.58	0.65
29/10/82	I	0.26	0.43	0.39	0.53	0.49	0.50	0.47	0.71
	II	0.38	0.32	0.40	0.44	0.81	0.61	0.60	0.59
	III	0.31	0.53	0.26	0.47	0.76	0.60	0.55	0.31
	Mean	0.32	0.43	0.35	0.48	0.69	0.57	0.54	0.54
5/11/82	I	0.18	0.27	0.21	0.44	0.27	0.52	0.50	0.47
	II	0.21	0.21	0.24	0.29	0.49	0.40	0.52	0.61
	III	0.17	0.20	0.16	0.37	0.42	0.51	0.64	0.52
	Mean	0.19	0.23	0.20	0.37	0.39	0.48	0.55	0.53
12/11/82	I	0.22	0.35	0.20	0.52	0.24	0.64	0.34	0.60
	II	0.25	0.23	0.32	0.41	0.61	0.40	0.48	0.57
	III	0.15	0.14	0.28	0.19	0.21	0.55	0.58	0.37
	Mean	0.21	0.24	0.27	0.37	0.35	0.53	0.47	0.51
19/11/82	I	0.17	0.24	0.19	0.39	0.36	0.42	0.41	0.63
	II	0.15	0.19	0.21	0.32	0.40	0.35	0.55	0.63
	III	0.18	0.16	0.17	0.22	0.27	0.61	0.69	0.48
	Mean	0.16	0.20	0.19	0.31	0.35	0.46	0.55	0.58
26/11/82	I	0.09	0.16	0.14	0.43	0.15	0.44	0.32	0.59
	II	0.19	0.13	0.16	0.21	0.35	0.36	0.43	0.51
	III	0.13	0.14	0.13	0.16	0.17	0.46	0.57	0.57
	Mean	0.14	0.14	0.15	0.26	0.23	0.42	0.44	0.56
3/12/82	I	0.10	0.13	0.12	0.20	0.13	0.32	0.27	0.53
	II	0.11	0.13	0.10	0.16	0.29	0.26	0.30	0.46
	III	0.12	0.08	0.12	0.17	0.19	0.51	0.56	0.45
	Mean	0.11	0.11	0.12	0.18	0.20	0.36	0.37	0.48
10/12/82	I	0.16	0.12	0.13	0.27	0.12	0.30	0.29	0.52
	II	0.11	0.13	0.12	0.12	0.27	0.23	0.28	0.49
	III	0.10	0.12	0.10	0.11	0.21	0.49	0.54	0.40
	Mean	0.12	0.13	0.12	0.17	0.20	0.34	0.37	0.47
17/12/82	I	0.09	0.09	0.16	0.28	0.11	0.21	0.21	0.35
	II	0.11	0.16	0.16	0.19	0.27	0.16	0.15	0.32
	III	0.09	0.12	0.09	0.10	0.13	0.44	0.48	0.30
	Mean	0.10	0.12	0.14	0.19	0.17	0.27	0.28	0.32
23/12/82	I	0.10	0.10	0.13	0.18	0.09	0.16	0.21	0.40
	II	0.18	0.10	0.14	0.15	0.29	0.19	0.19	0.51
	III	0.11	0.12	0.07	0.11	0.15	0.29	0.22	0.32
	Mean	0.13	0.11	0.11	0.15	0.18	0.22	0.21	0.41

Appendix IV 9.4 (continued)

Quadrat		1	2	3	4	5	6	7	8
Plant Density (plants/m²)		10.1	7.2	5.6	4.4	3.3	2.0	1.4	1.0
Date	Replicate	(14/9/82 Budburst)							
6/1/83	I	0.13	0.12	0.11	0.19	0.10	0.13	0.20	0.36
	II	0.17	0.08	0.19	0.16	0.18	0.28	0.21	0.30
	III	0.11	0.11	0.12	0.10	0.23	0.47	0.37	0.35
	Mean	0.14	0.10	0.14	0.15	0.19	0.29	0.26	0.34
20/1/83	I	0.08	0.11	0.06	0.13	0.10	0.12	0.10	0.17
	II	0.07	0.13	0.11	0.17	0.23	0.22	0.18	0.30
	III	0.10	0.08	0.11	0.07	0.15	0.18	0.40	0.32
	Mean	0.08	0.11	0.10	0.12	0.16	0.17	0.22	0.26
3/2/83	I	0.15	0.13	0.13	0.15	0.09	0.09	0.12	0.35
	II	0.14	0.18	0.21	0.09	0.25	0.31	0.19	0.13
	III	0.11	0.09	0.10	0.09	0.16	0.32	0.50	0.17
	Mean	0.13	0.13	0.15	0.11	0.17	0.24	0.27	0.21
17/2/83	I	0.10	0.19	0.08	0.16	0.06	0.20	0.26	0.50
	II	0.11	0.15	0.12	0.16	0.32	0.30	0.27	0.35
	III	0.12	0.16	0.08	0.12	0.14	0.28	0.52	0.32
	Mean	0.11	0.17	0.09	0.15	0.17	0.26	0.35	0.39
4/3/83	I	0.19	0.19	0.21	0.18	0.12	0.13	0.24	0.24
	II	0.20	0.16	0.32	0.16	0.25	0.24	0.34	0.44
	III	0.16	0.12	0.15	0.10	0.23	0.33	0.45	0.36
	Mean	0.18	0.16	0.23	0.15	0.20	0.23	0.34	0.35
18/3/83	I	0.20	0.20	0.23	0.29	0.12	0.26	0.23	0.50
	II	0.24	0.25	0.31	0.32	0.38	0.43	0.35	0.44
	III	0.20	0.23	0.18	0.24	0.23	0.45	0.51	0.31
	Mean	0.21	0.23	0.24	0.28	0.24	0.38	0.29	0.42
8/4/83	I	0.44	0.36	0.38	0.46	0.18	0.39	0.23	0.78
	II	0.27	0.51	0.50	0.47	0.37	0.73	0.48	0.52
	III	0.26	0.43	0.21	0.26	0.23	0.57	0.57	0.54
	Mean	0.32	0.44	0.36	0.40	0.26	0.56	0.43	0.61
29/4/83	Leaf fall								

10. <u>Appendix IV 9.4</u>   Percentage Leaf Cover

Quadrat		1	2	3	4	5	6	7	8
Plant Density (plants/m²)		10.1	7.2	5.6	4.4	3.3	2.0	1.4	1.0
Date	Replicate								
22/10/82	I	68.0	54.0	33.0	32.9	37.5	25.4	32.2	21.0
	II	60.2	53.4	35.0	34.9	36.1	23.0	35.0	20.6
	Mean	64.1	53.7	34.0	33.9	36.8	24.2	33.6	20.8
29/10/82	I	70.1	78.5	40.8	39.4	55.5	58.4	27.7	36.8
	II	65.0	71.2	68.4	47.4	29.3	22.1	57.1	27.9
	Mean	67.6	74.9	54.6	43.4	42.4	40.3	42.4	32.4
5/11/82	I	89.1	88.5	83.4	64.6	78.3	44.8	38.1	22.5
	II	99.2	90.7	85.2	63.5	47.0	29.1	23.1	44.0
	Mean	94.1	89.6	84.3	64.0	62.7	36.9	30.6	33.3
19/11/82	I	94.1	89.9	90.1	87.7	87.3	77.5	66.1	44.8
	II	96.0	87.7	86.3	80.1	57.0	75.6	89.1	51.4
	III	96.6	85.3	78.7	90.5	86.5	54.2	54.4	54.2
	Mean	95.6	87.6	85.0	86.1	76.9	69.1	69.9	50.1
26/11/82	I	100	100	90.7	77.7	89.4	63.4	66.4	34.8
	II	100	100	100	97.1	62.0	69.1	49.9	49.9
	Mean	100	100	95.4	87.4	75.7	66.3	58.2	42.4
10/12/82	I	100	100	96.0	87.0	93.0	67.0	71.0	43.0
	II	100	100	99.0	98.0	66.0	74.0	76.0	54.0
	Mean	100	100	98.0	92.0	80.0	71.0	74.0	49.0
23/12/82	II	100	100	92.0	86.0	91.0	88.0	78.0	49.0
	III	100	100	100	93.0	70.0	69.0	77.0	63.0
	Mean	100	100	96.0	90.0	80.0	78.0	77.0	56.0
20/1/83	I	100	100	100	100	100	52.3	53.5	71.1
	III	100	100	97.8	98.1	70.8	80.3	82.2	62.0
	Mean	100	100	98.9	99.0	85.4	66.3	67.9	66.5
17/2/83	I	100	100	94.7	91.3	85.7	53.2	90.5	74.8
	II	100	100	100	100	78.5	79.9	53.5	56.3
	Mean	100	100	97.3	95.6	82.1	66.5	72.0	65.6
18/3/83	I	100	100	100	100	83.6	44.7	40.7	46.0
	III	88.5	89.5	90.2	86.7	86.8	76.2	86.6	50.0
	Mean	94.3	94.8	95.1	93.4	85.2	70.5	63.7	48.0
8/4/83	I	93.0	85.4	83.0	84.9	65.7	47.0	75.9	47.8
	II	79.1	84.8	75.2	65.0	80.4	76.9	72.5	39.0
	Mean	86.0	85.1	79.1	89.2	73.1	62.0	74.2	43.4

10. <u>Appendix IV 9.4</u>   Estimated solar energy inputs ($\times 10^3$ MJ/m^2)

Quadrat	1		2		3		4		5		6		7		8	
Plant Density	10.1		7.2		5.6		4.4		3.3		2.0		1.4		1.0	
Date (weeks after budburst)																
5	0.19	0.19	0.20	0.20	0.23	0.23	0.21	0.21	0.25	0.25	0.17	0.17	0.22	0.22	0.18	0.18
6	0.09	0.28	0.08	0.28	0.09	0.32	0.07	0.28	0.04	0.29	0.06	0.23	0.06	0.28	0.07	0.25
7	0.13	0.41	0.12	0.40	0.13	0.45	0.10	0.38	0.10	0.39	0.08	0.31	0.07	0.35	0.08	0.33
8	0.13	0.54	0.12	0.52	0.12	0.57	0.10	0.48	0.11	0.50	0.08	0.39	0.09	0.44	0.08	0.41
9	0.10	0.64	0.09	0.61	0.09	0.66	0.08	0.56	0.08	0.58	0.06	0.45	0.05	0.49	0.05	0.46
10	0.13	0.77	0.13	0.74	0.13	0.79	0.11	0.67	0.12	0.70	0.09	0.54	0.09	0.58	0.07	0.53
11	0.14	0.91	0.14	0.88	0.14	0.93	0.13	0.80	0.13	0.83	0.10	0.64	0.10	0.68	0.08	0.61
12	0.10	1.01	0.10	0.98	0.10	1.03	0.09	0.89	0.09	0.92	0.07	0.71	0.07	0.75	0.06	0.67
13	0.15	1.16	0.14	1.12	0.14	1.17	0.13	1.02	0.14	1.06	0.12	0.83	0.12	0.87	0.11	0.78
14	0.11	1.27	0.11	1.23	0.11	1.28	0.11	1.13	0.10	1.16	0.10	0.93	0.10	0.97	0.08	0.86
16	0.27	1.54	0.28	1.51	0.27	1.55	0.27	1.40	0.25	1.41	0.22	1.15	0.23	1.20	0.21	1.07
18	0.26	1.80	0.25	1.76	0.25	1.80	0.25	1.65	0.23	1.64	0.23	1.38	0.22	1.42	0.21	1.28
20	0.27	2.07	0.27	2.03	0.26	2.06	0.27	1.92	0.26	1.90	0.23	1.61	0.23	1.65	0.24	1.52
22	0.25	2.32	0.23	2.26	0.26	2.32	0.24	2.16	0.23	2.13	0.21	1.82	0.18	1.83	0.17	1.69
24	0.19	2.51	0.20	2.46	0.18	2.50	0.20	2.36	0.19	2.32	0.18	2.00	0.16	1.99	0.15	1.84
26	0.18	2.69	0.18	2.64	0.17	2.67	0.16	2.52	0.17	2.49	0.14	2.14	0.16	2.15	0.13	1.97
29	0.17	2.86	0.14	2.78	0.16	2.83	0.15	2.67	0.18	2.67	0.11	2.25	0.14	2.29	0.10	2.07
32	0.11	2.97	0.09	2.87	0.11	2.94	0.10	2.77	0.12	2.79	0.07	2.32	0.10	2.39	0.07	2.14

11. <u>Appendix Section IV 9.5</u> Component key for compositional data
recorded for varietal selections at
various locations.

1	alpha-thujene
2	alpha-pinene
3	sabinene/beta pinene
4	myrcene
5	alpha-phellandrene
6	delta-3-carene
7	alpha-terpinene
8	beta phellandrene/limonene
9	cis beta-ocimene
10	trans beta-ocimene
11	gamma terpinene
12	alpha-terpinolene
13	non-an-2-one
14	unknown MW 152 (17)
15	terpin-en-4-ol
16	alpha terpineol
17	trans piperitol
18	carvone
19	unknown MW 182 (35)
20	borynl acetate
21	4-terpinyl acetate
22	beta-terpinyl acetate
23	beta elemene
24	beta caryophyllene
25	unknown MW 204 (39)
26	humulene
27	alloaromadrene
28	Germacrene-D
29	Gamma-elemene
30	gamma cadinene
31	caryophyllene epoxide
32	humulene epoxide
33	unknown (45)
34	unknown (46)

11. <u>Appendix Section IV 9.5</u> Percentage composition (peak area) data for varietal selections at various locations

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HORTICULTURAL RESEARCH CENTRE

1983 White Bud
1
1.44, 0.76, 25.06, 1.28, 0.44, 11.79, 0.96, 2.93, 1.05, 1.11, 1.16, 9.08, 0.55, 0.40, 0.28,
1.83, 0.75, 0.43, 0.33, 0.30, 0.75, 0.36, 0.34, 15.34, 0.48, 6.23, 0.05, 6.27, 1.65, 0.43,
1.37, 0.85, 0.34, 0.25.

Grahams No.1
White Bud
2
1.64, 2.43, 30.98, 1.50, .89, 17,84, 0.75, 2.25, 0.43, 0.70, 0.64, 10.54, 0.29, 0.22, 0.08,
1.11, 0.63, 0.38, 0.52, 0.30, 0.76, 0.34, 0.05, 6.99, 0.22, 2.31, 0.52, 5.71, 0.26, 0.22,
0.22, 0.33, 0.39, 0.20.

Super C
3
2.85, 1.07, 33.48, 1.92, 0.65, 18.64, 1.07, 2.82, 0.62, 1.96, 0.84, 9.61, 0.42, 0.25, 0.10,
1.81, 0.11, 0.44, 0.05, 0.05, 0.10, 0.35, 0.05, 8.05, 0.27, 3.16, 0.10, 3.61, 0.49, 0.35,
0.79, 0.56, 0.23, 0.20.

Baldwin
4
1.78, 1.05, 33.24, 1.50, 9,54, 15.88, 0.81, 2.40, 0.44, 1.22, 0.89, 7.70, 0.48, 0.35, 0.24,
1.95, 0.22, 0.22, 0.18, 0.20, 0.51, 0.38, 0.05, 10.84, 0.38, 4.34, 0.10, 4.28, 1.18, 0.38,
1.46, 0.95, 0.42, 0.24.

Goliath
5
0.00, 1.16, 39.55, 2.12, 0.57, 15.85, 1.65, 3.23, 1.13, 0.98, 0.57, 9.01, 0.25, 0.26, 0.18,
0.78, 0.23, 0.49, 0.15, 0.39, 0.26, 0.22, 0.08, 6.63, 0.24, 0.91, 0.00, 7.44, 0.30, 0.00,
0.57, 0.31, 0.21, 0.15.

Kerry
6
0.78, 1.28, 37.82, 1.82, 0.62, 15.85, 1.49, 3.56, 1.27, 1.00, 0.88, 10.34, 0.31, 0.30, 0.20,
0.96, 0.90, 0.2, 0.20, 0.22, 0.25, 0.31, 0.05, 7.76, 0.24, 1.16, 2.62, 7.48, 0.30, 0. 36,
0.74, 0.42, 0.20, 0.18.

Boskoop
Giant
7
0.41, 1.22, 5.94, 2.49, 1.13, 18.29, 1.87, 16.97, 0.87, 0.66, 0.25, 15.15, 0.29, 0.29, 2.11,
0.32, 0.32, 0.22, 0.46, 0.58, 0.40, 0.24, 13.16, 0.39, 3.95, 0.00, 0.10, 4.93, 1.52, 0.37,
1.48, 0.68, 0.26, 0.19.

Hatton
Black
8
2.25, 0.97, 27.09, 1.22, 0.33, 19.29, 1.14, 2.18, 0.61, 1.01, 0.58, 10.41, 0.32, 0.33, 0.21,
1.57, 0.58, 0.29, 0.28, 0.35, 0.73, 0.24, 0.10, 8.86, 0.09, 2.68, 0.05, 2.91, 0.48, 0.43,
0.93, 0.68, 0.23, 0.14.

Lees
Prolific
9
1.11, 0.79, 5.72, 2.30, 0.88, 31.31, 1.88, 3.17, 1.02, 1.92, 0.26, 16.88, 0.21, 0.35, 0.21,
1.60, 0.43, 0.40, 0.33, 0.35, 0.61, 0.50, 0.00, 9.48, 0.45, 3.47, 0.00, 6.96, 0.63, 0.44,
0.45, 0.34, 0.61, 0.30.

1982 Grahams No.1
White Bud
10
1.68, 1.12, 39.80, 1.49, 0.35, 16.90, 0.88, 2.68, 0.64, 0.78, 0.63, 8.11, 0.45, 0.65, 0.78,
0.68, 0.46, 0.37, 0.46, 0.27, 0.34, 0.27, 0.05, 5.44, 0.20, 1.95, 0.10, 3.47, 0.05, 0.20,
0.42, 0.59, 0.21, 0.25.

Goliath
11
1.45, 0.89, 39.16, 1.67, 0.39, 17.48, 0.87, 2.50, 0.61, 0.89, 0.51, 7.09, 0.35, 0.33, 0.68,
0.59, 0.37, 0.47, 0.46, 0.26, 0.26, 0.27, 0.25, 6.53, 0.20, 1.12, 0.10, 5.33, 0.05, 0.35,
0.61, 0.57, 0.00, 0.00.

Baldwin
12
1.48, 1.17, 32.31, 1.61, 0.39, 15.36, 0.70, 2.68, 0.57, 1.10, 0.87, 8.17, 0.64, 0.65, 1.19,
1.05, 0.64, 0.52, 0.23, 0.21, 0.61, 0.23, 0.20, 9.95, 0.24, 3.62, 0.00, 3.36, 0.00, 0.44,
1.53, 1.50, 0.45, 0.20.

Boskoop
Giant
13
1.81, 0.93, 10.81, 2.04, 1.47, 23.23, 0.81, 16.06, 0.00, 0.75, 0.30, 7.42, 1.05, 1.05, 2.82,
0.28, 0.39, 0.40, 0.61, 0.30, 0.32, 0.34, 0.02, 7.50, 0.24, 2.24, 0.00, 0.23, 0.39, 0.63,
1.59, 1.21, 0.40, 0.00.

Kerry
14
1.41, 1.03, 32.75, 1.29, 0.47, 14.49, 0.62, 4.07, 0.28, 1.79, 1.17, 5.93, 0.88, 0.66, 1.85,
2.03, 1.58, 0.79, 0.71, 1.11, 1.69, 1.21, 0.41, 7.91, 0.37, 1.08, 0.05, 0.86, 1.02, 0.52,
2.70, 1.20, 0.50, 0.25.

Lees
Prolific
15
1.09, 0.82, 29.59, 0.87, 0.28, 12.18, 0.56, 1.89, 0.37, 0.64, 1.02, 4.02, 1.18, 0.36, 1.47,
1.26, 0.98, 0.50, 1.19, 1.22, 0.86, 0.77, 0.26, 11.90, 0.52, 5.61, 0.24, 5.92, 0.59, 0.59,
2.54, 1.43, 0.79, 0.40.

1980 White Bud
16
1.56, 1.12, 28.14, 0.43, 0.36, 12.15, 0.36, 1.12, 0.93, 2.81, 0.46, 3.06, 0.32, 0.60, 5.90,
0.39, 1.85, 0.52, 0.82, 2.35, 0.67, 0.28, 0.21, 2.05, 0.10, 0.77, 0.05, 0.56, 0.23, 0.24,
3.06, 9.52, 4.48, 0.73.

Baldwin
17
1.08, 1.17, 38.56, 1.21, 0.40, 16.07, 0.30, 2.96, 0.00, 0.63, 1.20, 2.14, 1.54, 0.76, 3.44,
0.22, 1.09, 0.36, 0.23, 1.40, 0.65, 0.27, 0.05, 7.81, 0.08, 3.28, 0.07, 1.30, 0.10, 0.26,
2.38, 3.93, 1.45, 0.54.

Boskoop
Giant
18
0.56, 3.44, 14.81, 2.84, 1.23, 28.56, 0.25, 12.74, 0.35, 0.69, 0.52, 3.43, 0.25, 2.38, 0.43,
5.67, 0.32, 0.28, 0.28, 1.38, 0.23, 0.25, 0.25, 5.74, 0.25, 1.78, 0.21, 0.60, 0.45, 0.27,
1.95, 2.37, 0.30, 0.67.

Super C
19
0.49, 3.44, 10.63, 2.59, 1.00, 40.33, 0.20, 1.81, 0.28, 0.51, 0.23, 2.53, 1.20, 2.36, 0.75,
7.26, 0.28, 0.46, 1.18, 1.69, 2.55, 0.48, 0.22, 4.81, 0.22, 2.36, 0.25, 0.76, 0.39, 0.32,
3.27, 1.25, 0.28, 0.21.

Goliath 20
2.98, 1.34, 40.70, 0.85, 0.64, 17.09, 0.00, 2.02, 0.00, 1.24, 1.26, 1.36, 1.99, 0.69, 3.16, 0.25, 1.02, 0.44, 0.77, 0.23, 1.03, 0.34, 0.05, 4.97, 0.26, 1.03, 0.28, 0.82, 0.38, 0.62, 0.78, 3.57, 0.37, 0.28.

Kerry 21
1.85, 1.41, 30.77, 0.64, 0.26, 19.68, 0.27, 1.33, 0.00, 0.92, 8.08, 7.92, 0.64, 0.69, 0.45, 10.95, 0.25, 0.33, 0.20, 0.20, 0.31, 0.25, 0.08, 1.93, 0.27, 0.31, 0.10, 0.85, 0.48, 0.37, 2.13, 2.29, 0.23, 0.21.

HUON HORTICULTURAL RESEARCH STATION (GROVE)

1983 White Bud 22
0.91, 0.78, 27.96, 0.57, 0.82, 16.84, 0.81, 2.32, 0.60, 1.08, 0.82, 9.46, 0.47, 0.35, 0.28, 0.86, 0.74, 0.46, 0.19, 0.25, 0.46, 0.23, 0.32, 11.79, 0.37, 4.53, 0.25, 4.89, 0.10, 0.41, 1.87, 1.21, 0.44, 0.25.

Super C 23
0.87, 0.50, 6.47, 0.84, 0.85, 37.82, 2.04, 2.42, 1.35, 1.07, 0.27, 18.18, 0.48, 1.06, 1.06, 0.59, 0.46, 0.36, 0.20, 0.20, 0.18, 0.26, 0.10, 7.57, 0.21, 2.85, 0.00, 5.32, 0.00, 0.29, 0.43, 0.34, 0.13, 0.10.

Goliath 24
0.62, 0.33, 36.85, 0.26, 0.73, 18.02, 1.03, 2.75, 0.78, 1.74, 0.35, 9.65, 0.25, 0.00, 0.50, 0.49, 0.38, 0.39, 0.10, 0.10, 0.28, 0.31, 0.10, 6.96, 0.47, 1.41, 0.84, 4.90, 0.05, 0.38, 0.70, 0.37, 0.05, 0.10.

Hatton Black 25
0.81, 0.81, 28.30, 0.24, 1.17, 15.75, 0.89, 10.15, 0.39, 0.41, 0.00, 2.21, 0.27, 0.48, 0.75, 1.04, 1.70, 1.27, 0.87, 1.14, 1.04, 1.57, 0.10, 4.51, 0.30, 1.82, 0.00, 0.27, 0.05, 0.03, 0.27, 0.29, 0.00, 0.00.

Lees Prolific 26
0.43, 0.32, 7.96, 0.42, 1.38, 20.64, 0.68, 19.87, 0.24, 0.23, 0.23, 13.83, 0.00, 0.30, 0.28, 2.18, 0.30, 0.50, 0.21, 0.26, 0.21, 0.62, 0.36, 12.81, 0.00, 3.62, 0.00, 4.28, 0.13, 0.23, 1.45, 0.80, 0.37, 0.31.

Magnus 27
0.50, 0.52, 26.97, 0.22, 0.86, 16.33, 0.51, 1.49, 0.43, 1.17, 0.69, 8.73, 0.33, 0.21, 0.98, 0.89, 0.73, 0.40, 0.22, 0.33, 0.43, 0.26, 0.26, 11.62, 0.75, 5.00, 0.27, 4.55, 0.31, 1.11, 2.38, 1.50, 0.53, 0.65.

Boskoop Giant 28
0.86, 0.59, 13.95, 0.58, 1.27, 19.91, 1.33, 16.12, 0.30, 1.03, 0.29, 12.42, 0.23, 0.45, 1.15, 0.61, 0.61, 0.44, 0.23, 0.41, 0.47, 0.39, 0.08, 10.24, 0.39, 3.10, 0.00, 3.56, 0.14, 0.35, 1.67, 0.61, 0.17, 0.25.

Kerry 29
0.71, 0.52, 35.17, 0.29, 0.91, 16.10, 1.53, 7.13, 0.54, 0.78, 0.44, 9.25, 0.27, 0.25, 0.54, 0.68, 0.55, 0.42, 0.15, 0.44, 0.33, 0.20, 0.15, 5.21, 0.74, 2.47, 0.22, 3.27, 1.63, 1.16, 1.69, 1.16, 0.23, 0.21.

1982 Goliath 30
13.39, 1.22, 10.95, 1.61, 0.88, 22.78, 0.20, 2.40, 0.00, 8.23, 0.81, 7.24, 0.67, 0.47, 1.11, 2.68, 0.20, 0.23, 0.73, 0.60, 0.23, 0.47, 0.10, 8.82, 0.10, 0.93, 0.92, 0.78, 1.17, 1.56, 0.40, 2.28, 0.31, 0.66.

Boskoop Giant 31
2.79, 3.39, 3.00, 2.87, 2.01, 25.71, 0.00, 10.31, 0.00, 4.32, 0.32, 0.94, 1.91, 1.15, 0.56, 9.14, 0.47, 0.86, 0.63, 0.97, 1.46, 1.35, 0.32, 4.31, 0.30, 1.03, 0.03, 0.59, 0.42, 0.28, 2.29, 2.85, 1.06, 1.60.

Kerry 32
3.13, 1.11, 16.27, 1.19, 2.70, 10.16, 0.29, 0.25, 1.54, 2.56, 2.14, 1.28, 0.60, 0.98, 4.97, 3.28, 1.43, 1.92, 4.51, 2.03, 1.33, 1.12, 0.48, 2.44, 0.21, 0.49, 0.05, 0.81, 0.23, 0.36, 9.32, 1.50, 0.66, 1.00.

Magnus 33
5.64, 0.35, 3.41, 1.10, 0.66, 9.48, 0.10, 2.42, 0.00, 3.57, 2.36, 2.19, 2.93, 0.55, 4.08, 4.91, 0.53, 0.29, 1.60, 0.90, 1.00, 0.78, 0.13, 15.90, 0.29, 5.98, 0.51, 0.21, 0.28, 0.97, 4.65, 6.10, 2.25, 1.70.

Lees Prolific 34
3.79, 4.15, 2.52, 3.16, 2.14, 27.17, 0.00, 10.62, 0.00, 0.28, 4.17, 1.07, 1.52, 0.23, 0.47, 6.93, 0.36, 0.64, 0.93, 0.83, 2.40, 0.90, 0.64, 3.52, 0.26, 1.30, 0.21, 0.40, 0.28, 0.05, 2.63, 3.05, 1.17, 1.74.

Super C 35
0.34, 0.99, 2.86, 2.45, 0.84, 41.83, 1.50, 2.27, 0.93, 0.54, 0.21, 17.26, 0.80, 0.22, 2.32, 0.21, 0.46, 0.30, 0.31, 0.33, 0.27, 0.42, 0.20, 6.78, 0.28, 3.02, 0.83, 5.14, 0.37, 0.38, 1.11, 0.39, 0.26, 0.32.

MARION BAY

1980 White Bud 36
3.34, 0.22, 12.09, 0.57, 0.55, 10.79, 0.21, 1.49, 0.00, 2.10, 2.71, 2.43, 1.00, 0.81, 0.80, 10.87, 0.44, 0.35, 1.30, 2.18, 1.88, 0.28, 0.20, 7.09, 0.41, 3.57, 0.48, 0.33, 0.52, 0.48, 7.57, 4.90, 2.57, 1.33.

Goliath 37
4.64, 1.24, 37.84, 0.94, 0.74, 14.97, 0.22, 2.26, 0.00, 3.15, 1.47, 2.07, 2.42, 0.85, 1.85, 1.38, 0.83, 0.49, 0.28, 0.66, 0.45, 0.49, 0.25, 6.24, 0.22, 0.83, 0.73, 2.05, 0.70, 1.06, 1.16, 2.76, 0.21, 0.27.

11. <u>Appendix Section IV 9.5</u>   Latent vectors (coordinates) for principle coordinate analysis

37 x 34 oil components

	1	2	3	4
1	0.1534	0.0516	-0.0727	0.1222
2	0.1133	-0.0081	-0.0024	-0.0849
3	0.1337	-0.0065	-0.0556	-0.0490
4	0.1079	0.0320	-0.1032	-0.0047
5	0.2219	-0.0068	0.0774	-0.0917
6	0.2229	0.0298	0.0619	-0.0635
7	0.1620	-0.2386	0.1328	0.0838
8	0.1123	0.0223	-0.0288	-0.0232
9	0.2008	-0.1604	0.0221	0.1204
10	0.1034	0.0534	0.0167	-0.1130
11	0.1434	0.0502	0.0163	-0.1148
12	0.0709	0.0618	-0.0270	-0.0364
13	-0.0001	-0.1054	-0.0186	0.0185
14	-0.1093	0.0952	0.0772	-0.0311
15	0.0042	0.1372	-0.0290	0.0768
16	-0.2107	0.2499	0.2005	0.0517
17	-0.1151	0.1713	-0.0098	-0.0566
18	-0.1413	-0.2023	-0.0126	-0.1207
19	-0.2337	-0.2043	0.0018	-0.1224
20	-0.1045	0.1560	-0.0354	-0.1287
21	-0.0703	0.0135	-0.1165	-0.1806
22	0.1213	0.0755	-0.0333	0.0351
23	0.2296	-0.1209	0.0767	0.0920
24	0.1799	0.0828	0.0175	-0.0521
25	-0.0757	0.0783	0.1982	-0.0552
26	0.1025	-0.0949	-0.0277	0.1095
27	0.0789	0.1118	-0.0818	0.0895
28	0.1047	-0.0556	0.0155	0.0798
29	0.1405	0.0656	-0.0283	0.0217
30	-0.0948	-0.0275	-0.2101	0.0525
31	-0.3529	-0.2360	-0.0227	-0.0299
32	-0.3195	0.0657	0.3597	0.1179
33	-0.2990	0.0994	-0.1933	0.2489
34	-0.3441	-0.2609	-0.0202	-0.0627
35	0.1590	-0.1708	0.0385	0.0925
36	-0.3071	0.0629	-0.0934	0.1009
37	-0.0884	0.1327	-0.0903	-0.0925

Percentage variance
accounted for

cumulative	28.4	42.7	52.4	60.5
individual	28.4	14.3	9.7	8.1

11. Appendix Section IV 9.5   Latent Vectors (coordinates) for principle coordinate analysis

37 samples x 12 oil components

	1	2	3	4
1	0.2176	-0.1594	0.3102	-0.1665
2	0.1001	-0.1235	-0.0693	0.0737
3	0.0685	-0.1116	-0.0142	0.0157
4	0.0725	-0.1563	0.1244	-0.1032
5	0.2086	-0.1994	-0.2139	0.0476
6	0.2303	-0.2311	-0.1646	0.0143
7	0.3043	0.3107	-0.2199	-0.4043
8	0.0630	-0.0522	0.0026	0.0177
9	0.4108	0.1352	0.0489	0.0412
10	-0.0143	-0.1538	-0.1392	0.0877
11	0.0277	-0.1770	-0.1484	0.0782
12	-0.0025	-0.1298	0.0472	0.1141
13	-0.0042	0.2256	0.0239	-0.0153
14	-0.1316	-0.0655	-0.0314	-0.1544
15	0.0521	-0.1986	0.2132	0.0110
16	-0.2543	-0.0416	-0.1415	0.0176
17	-0.2120	-0.1546	0.0099	0.1010
18	-0.1699	0.2182	-0.0274	0.0158
19	-0.1739	0.2077	0.0016	0.0984
20	-0.2876	-0.1401	-0.0874	-0.0290
21	-0.1798	-0.0168	-0.1679	-0.0312
22	0.1026	-0.1258	0.1139	0.1228
23	0.3723	0.1895	-0.0560	0.1848
24	0.0776	-0.1548	-0.1376	0.0677
25	-0.1770	0.0404	-0.1257	0.0618
26	0.1657	0.2254	0.1174	0.1117
27	0.0534	-0.1332	0.2099	0.0151
28	0.1683	0.1646	0.0270	0.0623
29	0.1045	-0.0875	-0.0068	-0.3477
30	-0.1385	0.1058	0.1360	-0.2221
31	-0.2435	0.2889	-0.0187	0.0152
32	-0.2742	0.0303	-0.0916	0.0071
33	-0.1832	0.0537	0.4276	0.0532
34	-0.2445	0.3063	-0.0431	0.0676
35	0.3189	0.2266	-0.0108	0.1187
36	-0.2179	0.0412	0.1369	-0.0148
37	-0.2097	-0.1575	-0.0352	-0.1338

Percentage
variance
accounted for

cumulative	29.6	51.1	66.1	77.9
individual	29.6	21.5	15.1	11.8

12. <u>Appendix Section IV 10</u>   Blackcurrant Budget Assumptions

1. <u>Yields</u> have been obtained from a plant density experiment carried

    out at Bushy Park (see Section IV 9.1), and further plantings of

    first year cuttings at Glen Huon, Southern Tasmania. Maximum yield

    is achieveable in the fourth year.   The percentage yield of concrete

    has been determined experimentally with a pilot extraction plant.

2. <u>Price</u> Current market information indicates a price of $1000 per

    kilogram of concrete.

3. <u>Capital Expenditure</u>

    (i)   Land @ $2000/ha in close proximity to irrigation water

    (ii) An irrigation scheme is installed at a cost of $2000/ha.

4. <u>Establishment Costs</u>

        (i)   Site preparation

            - ploughing x 1.5 hr/ha @ $26/hr   39

            - discing x 2 x 1 hr/ha @ $26/hr   52

            - chisel plough x 1 hr/ha @ $26/hr   26

            - harrowing x ½ hr/ha @ $20/hr   10
                                                    _____
                                                    $127/ha

        (ii) Planting material

            borer free, unrooted hardwood cuttings at a cost

            of 6 cents each

            $\therefore$ 2.8 plants/m^2 = 2.8 x 0.06 x 10,000 = $1,680/ha

        (iii) Planting labour

            55 hrs/ha/10,000 cuttings at $6/hr

            $\therefore$ 55 x 2.8 x 6 = $924/ha

(iv)　Shelter　-　Wattles　-　$200/ha

(v)　Fencing　-　$140/ha

(vi)　Fertilizers　-　Peppermint and hops which are other crops

with a high nitrogen demand e.g. 300 kg N/ha.

From this and the known response of blackcurrants to high

levels of nitrogen apply

1000 kg/ha Ammonium Nitrate @ $348/tonne　　348

1200 kg/ha 0-7-12 @ $161/tonne　　　193.2

Cartage @ $15/tonne　　　　33

Spreading 1.5 hr/ha @ $21.45/250 kg/ha　　188.8
　　　　　　　　　　　　　　　　　　　　　　—————
　　　　　　　　　　　　　　　　　　　　　　$763/ha

(viii) Weed Control

Caraguard　6ℓ/ha @ $8/ℓ　　　48

Spot spraying $10/ha materials)
　　　　　　　　　　　　　　)　　30
　　　　　　　$20/ha labour　)

Application 2 hr/ha @ $26/spray/hr　　52
　　　　　　　　　　　　　　　　　　————
　　　　　　　　　　　　　　　　　　$130/ha

(ix)　Disease Control

Cuprox @ 1,150 ℓ/ha x 200 g product/100 ℓ x $4.00/kg product

= 11.5 x 0.2 x 4　　　　　　9.20

Captan @ 1,150 ℓ/ha x 125 g product/100 ℓ x $7.00/kg product

= 11.5 x 0.125 x 7　　　　　10.06

Application 2 x 2 hr/ha @ $26/hr　　104
　　　　　　　　　　　　　　　　　————
　　　　　　　　　　　　　　　　　$124.00/ha

(x)　Tractor Operating Costs

Tractor usage = 13.5 hrs/ha @ $4/ha

plus 11.5 hrs/ha general use

= 25 x 4　　　　　　　　$100/ha

(xi) Equipment Repairs and Maintenance

3% of capital value 0.03 x 9000   = $270/ha

(xii) Irrigation running costs   = $100/ha

(xiii) Tools, consumables, freight, protective clothing

= $300/ha

Operating Costs

(i) Fertilizers as per Establishment year $763/ha

(ii) Herbicides Caroguard @ 6 ℓ/ha x $6/ℓ   36

Roundup @ 4 ℓ/ha x $20/ℓ   80

Miscellaneous sprays $20/ha   20

Application 2 x 2 hr/ha @
$26/hr   104
————
$240/ha

(iii) Disease control as per establishment year

(iv) Tractor operating costs   "   100

(v)   Repairs and maintenance   "   270

(vi) Irrigation running costs   "   100

(vii) Tools and consumables   "   300

Harvest and Extraction Costs

(1) (a) Handpicking buds - 0.65 kg buds/hr at $6/hr

(b) Machine harvester - 3 row cutter bar at 1 km/hr

plant density x 3.33 = total row length

$\therefore$ cost = $26 x 3.33 x plant density

Royalty - 1½% of Farm Gate value of produce

(2) Transport and storage $0.5/kg buds

(3) Extraction labour @ 16 hrs/50 kg buds @ $10/hr

(4) Solvent Loss 25 ℓ per 50 kg buds $\therefore$ $100/50 kg buds

(5) Hire of extractor   15% of $70,000/2 days/50 kg buds $58

   i.e. $29/day

   includes hire of mincer to crush buds

Sale Overheads

(i)	Packaging and freight	$30/kg concrete	
(ii)	Advertising	2% of revenue	
(iii)	Administration	1%	"
(iv)	Research and development	2%	"
(v)	Agent's Commission	5%	"

13. Appendix Section IV 9.2   Oil composition of dormant buds at harvest 1982

Density

	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16
Alpha-thujene	0.88	0.80	0.63	0.43	3.40	1.95	1.79	2.01	1.94	1.60	1.06	2.10	1.44	2.05	1.78
Alpha-pinene	1.38	1.35	0.76	0.75	2.73	1.67	1.44	1.69	1.61	1.19	1.24	1.98	1.44	2.03	1.50
Sabinene/beta-pinene	18.6	18.2	23.6	18.2	33.1	33.5	31.9	35.2	33.5	28.6	23.6	36.3	27.1	33.5	18.7
Myrcene	1.49	1.52	0.45	0.39	3.05	1.49	1.40	1.75	1.69	1.53	1.01	1.50	1.33	1.33	1.68
Alpha-phellandrene	0.80	0.14	0.10	0.11	0.44	0.26	0.42	0.24	0.22	0.74	1.08	0.15	0.40	0.13	1.13
Delta-3-carene	12.7	12.2	12.6	8.46	18.1	15.9	17.1	16.9	16.8	14.3	11.9	20.2	15.4	17.5	13.8
Beta-phellandrene	0.30	0.23	0.20	0.20	0.80	0.60	0.82	0.79	0.72	1.26	0.34	0.58	0.72	0.44	0.49
Limonene	1.73	1.39	1.17	4.77	10.4	7.13	8.16	2.15	2.23	6.18	5.04	1.67	9.56	1.40	7.66
Cis beta-ocimene	0.24	0.21	0.00	0.00	0.19	0.18	0.17	0.64	0.54	0.51	0.95	0.51	0.20	0.48	2.55
Trans beta-ocimene	0.58	0.28	0.63	0.35	1.09	0.52	0.38	0.37	0.38	0.74	2.01	0.25	0.56	0.27	0.31
Gamma-terpinene	1.35	2.77	1.41	2.09	0.70	0.53	0.42	0.55	0.70	0.66	1.99	0.34	0.67	0.40	0.44
Alpha-terpinolene	7.77	4.59	3.45	3.18	11.0	9.18	9.16	8.82	7.64	8.95	9.08	9.79	10.3	9.07	8.15
Non-an-2-one	0.68	3.82	2.55	2.96	0.43	0.13	0.14	0.21	0.36	0.45	0.58	0.30	0.37	0.16	0.20
Unknown MW 152 (17)	1.22	1.96	1.07	1.13	0.53	0.34	0.33	0.38	0.51	0.41	0.51	0.26	0.41	0.28	0.44
Terpinen-4-ol	0.33	0.61	0.35	0.41	0.19	0.59	0.52	0.62	1.03	1.18	1.35	0.37	0.91	0.42	1.88
Alpha-terpineol	3.26	9.97	4.13	4.44	1.56	0.94	0.76	0.84	1.07	0.91	1.25	0.70	0.93	0.83	0.74
Trans piperitol	2.01	0.44	1.76	2.07	0.99	0.73	0.60	0.70	0.82	0.78	1.14	0.73	0.96	0.97	0.87
Carvone	0.97	0.43	0.76	0.88	0.65	0.59	0.49	0.53	0.57	0.57	0.65	1.06	0.60	1.35	0.92
Beta terpinyl acetate	1.23	1.49	0.83	1.00	0.81	0.51	0.48	0.51	0.61	0.54	0.63	0.79	0.52	1.03	1.08
Beta elemene	0.10	0.12	0.15	0.43	0.07	0.02	0.07	0.06	0.56	0.25	0.23	0.14	0.24	0.10	0.61
Beta-caryophyllene	19.2	17.7	16.1	18.9	7.90	9.64	9.63	10.6	11.0	9.58	12.6	7.37	9.95	9.34	9.64
Humulene	7.57	6.87	7.24	8.50	2.77	3.77	3.81	4.29	4.63	3.77	5.20	3.18	3.56	4.03	3.76
Germacrene-D	6.15	4.08	4.39	6.57	2.95	4.49	5.12	5.43	4.41	4.68	4.05	3.82	3.68	5.08	2.36
Gamma-elemene	0.25	0.30	0.85	0.17	0.04	0.06	0.06	0.04	0.09	0.31	0.05	0.05	0.05	0.05	0.53
Caryophyllene epoxide	6.66	5.90	4.22	3.74	0.05	0.72	0.30	0.28	0.30	2.29	2.64	0.21	1.19	0.19	1.39
Humulene epoxide	0.25	0.31	2.94	2.60	0.03	0.03	0.05	0.06	0.30	1.02	1.73	0.40	0.80	0.43	0.83

13. <u>Appendix Section IV 9.2</u> Oil composition of dormant buds at harvest 1983

	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16
Alpha-thujene	0.86	1.38	0.91	1.32	1.02	1.23	0.85	0.63	1.42	0.71	1.40	1.21	1.43	1.93	1.90
Alpha-pinene	1.46	1.13	1.51	1.52	1.92	1.44	1.57	1.86	1.47	1.38	1.31	1.30	0.98	0.92	1.13
Sabinene/beta-pinene	39.8	38.9	41.7	34.5	36.1	38.4	32.7	33.2	42.0	38.0	42.4	42.3	34.2	32.6	38.9
Myrcene	1.80	1.82	1.40	1.43	2.59	2.21	2.41	2.53	1.50	2.12	1.19	1.76	1.41	0.63	0.36
Alpha-phellandrene	0.65	0.61	0.66	0.86	0.80	0.69	0.84	0.88	0.69	0.77	0.66	0.60	0.57	0.70	0.88
Delta-3-carene	20.3	16.2	18.3	18.5	18.2	17.2	18.6	18.1	18.8	18.4	18.6	17.9	16.2	16.8	19.4
Beta-phellandrene	0.77	1.91	1.44	1.65	2.06	2.20	2.60	2.23	1.37	1.60	1.48	1.67	1.57	1.21	1.32
Limonene	2.24	3.66	3.10	7.20	7.32	5.18	7.52	8.56	3.12	3.19	3.03	2.93	2.77	2.67	3.11
Cis beta-ocimene	0.31	1.27	0.92	0.66	0.93	1.49	1.84	0.94	0.82	0.97	0.96	1.06	1.03	0.90	1.03
Trans beta-ocimene	0.74	0.72	0.59	0.52	0.49	0.56	0.53	0.45	0.43	0.46	0.39	0.45	0.59	0.46	0.35
Gamma-terpinene	9.29	8.83	9.15	9.57	9.38	9.70	11.2	10.2	8.80	9.49	9.11	8.80	9.62	9.24	9.94
Alpha-terpinolene	0.23	0.32	0.34	0.19	0.23	0.25	0.26	0.20	0.21	0.24	0.20	0.27	0.37	0.28	0.15
Non-an-2-one	0.24	0.31	0.38	0.29	0.27	0.22	0.26	0.21	0.18	0.19	0.19	0.19	0.26	0.27	0.21
Unknown MW 152 (17)	0.18	0.17	0.23	0.12	0.12	0.12	0.11	0.10	0.13	0.14	0.10	0.10	0.15	0.16	0.11
Terpinen-4-ol	0.99	1.18	1.15	0.71	1.04	1.05	0.97	1.20	0.75	0.97	0.85	0.85	1.11	1.22	0.85
Alpha-terpineol	0.56	0.46	0.34	0.34	0.40	0.37	0.37	0.43	0.33	0.32	0.21	0.19	0.11	0.29	0.23
Trans piperitol	0.13	0.32	0.34	0.30	0.33	0.36	0.39	0.39	0.21	0.30	0.26	0.32	0.38	0.33	0.24
Carvone	0.00	0.00	0.13	0.09	0.21	0.18	0.16	0.17	0.10	0.11	0.10	0.08	0.11	0.15	0.08
Unknown MW 182 (35)	0.00	0.23	0.27	0.21	0.24	0.28	0.22	0.26	0.22	0.19	0.25	0.24	0.29	0.24	0.16
Bornyl acetate	0.51	0.48	0.50	0.41	0.44	0.44	0.40	0.43	0.36	0.33	0.38	0.37	0.63	0.68	0.32
4-terpinyl acetate	0.24	0.23	0.11	0.24	0.11	0.20	0.26	0.22	0.13	0.10	0.23	0.15	0.27	0.51	0.41
Beta terpinyl acetate	0.10	0.16	0.21	0.12	0.09	0.11	0.05	0.08	0.05	0.21	0.15	0.15	0.10	0.08	0.10
Beta-caryophyllene	8.85	7.82	7.61	7.55	6.57	6.33	6.01	6.73	6.05	7.06	6.16	6.40	9.67	10.6	6.92
Unknown MW 204 (39)	0.16	0.15	0.11	0.17	0.10	0.14	0.24	0.22	0.13	0.15	0.10	0.13	0.17	0.22	0.19
Humulene	3.48	3.04	1.44	2.86	1.89	2.32	1.99	2.50	2.15	2.75	2.02	2.48	3.75	3.79	2.78
Alloaromadrene	0.27	0.08	0.10	0.08	0.05	0.05	0.24	0.10	0.23	0.10	0.21	0.20	0.20	0.29	0.15
Germacrene-D	3.29	3.19	1.04	3.10	2.45	2.85	2.62	2.96	2.99	3.24	3.51	3.26	4.92	4.69	2.47
Gamma-elemene	0.50	0.40	0.50	0.45	0.37	0.36	1.61	0.42	0.23	0.81	0.19	0.21	0.32	0.45	0.29
Gamma-cadinene	0.24	0.19	0.27	0.21	0.15	0.16	0.21	0.25	0.17	0.16	0.19	0.17	0.27	0.38	0.31
Caryophyllene epoxide	0.80	0.65	0.50	0.52	0.58	0.52	0.62	0.55	0.47	0.73	0.46	0.51	0.89	0.80	0.37
Humulene epoxide	0.55	0.45	0.34	0.31	0.39	0.30	0.25	0.36	0.32	0.40	0.29	0.35	0.61	0.46	0.30
Unknown (45)	0.28	0.20	0.27	0.15	0.16	0.12	0.10	0.26	0.14	0.24	0.12	0.24	0.31	0.32	0.16
Unknown (46)	0.18	0.11	0.09	0.09	0.09	0.08	0.08	0.10	0.09	0.11	0.11	0.11	0.26	0.25	0.14

13. Appendix Section IV 9.2

Amount of volatile oil present in blackcurrant buds at various harvest dates during the growing season (µℓ/g bud fresh weight)

Plant density (plants/m²)	1.1			1.6			2.2			3.0		
Harvest date	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
12/11/82	6.99	0.69	3.84	0.86	2.72	1.79	4.88	0.02	2.45	0.00	3.35	3.35
19/11/82	2.88	3.74	3.31	3.09	2.67	2.28	1.77	2.56	2.17	2.97	3.32	3.15
3/12/82	6.53	2.26	4.40	2.54	5.88	4.21	2.62	1.42	2.02	3.18	2.36	2.77
10/12/82	2.90	3.28	3.09	6.52	1.57	4.05	1.59	4.62	3.10	3.57	4.56	4.07
17/12/82	3.18	3.64	3.41	3.71	3.59	3.65	1.56	4.37	2.97	2.79	2.09	2.44
23/12/82	8.93	6.44	7.69	4.65	-	4.65	4.11	6.04	5.08	4.43	4.96	4.70
6/1/83	4.46	8.84	6.66	2.71	4.61	3.68	6.17	6.02	6.09	4.36	7.21	5.79
20/1/83	7.52	6.82	7.20	4.71	8.14	6.43	3.54	6.69	5.12	9.92	6.50	8.21
3/2/83	5.76	8.18	6.97	4.89	7.30	6.10	4.48	9.89	7.19	7.02	6.84	6.93
18/3/83	11.4	6.48	8.94	5.26	8.58	6.92	6.72	10.80	8.76	12.94	4.92	8.86
8/4/83	10.74	13.54	12.14	11.69	9.49	10.59	6.74	13.94	10.84	6.89	12.16	9.53
29/4/83	12.25	13.46	12.86	7.02	12.16	9.59	8.07	10.40	9.24	10.67	12.25	11.46

Plant density (plants/m²)	5.2			7.2			10.1		
Harvest date	I	II	Mean	I	II	Mean	I	II	Mean
12/11/82	1.80	3.55	2.68	6.53	2.23	4.38	5.17	0.03	2.59
19/11/82	4.14	-	4.14	6.03	0.96	3.50	3.92	1.17	2.55
3/12/82	3.23	2.82	3.03	1.55	3.35	2.45	3.15	2.61	2.88
10/12/82	4.12	2.84	3.48	1.69	4.30	3.00	2.74	3.03	2.88
17/12/82	1.68	2.18	1.93	0.96	5.11	3.03	0.00	3.82	3.82
23/12/82	8.94	5.91	7.43	4.65	-	4.65	7.47	9.30	8.39
6/1/83	5.80	-	5.80	-	-	-	-	-	-
20/1/83	6.09	7.52	6.81	9.14	7.09	8.12	4.68	11.91	8.30
3/2/83	6.34	9.28	7.81	9.20	5.44	7.32	11.13	9.28	10.21
18/3/83	5.14	8.56	6.86	10.35	9.90	10.13	11.56	14.04	12.80
8/4/83	9.38	6.40	7.89	7.29	7.24	7.27	8.91	18.69	13.80
29/4/83	9.75	8.77	9.26	6.33	11.19	8.76	11.63	11.72	11.68

14. **Appendix Section IV 8.1 and 9.3**

Organoleptic Calibration of Sensory Assessor

This calibration was carried out using the triangle test described by Larmond (1977), in a cool, quiet environment free from any noticeable odours. The three samples chosen for this test were from the field bud burst experiment.

Sample 1 and 2 - identical samples from harvest on 26/8/83.

Sample 3 - sample of White Bud from 12/8/83.

	Sample	1	2	3	Correct Score
Tests	1	0	0	1	✓
	2	0	0	1	✓
	3	0	1	0	x
	4	1	0	0	x
	5	0	0	1	✓
	6	0	0	1	✓
	7	0	0	1	✓
	8	0	0	1	✓
	9	0	0	1	✓
	10	0	1	0	x
	11	0	0	1	✓
	12	0	0	1	✓
	13	0	0	1	✓
	14	0	0	1	✓
	15	1	0	0	x
	16	0	0	1	✓
	17	0	0	1	✓
	18	0	0	1	✓
	19	0	1	0	x
	20	1	0	0	x
	21	0	1	0	x

Total correct scores = 14 out of 21. The probability (from Larmond 1977) that this result is due to chance is 1%; therefore it is accepted that the sensory assessor is able to discern these aroma differences.