A study of post-harvest changes in watercress: Rorippa nasturtium-aquaticum (L.) Hayek.

Spence, R. M. M.

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A STUDY OF POST-HARVEST CHANGES IN WATERCRESS

Rorippa nasturtium-aquaticum (L.) Hayek

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R-M.M. SPENCE

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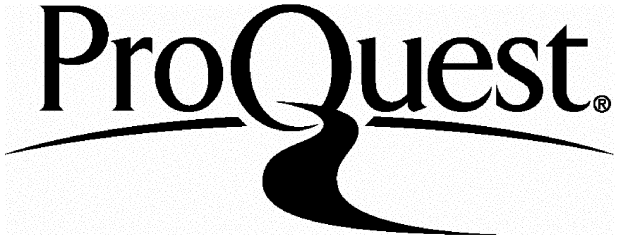
R-M M Spence



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ABSTRACT

Watercress is a highly perishable crop and this thesis is a study of post-harvest changes in watercress which will aid an understanding of deterioration processes during storage and transport, and of conditions which affect senescence.

The effects of three different daylengths (8, 12 and 16 h), corresponding to winter, spring and summer conditions, on growth, yield and shelf-life of plants were studied.

The commercial distribution chains of watercress were studied over a five month period to determine the post-harvest conditions to which it is exposed and to measure both physiological and sensory changes occurring. Comparisons were made between long and short haul distribution chains, refrigerated and unrefrigerated transport, and two types of prepacks.

The results of these commercial distribution chain studies led to the development of a sensory profile for watercress. This type of assessment proved of value in determining changes in quality which could not be detected by the physiological methods used. Aroma was found to be a reliable indicator of watercress freshness and volatiles were collected and compounds identified for use as odour reference samples.

Volatiles emitted from the plant material were collected using headspace apparatus and a number of compounds not previously reported in watercress were identified. Their detection is accounted for by the use of non-destructive methods of collection, resulting in minimum glucosinolate degradation. Few isothiocyanates or thiocyanates were collected from

fresh material, but as the plant deteriorated the amounts of sulphur compounds in samples increased as glucosinolate degradation occurred. Changes in the production of watercress volatiles under different storage conditions were monitored using a dual headspace apparatus. Storage in the dark at 10^{o}C in a current of air kept the watercress fresh and crisp apart from yellowing of leaves, which was prevented by cytokinin treatment.

A preliminary examination was made of watercress microflora for the presence of microorganisms which might be responsible for the large quantities of acetic acid found in the samples of volatiles. The epicuticular wax was analysed to determine whether it retains aroma components liberated from within the plant, or whether it contributes to the aroma itself. The wax surface was examined using scanning electron microscopy and found to be an amorphous layer without any structures capable of retaining flavour compounds.

GENERAL INTRODUCTION

Watercress is a cruciferous freshwater emergent macrophyte, growing in shallow, slow-running, calcareous water at the edges of rivers and streams. It is a creeping, extensively branched, perennial herb with hollow stems; in shallow water the plant may be floating (Howard and Lyon, 1952). The leaves are pinnate with three to six pairs of well-separated ovate or orbicular glabrous leaflets with slightly sinuate margins. Adventitious roots develop exogenously in leaf axils and, in highly humid conditions, well above the soil or water level. Seedling roots develop into a basal or anchorage system, whereas most adventitious roots are free-floating; both types of roots participate in the uptake of nutrients (Cumbus and Robinson, 1977a). The watercress inflorescence is a short raceme of small white flowers (6.0 mm diameter). Cross pollination by insects, such as Coleoptera, Diptera and Hymenoptera, is the usual method of fertilising flowers, although in rainy weather compatible self-pollination occurs in closed flowers. The fruits are borne on spreading, slightly upcurved pedicels, and a double row of seeds in each half of the siliqua is a marked taxanomic character. Vegetative reproduction also occurs by the rooting of adventitious roots when stems fall over. Watercress is phenotypically a very plastic plant and although it can tolerate dry conditions, its growth is related to the amount of water available (Howard and Lyon, 1952).

Watercress has been used as a medicinal plant and for culinary purposes from the first century A.D. to the present time. Its characteristic taste was mentioned in Dioscorides' Materia Medica (c. A.D. 77) (Howard, 1959) and its Latin name, Nasturtium officinale

R. Br., is said to be derived from nasus, the nose, and tortus, twisted, due to the pungent sensation on eating the plant (Henslow, 1905).

In Pliny's time watercress was regarded as efficacious for brain disorders such as insanity, which is reflected in its Greek name 'Kardamon', meaning "head-subduer" (Henslow, 1905). In medieval and Anglo-Saxon herbals, for example the Herbarium of Apuleus Platonicus (Cockayne, 1864), watercress, mixed with vinegar, was said to be good for those who were dull and lethargic, and a watercress pottage was recommended to cleanse the blood in spring. In the seventeenth century watercress was crushed and applied to the face to try to remove freckles, which were considered unfashionable, and it was also thought to cure toothache and hiccups (MacCarthy, 1976). From mention in herbals, the plant passed to early pharmacopaeias of many countries, including England, France, Scotland and Denmark, where it was valued as an antiscorbutic. Although watercress is rich in salts and vitamins (calcium, phosphorus, iron, vitamins A and C - Paul and Southgate, 1978), its use as a medicine became superfluous with improved medicinal and dietary standards, and it was excluded from the re-issue of the pharmacopaeias of London and Edinburgh in 1809 (Manton, 1935).

The use of watercress as a food was known to the Greeks, when 'Kardamon' was used as a salad as well as a medicine. In the fourteenth century "cresson de fontaine" was one of the street cries of Paris, and in this country it was known as food for the poor, (Dorveaux, 1917, quoting Bonfons, 1545). In the eighteenth century public demand for watercress as food increased considerably, but in spite of many centuries of exploitation, the marketed produce was

still the wild, uncultivated plant. This was collected from streams and ditches until the beginning of the nineteenth century when commercial cultivation as we know it today began.

The Distribution and Cultivation of Watercress

Watercress is widely distributed throughout the temperate regions of the world. Plants from all over Europe were collected by Manton (1935) in order to determine the natural occurrence of the three types of watercress, Rorippa nasturtium-aquaticum (L.) Hayek, R. microphylla (Boenn.) Hyland, and their hybrid R. nasturtium-aquaticum x R. microphylla. Although the number of plants collected was limited, Manton suggested that all watercress in southern Europe is R. nasturtium-aquaticum, whereas R. microphylla is only found in northern Europe i.e. England, Scotland, Ireland, Holland and Denmark. However, Clapham, Tutin and Warburg (1962) state that R. nasturtium-aquaticum is native to Great Britain, Ireland, Channel Islands, southern and central Europe, northwards to southern Sweden and Denmark, and eastwards to Posen, Lower Silesia and the Carpathians; North Africa and West Asia. R. microphylla is native to Great Britain, Ireland, Europe and West Asia, being more abundant than R. nasturtium-aquaticum in Scotland. The sterile hybrid is native to North England, Scotland and Ireland.

Watercress has been introduced into America, South Africa, Australia and New Zealand, and is cultivated on a small scale in several parts of the tropics and subtropics. In New Zealand it is a serious river weed (Clapham et al, 1962). It is mainly a lowland species found at the edges of rivers, streams, ditches and springs, where water is shallow (7 - 15 cm deep) and plants can grow with ascending leafy shoots just above the water level.

The cultivation of watercress as a food crop is recorded 400 years ago (Dorveaux, 1917), but commercial cultivation dates from the eighteenth century in Germany and the nineteenth century in England. In Britain the first watercress beds were built in 1808 in Kent. One of the earliest accounts of watercress cultivation was written by Glenny (1897) and later the Ministry of Agriculture, Fisheries and Food published a paper on cultivation (Hoare, 1924). The methods of cultivation used today are very similar to those of a hundred years ago, and still one of the major concerns of growers is the short post-harvest life of cress, which prompted the growers in the 1890s to suggest putting blocks of ice in the middle of boxes of watercress transported over long distances (Glenny, 1897).

Watercress is grown commercially in irrigated beds as an annual leafy salad crop producing several harvests. The yield and number of harvests depends on the variability of local seasonal climatic conditions. The plant grows on gravel, sand, silt or clay substrates and is intolerant of stagnant water and acid or alkaline peat. Commercial cress holdings are usually located in predominantly soft limestone areas, for example Jurassic oolitic limestone and Cretaceous greensands and chalk. These geological formations often form slight to moderately high ground and become aquifers because of their porous structure. Holdings are sited in broad, shallow valleys and irrigated either by natural springs rising from an impervious layer adjacent to the aquifer, or by water in the aquifer being pumped up through bore-holes. The major areas of production in England follow the chalk and soft limestone areas in Dorset, Wiltshire, Hampshire and Surrey (Lyon and Howard, 1952).

Commercial Beds

Commercial watercress beds are usually rectangular in shape, although many of the older ones follow the contours of the land. They are enclosed by low concrete walls and the bed substrate has a basal layer of compacted chalk, flint or brick rubble covered with a 7 - 10 cm gravel layer. The gravel is raked across and down the bed to produce an even depth of water flowing down a slight gradient. If possible, beds should be sited in south or south-east open aspects to obtain maximum daylength in short-day winter conditions, and sheltered from cold north and east winds (Rhodes, 1967).

The irrigating water at source has a constant temperature of 9 - 10^{o}C and this heat protects plants against frost damage in winter. Although the water temperature decreases towards the outlets, a maximum bed length of 100 m has now been established. The flow of water is regulated to prevent seeds washing away and seedlings drowning, but "hidden hunger" and reduced growth caused by insufficient irrigation must also be avoided. During winter months the water level is raised to provide protection from frost damage and cold wind "leaf scorching".

Propagation

Growers are increasingly propagating watercress from seed as an annual crop to avoid the spread of virus infection by continual propagation from cuttings. Seed is chitted (pre-germinated) by mixing with moist sand or peat and incubating at 18^{o}- 20^{o}C until radicle emergence after 24 - 48 h. Sowing rates depend on whether the bed is to be used for production or for stocking (nursery beds). Production beds are sown 10 - 15 lb ac^{-1} (11.2 - 16.8 kg ha^{-1}) which, assuming a 60% survival rate, gives a final crop density of 2 - 3 x 10^{3} plants yd^{-2} (1.67 -

2.76×10^3 plants m^{-2}). Stock beds are sown at double the production bed rate. Direct sowing into open beds, by hand or machine, accounts for high seedling mortality, which is compensated by high sowing rates and transplanting from stock beds to produce a dense crop sward. Some growers cover seed beds with frames to avoid damage by rain and they are kept moist by over-head spray lines or small channels of water running down the sides of beds. However, increasing seed costs have led to the introduction of propagation houses where seeds are sown on trays of peat and germinated under mister units. In heated houses a six day sowing programme operates and the seedlings are planted out when 3 cm tall by broadcasting clumps of seedlings by hand. Growers are currently developing machinery to mechanise this operation in the future. As the plants become established they are often "topped" to encourage shoot growth, and the cuttings are left to root and fill gaps in the beds.

In order to reduce the time needed to produce a harvestable crop of watercress, protective coverings of different types have been used on experimental beds by A.D.A.S. at Fobdown Farm, Alresford, Hampshire. Both steel-framed polythene tunnels and inflated "bubbles" are currently used by one grower.

Harvesting and Marketing

Watercress is cut with a knife when stems are 15 - 20 cm long, stacked in large plastic bins and transported to the packing shed. In winter the crop is usually too short for cutting, and is pulled and the roots trimmed off. One grower is developing a mechanical harvester to offset increasing labour costs and the shortage of workers. However, this machinery does not cut the stems a suitable length for bunching, but shorter sprigs which are marketed in a

"jumble pack". After harvesting, axillary buds in the remaining stubble develop lateral shoots which produce the second and subsequent crop harvests.

Most watercress is sold in the traditional bunch which varies in weight depending on the packer, but the average bunch weighs 4 oz (112 g). Stems are fastened with an elastic band and their ends trimmed. Fifteen or twenty bunches are packed in a waxed cardboard box, chilled by hydrocooling, and stored in a cold room until despatched to market. The grower with the largest business in this country also markets cress in two types of supermarket prepacks; the dew pack and jumble pack. Watercress for dew packs is cut to a standard length and placed in boat-shaped plastic containers which are covered with shrink-film. Condensation within the pack should maintain the turgidity of the plant material. The mechanically harvested cress for jumble packs is washed, hydrocooled, shaken dry on an agitating conveyor belt and packed in acetate bags which are sealed, weighed and priced for the convenience food market. This packaging was introduced to provide a standard, high quality, table-ready food which complies with rigorous hygiene specifications and control checks laid down by the retailer.

Types of Watercress and Problems of Cultivation

Research on watercress began in this country in the 1930s. Manton (1932) published results of cytological work showing that the type grown was still the wild species because watercress had only been cultivated on a large scale for a hundred years. This species was found to contain three polyploid forms with 32, 48 and 64 somatic chromosomes, and work by Jaretzky (1932) confirmed these findings. All three types were found to occur in Britain and Europe and,

although morphologically very similar, the 48 chromosome triploid form is sterile, whereas the other two types are fertile. The sterile form is known as brown cress (R. nasturtium-aquaticum x R. microphylla) and Manton (1935) suggested that this could be the hybrid between a native brown tetraploid (R. microphylla) and an imported green diploid cress (R. nasturtium-aquaticum) which was only cultivated in England after 1875 when the practice of alternating between summer green cress and winter brown cress was introduced. Studies by Howard (1947) confirmed the brown cress as a hybrid of the diploid and tetraploid species; but within the two cultivated types of watercress there are varieties which show differences in leaf shape, plant height and degree of flowering. Herbarium records provided Howard and Lyon (1952) with sufficient evidence to dismiss Manton's suggestion that the hybrid was not found in Britain before 1875.

The appearance of watercress mosaic virus (a strain of cabbage black ringspot virus) destroyed the possibility of increased production of brown watercress and the development of new varieties by crosses with the brown type, since only plants grown from seed were virus-free (Tomlinson, 1956). As well as watercress mosaic virus, turnip mosaic virus (TuMV) and turnip yellow mosaic virus (TYMV) both reduced yields in watercress and caused blemishes on the leaves. TuMV is spread by aphid vectors, but not TYMV, which is thought to be transmitted by flea beetles (Tomlinson, 1974).

Crook root disease, caused by the water-borne fungus Spongospora subterranea f.sp. nasturtii (Howard and Lyon, 1950) also reduced yields and threatened the industry, then growing the green type, although brown cress proved more susceptible to the disease (Spencer and Glasscock, 1953). Extensive research by Tomlinson (1958 a,b,c)

showed that the zoospores of the crook root fungus are killed by solutions containing 1.0 mg l^{-1} zinc. He suggested a method for controlling crook root disease by applying zinc frit to the watercress bed substrate. A drip-feed method has been developed to supply zinc sulphate in the inlet water to give a constant concentration of 0.1 mg l^{-1} zinc, which has proved effective in controlling the disease.

The production of brown watercress was stopped in the 1950s because of continued infection by cabbage black ringspot virus through vegetative reproduction, its higher susceptibility to crook root disease and the increasing popularity of green cress. However, a disadvantage with the green type was found to be its early flowering, thus shortening the harvesting season. The effect of daylength on the flowering of commercial watercress strains was investigated by Bleasdale (1964). He found that daylengths greater than 13 hours caused flowering and stem elongation, so that as a result of normal sowing in the autumn, the crop could be expected to flower in spring the following year. Stem elongation accounts for the different seasonal harvesting techniques used; during winter the cress is short and the stems are pulled; in spring and autumn the longer stems are cut for bunching. The techniques of seeding the beds in spring and autumn, and not growing on from stubble, plus selection of the latest flowering plants for seed production have alleviated the early flowering problem.

The recognised types of watercress are summarized below : -

1) R. nasturtium-aquaticum (L.) Hayek, syn. N. officinale R. Br, diploid (2n = 32) and cultivated as green or summer cress.
2) R. microphylla (Boenn. Hyl), syn. N. microphyllum (Boenn.) Rchb.

and N. uniseriatum Howard and Manton, tetraploid (2n = 64); apparently an allotetraploid hybrid of R. nasturtium-aquaticum, possibly with a Cardamine sp. (Clapham et al, 1962). This type is not cultivated.

3) R. nasturtium-aquaticum x R. microphylla, triploid (2n = 48) with leaves and stems which turn purple-brown in cold weather, formerly cultivated as brown or winter cress.

4) Florida 'Strain' flown from Florida,U.S.A. to England in 1976 and sent to the National Vegetable Research Station, Wellesbourne, Warwick, for virus identification and eradication, and to Kew Gardens for chromosome count; triploid (2n = 48). It has a spreading growth habit, develops a purple leaf tinge in cold weather and does not produce viable seed. Growers consider it to be the original British brown cress, introduced into Florida.

Research into the "Hotness" of Watercress

In the nineteenth century researchers began to extract and identify the compounds responsible for the characteristic odour and hot flavour of watercress. Hofmann (1874) distilled uncut material and concluded that the main constituent of watercress essence was phenyl propionitrile. However, Gadamer (1899) crushed the watercress before distillation and identified 2-phenethyl isothiocyanate as the major flavour component. He also extracted the glucosinolate from watercress seeds.

Isothiocyanates are the compounds responsible for the strong, hot taste in horse-radish, mustard and several other plants. They are liberated from the corresponding glucosinolate by the hydrolysing enzyme myrosinase, which is contained in specific cells in the plant tissue and released by cell disintegration during crushing or chewing. β-phenethyl isothiocyanate was detected in watercress seeds by Kjaer,

Conti and Larsen (1953) and Gadamer had previously identified this compound from fresh watercress plants. These results have been confirmed by more recent work which has shown β-phenethyl isothiocyanate to be present in watercress leaves (Anon., 1964; MacLeod and Islam, 1975).

Nutritional Studies

Being semi-aquatic, the watercress plant derives its nutritional requirements mainly from the water supply. To support a dense crop 500,000 to 1,000,000 gallons of flowing water are needed per acre (5,165,000 - 11,233,000 l ha^{-1}) per day. One of the first nutritional studies into watercress production was by Barbier and Marcel (1938). They concluded that in France, while irrigating waters normally supply sufficient nitrate and potassium, the amount of phosphate was inadequate to meet plant requirements. They recommended the application of phosphatic fertilizers to the watercress bed substrate. Hamence and Taylor (1947) discovered a nitrate deficiency in the water supply of cress beds in Lincolnshire which was rectified by the addition of sodium nitrate. This shows that areas are subject to regional differences. Nutrient levels in water from chalk, limestone and greensand areas in England were examined by Howard and Lyon (1952) and they concluded that the Lincolnshire case was unusual, and that in other districts of England there is an adequate level of nitrate for growth. They also found the low nutrient levels in irrigating water were compensated by the enormous volumes passing through the beds. Irrigating waters, apart from those in greensand aquifers, are usually supplemented with additional phosphate either as super-phosphate or basic slag. A balance sheet for the flow of elements into and out of a cress bed was compiled by Crisp (1970) and he suggested drip-feed

application of phosphate and potassium fertilizers at the inlet to beds being more economical than direct applications which are normally leached away within 24 hours.

The importance of sulphur nutrition to the flavour of watercress was shown by Freeman and Mossadeghi (1972). When the sulphur supply is limiting, the synthesis of sulphur-containing proteins and metabolites for growth occurs exclusively. However, when the sulphate concentration exceeds a critical value (0.25 meq l^{-1}) production of the flavour precursor occurs in addition to the synthesis of proteins and metabolites. The increase in flavour with increased sulphate concentration was shown both by sensory tests and an increase in the β-phenethyl isothiocyanate concentration. Flavour preferences vary greatly among consumers and altering the concentration of sulphate in the water supply may meet these preferences if it is considered a viable economic proposition.

The most recent nutritional studies on watercress by Robinson and Cumbus (1977) and Cumbus and Robinson (1977a) have established critical levels of nitrogen, potassium and phosphorus by plant tissue analysis, and elucidated the relative contributions of the free-floating root system and the basal system. It was found that basal roots can absorb ions, thus making the bed substrate a potential source of nutrients. Further work (Cumbus and Robinson, 1977b; Cumbus, Hornsey and Robinson, 1977) suggests that chlorosis in watercress plants may be due to the different contributing factors of two systems : 1) enhanced absorption of zinc coupled with reduced manganese uptake, and 2) iron deficiency caused by the inhibition of iron translocation by increasing levels of phosphorus, zinc and manganese.

Standards of Hygiene and a Code of Practice for Cultivation

Since watercress is primarily a salad crop, it is only washed before consumption, not cooked. Standards of hygiene during production and marketing have caused concern, and culminated in the "Approved Code of Practice" drawn up by the Watercress Branch of the National Farmers Union in 1975 (revised in 1979) under which growers can apply for registration. The code controls growing conditions in and around watercress beds to eliminate contamination by livestock and ensure a pure water supply. After registration a grower can display the Code of Practice symbol to advertise the fact that a particular watercress holding complies with the required standards. As yet registration is only voluntary.

Early watercress production was merely an elaboration of stream-growing and bacteriological studies were concerned only with finding measures to eliminate coliform infection on the cress (Walters et al, 1957; Tee, 1962), and it was suggested that packed cress be immersed in chlorinated water containing 40 - 50 mg l^{-1} chlorine. In 1966 the Ministry of Health found little evidence that watercress is a vehicle of enteric organism infection as was previously thought (Tee, 1966), but that contamination of watercress beds with water from nearby meadows can cause an infestation of snails, possibly carrying liver fluke. Correct conditions of cultivation, as required by the Code of Practice, completely eliminate the possibility of liver fluke and coliform contamination. The hydrocooling and chlorination of vegetables other than watercress have proved advantageous (Scarlett, 1963) and watercress growers are advised to chill boxed bunches of cress with water containing 50 mg l^{-1} chlorine. However, doubts have been expressed as to the value of chlorination since current production methods should eliminate the danger of coliform infection. The effects

of chlorine treatment on flavour and texture, and on the microbial flora in prepacks have not been investigated.

Post-Harvest Changes

Watercress growers aim to produce high quality cress which will reach the market or shop with the least amount of damage or deterioration. The conditions to which watercress is subjected after harvesting affect its state when it reaches the shop, and hence its shelf-life. Increased production means marketing in areas far from watercress beds and rapid distribution is necessary because of its short shelf-life.

Since the value of the watercress crop in Britain is only 0.57% of the total for all vegetables (M.A.F.F., 1979) very little research has been carried out into the changes occurring in cress during storage and transport, and establishing the optimum conditions to maximise its shelf-life. The rates of carbon dioxide production and water loss over a range of temperatures for thirty commodities, including watercress, were measured by Robinson, Browne and Burton (1975). They concluded that watercress should be stored at or near to $0^{o}C$ without causing freezing damage, and above 95% relative humidity, which reduces water loss by evaporation, but may encourage microbially induced rotting. Under these conditions they found that watercress could be stored for 4 - 7 days, and these results were confirmed by Tomalin, Robinson and Browne (personal communication) who also found similar storage characteristics for watercress prepacked in shrink-film covered plastic containers. They recommend keeping cress at temperatures below $5^{o}C$ and extending the cold chain as far as possible. Most growers aim to hydrocool the bunched watercress at $2^{o}C$ and then transfer to a cold store room at $6^{o}C$ before transportation. Only the largest grower at present can afford refrigerated transport, and until

there is more control by the grower over retail storage, the smaller growers consider the financial outlay for refrigerated lorries unwarranted. Unless the cold chain is extended to the consumer, the initial exposure to very low temperatures during hydrocooling and storage (2^{o}- 6^{o}C) followed by exposure to high temperatures during unrefrigerated transport and distribution (15^{o}- 20^{o}C) may prove more harmful to the plant than keeping it at a constant temperature throughout the chain.

An increasing number of vegetables are prepacked for supermarkets and are very popular with consumers. The watercress industry is beginning to follow this trend, particularly as the traditional bunching and boxing of cress increases the number of damaged sprigs from 35% to 75% and increases the rate of deterioration (Tomalin et al, personal communication). At present most watercress is still marketed in bunches, but a small proportion is also sold in two different pre-packs - dew packs and jumble packs. However, very little research has been done into the environmental conditions within these packs and their effect on the watercress and its associated microflora.

Aims of the Thesis

The main aims of this thesis are to determine the conditions during post-harvest processing, storage and distribution to which watercress is subjected, and to measure quality changes by sensory assessment and physico-chemical methods; to link sensory results and physiological deterioration with the production of volatiles; and finally to suggest optimum storage and transport conditions for maximum shelf-life.

Section 1 describes an experiment to determine the effects of daylength on the growth and development of watercress. This study was made in

response to a request from growers who observe that in winter watercress plants are tough and hardy compared with the more delicate, quicker growing summer cress.

Section 2 is a study of the post-harvest conditions to which watercress is exposed, and the physiological and sensory changes occurring.

Section 3 is concerned with the development of a sensory profile for watercress to assess the different attributes contributing to its quality.

Section 4 contains an analysis of the volatile components of watercress and the changes occurring in these compounds under different storage conditions. This section also contains a preliminary examination of watercress microflora.

Section 5 describes a study of watercress cuticular wax to determine its importance in volatile production.

SECTION 1

Introductory Experiments to Determine the Effects of 8, 12 and 16 hour Daylengths on the Growth and Development of Watercress

1.1 Introduction

The possibility that daylength might influence plant distribution and development was first considered in the nineteenth century (Henfrey, 1852). The recognition of daylength as a major factor controlling the types and degree of responses, such as flowering, dates from the classic paper of Garner and Allard (1920) in which they discuss the effects of day and night periods on growth and reproduction in plants. They also introduced the terms photoperiod for daylength, and photoperiodism for the response to relative lengths of night and day.

The effect of light on the germination of watercress was studied by Howard and Lyon (1951) and Bleasdale (1964) established daylength as the major factor affecting the flowering of watercress. R.nasturtium-aquaticum was shown to be a long-day species with internodes of the main stem elongating more and with fewer branches developing in long days than in short days. The number of pinnae per leaf was not significantly affected by daylength.

Growers have observed significant seasonal differences in the crop yield and shelf-life of watercress. Winter harvested cress is reported to be tougher, hardier and have a longer shelf-life than summer grown cress which is quicker growing, more fragile and highly perishable. An investigation was carried out to determine the effects of 8 h (winter), 12 h (spring) and 16 h (summer) daylengths on the growth, crop yield and post-harvest shelf-life of watercress grown in controlled environment watercress bed simulation tanks. Two experiments were made at different seasons in 1978 :

Experiment 1 April - May

Experiment 2 September - November

1.2 Watercress Bed Simulation Tanks

Watercress plants are capable of growing on land as well as in their natural environment. However, dry land conditions promote extensive basal root development, leaves are smaller, and the whole plant is generally tougher and more fibrous (Cumbus, 1975). To reproduce commercial bed conditions, in order that experimental results would be relevant to the commercial situation, small scale tank units were constructed into which seedlings were transplanted and continuously irrigated with a low concentration aqueous nutrient solution.

Watercress was grown in two types of simulation tanks described in 1.2.1 and 1.2.2. The controlled environment tanks were used for the investigation of the effects of different daylengths on growth, described in this section, and both types of tank were used to grow plant material for work carried out in Sections 3, 4 and 5.

1.2.1 Controlled Environment Simulation Tanks

Six tanks (170 x 40 x 25 cm high) were constructed with 15 mm marine plywood. Two interior plywood weirs, 15 and 13 cm high, were placed 12 and 17 cm from each end, respectively. Between the lower weir and the end of each tank two 5.0 cm diameter holes were fitted with outlet tubes leading into a blackened 40 l polythene nutrient reservoir. A 3.0 cm diameter hole in the side of the tank was fitted with perspex tubing as a bleed valve. All tank surfaces were painted with clear polyurethane varnish ("Blackfriar", E. Parsons and Sons Ltd.) and the interior coated with non-phytotoxic bituminous paint ("Bitumastic", Wailes Dove Bitumastic Ltd.). Each tank contained a gravel substrate (6 mm water-washed pea gravel) to a depth of 11.0 cm.

Nutrient solution (1.2.3) was circulated at 3 l min^{-1} through each tank, via four inlet tubes, by an electric centrifugal water pump (Stuart Turner Ltd., Type 16). The solution temperature was controlled by a refrigeration unit set at 10°C, through which the circulating nutrient solution was passed.

Each tank was enclosed in a 3 mm hardboard cabinet (2 m x 80 x 95 cm high), supported on Dexion framework and ventilated by an electric centrifugal blower fan unit (8 x 4.5 cm, Smiths Ltd.) positioned in an end wall of the cabinet. Lighting was provided by a bank of six parallel 2 m white 75/85 watt fluorescent reflective tubes suspended 50 cm above the substrate level. In addition, two 60 cm white 20 watt fluorescent tubes were suspended across each end of the light bank to compensate for decreasing light intensity at the tank ends. The lights, combined with reflective silver foil on the cabinet sides, gave illumination of 18.0 watts m^{-2} at substrate level. Lighting periods were controlled by electric time switches.

The units were housed in an unheated, well-ventilated glasshouse. Low air temperatures during winter months were raised to 10°C with an electric centrifugal hot air fan blower, but high temperatures were uncontrolled. One tank, with a cabinet side removed, is shown in Plate 1.1.

1.2.2 Natural Environment Simulation Tanks

Six tanks (180 x 60 x 33 cm high) were constructed with 19 mm marine plywood. A 17 cm high plywood weir, placed 8 cm from one end of each tank separated the 14 cm deep substrate layer (6 mm water-washed pea gravel) from five 2.5 cm diameter outlet holes (three in the base and

Plate 1.1 Controlled environment watercress bed simulation tank with side of cabinet removed, showing :

1 - gravel substrate

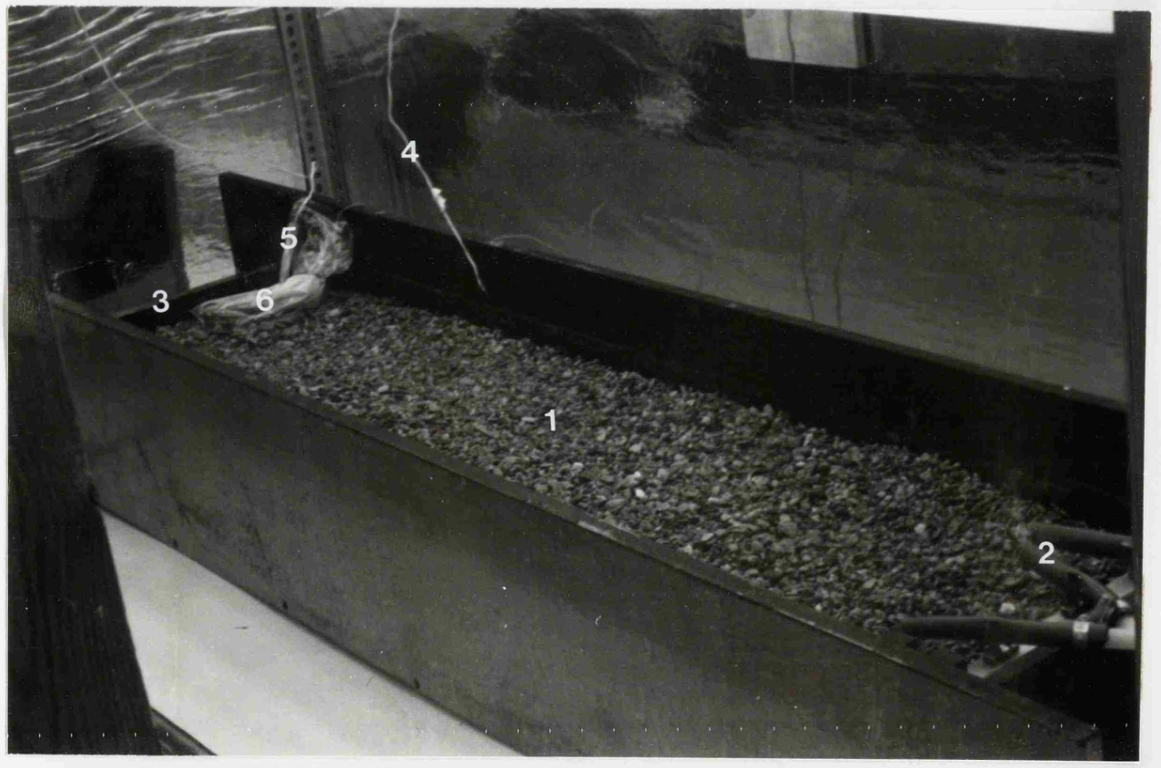
2 - inflow pipes for nutrient solution

3 - weir for outflow of solution

4 - thermocouple monitoring air temperature

5 - thermocouple monitoring water temperature

6 - thermostat for water cooling system



two in the end wall) with tubes leading into 200 l black polypropylene reservoirs sunk to soil level and covered with plywood lids. The tanks were painted with clear polyurethane varnish with an additional coat of non-phytotoxic bituminous paint on the inside. Nutrient solution (1.2.3) in each reservoir was circulated at 5 l min^{-1} by an electric centrifugal water pump (Stuart Turner Ltd., Type 16), through two inlet pipes, on to a plastic tray (55 x 5.0 x 2.0 cm high) at one end of the tank. This functioned as a weir, allowing a flow of solution down the tank, over the bottom weir and into the reservoir for recirculation.

The tanks were situated at the Bath University Field Station (Claverton Down). During severe weather conditions the watercress plants were protected by placing light wooden frames covered with clear PVC (180 x 60 x 25 cm high) on top of the tanks.

1.2.3 Nutrient Solution

Seedlings were transplanted to the watercress bed simulation tanks 14 days after sowing, and irrigated by tap water supplemented with nutrient solution. Analysis of the tap water was carried out in 1977 and compared with previous results obtained in 1972 (Robinson and Cumbus, 1977) (Table 1.1).

Table 1.1 Nutrient composition of a commercial watercress water supply and Bath University tap water.

Source	pH	Elements ppm (mg l^{-1})							
		N	P	K	Ca	Mg	Fe	Mn	Zn
Commercial supply	7.1	10.0	0.014	1.1	104	1.6	0.03	0.01	0.02
Bath university 1972	7.2	0.03	0.017	2.8	95	9.4	0.02	0.01	0.10
Bath university 1977	7.1	4.45	0.040	3.2	104	8.22	0.02	0.001	0.026

The macro elements, N, P, K, Ca and Mg were determined according to methods described by Mackereth (1963), and micro nutrients (Fe, Mn and Zn) by atomic absorption spectrophotometry. Differences in levels of nutrients in tap water in 1977 compared with 1972 were insignificant considering seasonal and annual fluctuations due to variation in rainfall (Wessex Water Authority 1977 - personal communication). Therefore, tap water was supplemented with two macro nutrient solutions (A and B) and one micro, as used by Cumbus (1975) and shown in Tables 1.2 and 1.3.

Table 1.2 Chemical composition of macronutrient stock solution (S.S) and final dilute solution (D.S) of 10 ml in 40 l reservoirs.

Solution	Chemical compound	Formula	S.S $g\ l^{-1}$	D.S $mg\ l^{-1}$		ppm
A	Calcium nitrate	$Ca(NO_3)_2.4H_2O$	250.32	62.58	N Ca	7.42 14.30
	Potassium nitrate	KNO_3	74.64	18.66	N K	2.58 7.20
B	Sodium dihydrogen orthophosphate	$NaH_2PO_4.2H_2O$	100.50	25.125	P	5.0

Table 1.3 Chemical composition of micronutrient stock solution (S.S) and final dilute solution (D.S) of 10 ml in 40 l reservoirs.

Chemical compound	Formula	S.S $g\ l^{-1}$	D.S $mg\ l^{-1}$		ppm
Boric acid	H_3BO_3	1.140	0.285	B	0.050
Cupric sulphate	$CuSO_4.5H_2O$	0.080	0.020	Cu	0.005
Manganese sulphate	$MnSO_4.4H_2O$	1.620	0.405	Mn	0.100
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.040	0.010	Mo	0.005
Zinc sulphate	$ZnSO_4.7H_2O$	0.440	0.110	Zn	0.025
Ferric EDTA	$C_{10}H_{12}O_8N_2FeNaH_2O$	28.000	7.000	Fe	1.000
Potassium chloride	KCl	2.100	0.525	K Cl	0.275 0.250

The nutrient solutions in the reservoirs of the controlled environment simulation tanks were initially renewed at 48 h intervals. As a result of monitoring potassium content in the irrigating solution as an indication of the overall rate of depletion of nutrients during plant growth, the solutions were renewed every 24 h after seedlings had been in the tanks for 21 days. The solutions in the reservoirs of the natural environment simulation tanks were not drained, but kept full with tap water supplemented with 25 ml of each nutrient stock solution once a week, to maintain growth with plants showing no deficiency symptoms.

1.2.4 Seed Material

The commercially produced green watercress (R.nasturtium-aquaticum) was used in all studies. There are no named varieties of green watercress, but various stocks have been established by different growers. The seed used in these experiments was obtained from Hampshire Watercress Ltd., Fobdown, Alresford, Hampshire in 1977. Florida watercress, used in Sections 4 and 5, was also obtained from Fobdown, from the A.D.A.S. experimental watercress beds.

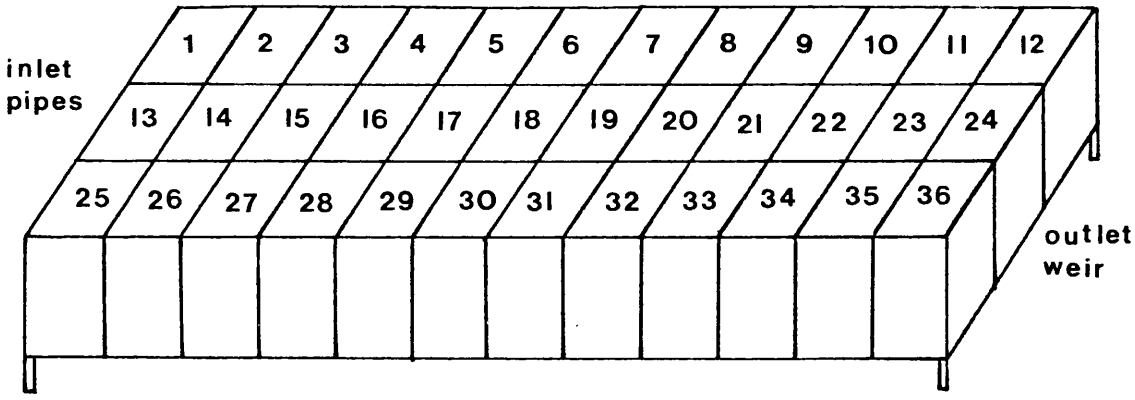
1.3 Materials and Methods

A mixture of 0.5 g (c. 2,000) seeds and 5 g sand was sown in each of twelve seed trays filled with moist Fisons Levington Universal compost. The trays were placed in a mist propagation unit in a heated glass-house (20^{o}C) and germination occurred within 2 days. After 14 days, two trays of seedlings were transplanted into each tank which was filled with dilute nutrient solution to the substrate surface. A

guard row of single plants was made around the tank perimeter, using half a tray of seedlings per tank, and the rest randomly transplanted into the remaining area. Two units were illuminated for 8 h, two for 12 h, and two for 16 h; comparable with winter, spring and summer daylengths, respectively. The lighting was switched on at 17.00 h each day to avoid increasing the relatively high day air temperatures with heat from the fluorescent tubes.

A 6 mm guage perspex grid (30 x 120 x 18 cm high) on short legs, divided into 10 cm^2 boxes (Fig. 1.1) was positioned 2.0 cm above the substrate in each tank after transplanting. The grids were designed so that the data obtained was suitable for statistical analysis.

Fig. 1.1 Diagram of perspex grid with numbering of boxes for harvesting.



Two days after transplanting the nutrient solution level was raised 1.5 cm above the substrate, and after another two days the tanks were irrigated. The nutrient solution (1.2.3) was renewed every 48 h initially, and then every 24 h 21 days after transplanting.

1.3.1 Harvesting Procedure

The crop in each tank was harvested when plants in half the boxes had grown 20 cm to the top of the grid (standard commercial harvesting height).

Day 1

Box 18 (Fig.1.1) was harvested and the following measurements taken :

plant number

plant height

leaf number

chlorophyll content

fresh weight

dry weight

A box adjacent to 18 was harvested in the other tanks and plant numbers, fresh weight and dry weight measured.

Day 2

Twelve boxes (selected at random) were harvested and plant numbers and fresh weight measured. Chlorophyll content was determined of plants from six boxes (1.3.2) and weight loss of plants from the other six (1.3.3).

Day 3

Three boxes (chosen at random) were harvested for mineral analysis (1.3.4).

1.3.2 Determination of Chlorophyll Content

The post-harvest breakdown and loss in chorophyll during senescence may be a measure of the shelf-life of watercress. The method described below was suggested by Cumbus (personal communication 1977).

One disc was cut with a 1.0 cm cork borer from the first fully expanded leaf below the apex of fifty plants. Two replicate samples of ten discs were placed in 15 ml glass specimen bottles, with snap-on plastic lids, containing 10 ml 80% (v/v) aqueous acetone and stored in the dark at $4^{o}C$ for 7 days. Three replicates of ten discs were floated, adaxial surface uppermost, in three 9.0 cm petri dishes on 5.0 ml deionised water. The dishes were placed in a $20^{o}C$ incubator with continuous illumination (2.0 watts m^{-2}) for 72 h. The discs were then transferred into three specimen bottles containing 80% (v/v) aqueous acetone and dark-stored at $4^{o}C$ for 7 days. Values for the chlorophyll extracts were read against an 80% (v/v) aqueous acetone blank at 663 nm and 645 nm on a Unicam SP500 spectrophotometer. Total chlorophyll was calculated using the following equation (Arnon, 1949).

$$\frac{E_{663} \times 8.02 + E_{645} \times 20.2}{7.85 \text{ (area of 10 discs, } cm^2) \times 100} = \text{total chlorophyll } mg\ cm^{-2}$$

The difference in chorophyll content between light and dark-treated leaf discs is an indicator of shelf-life.

1.3.3 Determination of Fresh Weight Loss

Six bunches were made from plants harvested from six boxes and the initial fresh weight of each bunch taken. Three bunches were hung in a growth cabinet under continuous light (60.0 watts m^{-2}) at $20^{o}C$ and $>$ 70% relative humidity (RH). Three bunches were hung in a $4^{o}C$ cold room at $>$ 70% RH with continuous illumination (2.0 watts m^{-2}). Temperature and humidity measurements were recorded on a thermohydrograph. The bunches were reweighed at periodic intervals over 24 h and fresh weight losses calculated as a percentage of initial fresh

weight as an indication of shelf-life (Cumbus 1977 - personal communication).

1.3.4 Mineral Nutrient Analysis of Plant Tissue

The plant material for mineral analysis was separated into leaves and stems, placed in muslin bags and dried at 80°C for 24 h, crushed with a pestle and mortar and stored in glass specimen tubes. Prior to analysis, the ground tissue was dried for 1 h at 100°C, cooled in a desiccator and duplicate 50 mg samples transferred into 20 ml calibrated Exelo tubes for wet digestion by the method described by O'Neill and Webb (1970). Nitrogen was measured as the indophenol blue complex and phosphorus as the yellow phosphovanadate complex on a Unicam SP500 spectrophotometer, at 630 and 420 nm respectively, and potassium by flame photometry (O'Neill and Webb, 1970).

1.3.5 Analysis of Results

The results for chlorophyll loss were analysed using Minitab II statistics package (Penn State University) available on the Multics computer, Avon Universities.

1.4 Results and Discussion

The number of days from sowing to harvest for plants grown under the three daylength treatments is shown in Table 1.4. Under 8 h days growth was slower and the crop harvested after 7 weeks compared with 6½ weeks under 12 and 16 h days. The difference of 4-5 days to harvesting between short (winter) daylengths and long (summer)

daylengths may be significant in contributing to the higher price of watercress in the winter months. However, commercially grown cress is exposed to low air temperatures in winter, and these probably explain the greater differences in time to harvest between summer and winter grown cress on watercress holdings (6 weeks mid June, 13 weeks mid February, Stevens, 1975), compared with the results in this study where there was no temperature difference between treatments.

Table 1.4 Number of days (mean value) from sowing to harvest.

Expt	Daylength (h)		
	8	12	16
1	50	46	45
2	49	44	44

Mean values from the two experiments (Table 1.5) show an increase in plant height, leaf number, fresh weight, chlorophyll content and yield with longer daylengths in Experiment 1. This is in agreement with work by Bleasdale (1964), who found that longer daylengths caused elongation of the internodes and more rapid growth. In Experiment 2 the results for plant height and leaf number appear to be anomalous for the 16 h treatment; the reason for this is unknown. The other results follow the same pattern as in Experiment 1. The plants growing more slowly in 8 h days had thicker stems and larger dry weights than plants in longer days. Lateral shoots were not produced in any daylength treatment. The density of plants was smaller in Experiment 2, and may have been due to a decrease in seed viability, variation in seed weight, or seedling death. However, there was no difference in density between treatments for both

experiments.

Table 1.5 Mean values from two experiments determining the effects of winter (8 h), spring (12 h) and summer (16 h) daylengths on growth of watercress. Measurements taken were plant height (cm), number of leaves/plant (leaf number), fresh weight/plant (F.W. g), dry weight/plant as a percentage of fresh weight (D.W.% F.W.), chlorophyll content (mg cm^{-2}), crop yield (F.W. m^{-2}) and plant density (plant numbers m^{-2}).

Growth parameter	Expt.	Daylength (h)		
		8	12	16
Height (cm)	1	17.314	19.090	24.787
	2	15.826	19.176	17.375
Leaf number	1	10.814	12.194	16.524
	2	12.246	15.550	14.286
F.W. (g)	1	0.486	0.674	1.063
	2	0.766	1.279	1.457
D.W. (% F.W.)	1	4.505	4.012	4.013
	2	4.104	3.521	3.840
Chlorophyll (mg cm^{-2})	1	0.0243	0.0354	0.0397
	2	0.0280	0.0313	0.0316
Yield (g m^{-2})	1	1764	2412	3286
	2	1771	3072	3650
Density (m^{-2})	1	3600	3600	3100
	2	2300	2400	2500

The analysis of variance (Table 1.6) of data from both experiments showed significant differences between daylengths for plant height, leaf number, fresh weight and dry weight. The chlorophyll content in Experiment 1 was also significantly different between daylengths. As a result of these findings further analyses were carried out and described below.

Table 1.6 F ratios of analysis of variance for growth data for watercress grown under three different daylengths in two experiments, showing significant values (*) at 5% level (Elementary Statistical Tables, University of Bath, School of Mathematics, 1971).

	F ratio	
Growth parameter	Expt.1	Expt.2
Height (cm)	31.439 *	5.730 *
Leaf number	14.935 *	3.841 *
F.W. (g)	16.939 *	9.797 *
D.W. (% F.W.)	3.845 *	5.786 *
Chlorophyll (mg cm^{-2})	52.301 *	2.086

The differences in means (Table 1.7) for Experiment 1 show significant differences between the 16 h and 12 h means and 16 h and 8 h means for plant height, leaf number and fresh weight. Differences between 12 h and 8 h means for plant height and leaf number were not significant. The results for dry weight and chlorophyll content show the 8 h mean to be significantly different from the other treatment means. The Experiment 1 results show that 16 h long days produced plants significantly different in height, leaf number and fresh weight from the other two daylengths, and that 8 h short day plants were

significantly different in dry weight and chlorophyll content. In Experiment 2 there were only significant differences between the 12 h and 8 h means for plant height and leaf number, but differences between all daylength means were significant for fresh and dry weights. There was no difference between treatment means for chlorophyll content in Experiment 2.

Table 1.7 Differences between means of growth data for watercress grown under three different daylengths (8, 12 and 16 h) in two experiments, showing significant differences (*) at 5% level.

Growth parameter	Expt.	Treatment means (h)		
		12 and 8	16 and 8	16 and 12
Height (cm)	1	1.698	7.348 *	5.924 *
	2	3.381 *	1.605	-1.734
Leaf number	1	1.193	5.076 *	4.066 *
	2	2.708 *	1.716	-0.988
F.W. (g)	1	1.774 *	5.602 *	3.969 *
	2	3.052 *	4.221 *	1.010 *
D.W. (% F.W.)	1	-2.423 *	-2.481 *	0.005
	2	-3.399 *	-1.572 *	1.772 *
Chlorophyll ($mg\ cm^{-2}$)	1	7.129 *	9.910 *	2.781
	2	2.168	2.368	0.200

Table 1.8 gives the standard deviations of the data for the different daylength treatments. In Experiment 1 variation between treatments for all growth parameters increased with longer daylength, indicating that 8 h days produced more uniform plants than 12 and 16 h days. These results agree with the Experiment 1 height range data (Table 1.9) which show a smaller range in the 8 h day treatment. In Experiment 2 variation was greater in the 12 h daylength for plant height, leaf number and fresh weight than the other two treatments, and this was also evident in the height range results. A further experiment would possibly indicate which results are anomalous.

Table 1.8 Standard deviations (square root of variance) of data for watercress grown under three different daylengths in two experiments.

Growth parameter	Expt.	Daylength (h)		
		8	12	16
Height (cm)	1	5.078	5.835	6.609
	2	5.766	5.784	4.358
Leaf number	1	3.683	5.840	8.549
	2	5.566	8.023	6.361
F.W. (g)	1	0.308	0.499	0.816
	2	0.640	1.054	1.043
D.W. (% F.W.)	1	0.875	1.190	1.222
	2	1.030	0.708	0.967
Chlorophyll ($mg\ cm^{-2}$)	1	0.00231	0.00201	0.00236
	2	0.00151	0.00182	0.00418

Table 1.9 Height range (cm) and differences between tallest and shortest plants grown under three different daylengths in two experiments.

	Daylength (h)					
	8		12		16	
	1	2	1	2	1	2
Tallest	26.0	25.5	31.5	29.5	34.5	24.0
Shortest	4.0	6.0	8.0	8.0	8.0	5.5
Difference	22.0	19.5	23.5	21.5	26.5	18.5

Mineral nutrient analysis of leaves and stems from plants in Experiment 1 showed larger amounts of nitrogen and phosphorus and smaller amounts of potassium in leaves compared with stems (Table 1.10). These results are in agreement with those of Robinson and Cumbus (1977), although rather high levels of potassium in the stems were obtained in this study.

Table 1.10 Percentages of nitrogen (N), phosphorus (P) and potassium (K) (dry matter basis) in leaves (L) and stems (S) of watercress grown under three different daylengths in Experiment 1.

Element	Tissue	Daylength (h)		
		8	12	16
N	L	5.98	5.92	5.55
	S	3.76	3.17	3.41
P	L	0.92	0.82	0.86
	S	0.68	0.58	0.69
K	L	5.87	4.38	3.34
	S	16.76	12.20	9.88

The amounts of nitrogen and potassium in leaves and stems were less in plants grown under longer daylengths, but phosphorus levels were the same for all treatments. The higher levels of nutrients in the 8 h daylength plants may be associated with a larger uptake of nutrients over a longer growing period, rather than with daylength.

The shelf-life of watercress grown under different daylengths was measured by fresh weight loss and chlorophyll breakdown. The fresh weight losses of bunches of plants grown under the different daylengths were not significantly different (Fig. 1.2), although the weight loss of 16 h day cress from Experiment 1, stored at 20°C, was less than the losses from the 8 and 12 h day cress. Losses in fresh weight for all daylength treatments were less at 4°C storage than at 20°C, due to lower rates of evaporation and dehydration at the lower temperature. The initial and final fresh weight losses are given in Table 1.11 and confirm the results in Fig.1.2.

Chlorophyll content of leaves from plants grown under different daylengths is shown in Table 1.12. The difference between the initial chlorophyll content (0 h) of leaf discs and after 72 h exposure to light at 20°C may give an indication of the shelf-life and consequently an analysis of variance was carried out on the data. The data were analysed for differences both within and between treatments using the following transformations to measure direct (i) and proportional (ii) loss of chlorophyll after 72 h exposure to light compared with the initial values (0 h).

i) Direct loss $\quad$ observation (72 h) - control (0 h) mean

ii) Proportional loss $\quad \dfrac{\text{observation (72 h) - control (0 h) mean}}{\text{control (0 h) mean}}$

Fig. 1.2 Percentage fresh weight losses for bunches of watercress grown under three different daylengths and stored at 4° and 20°C over a 24 h period.

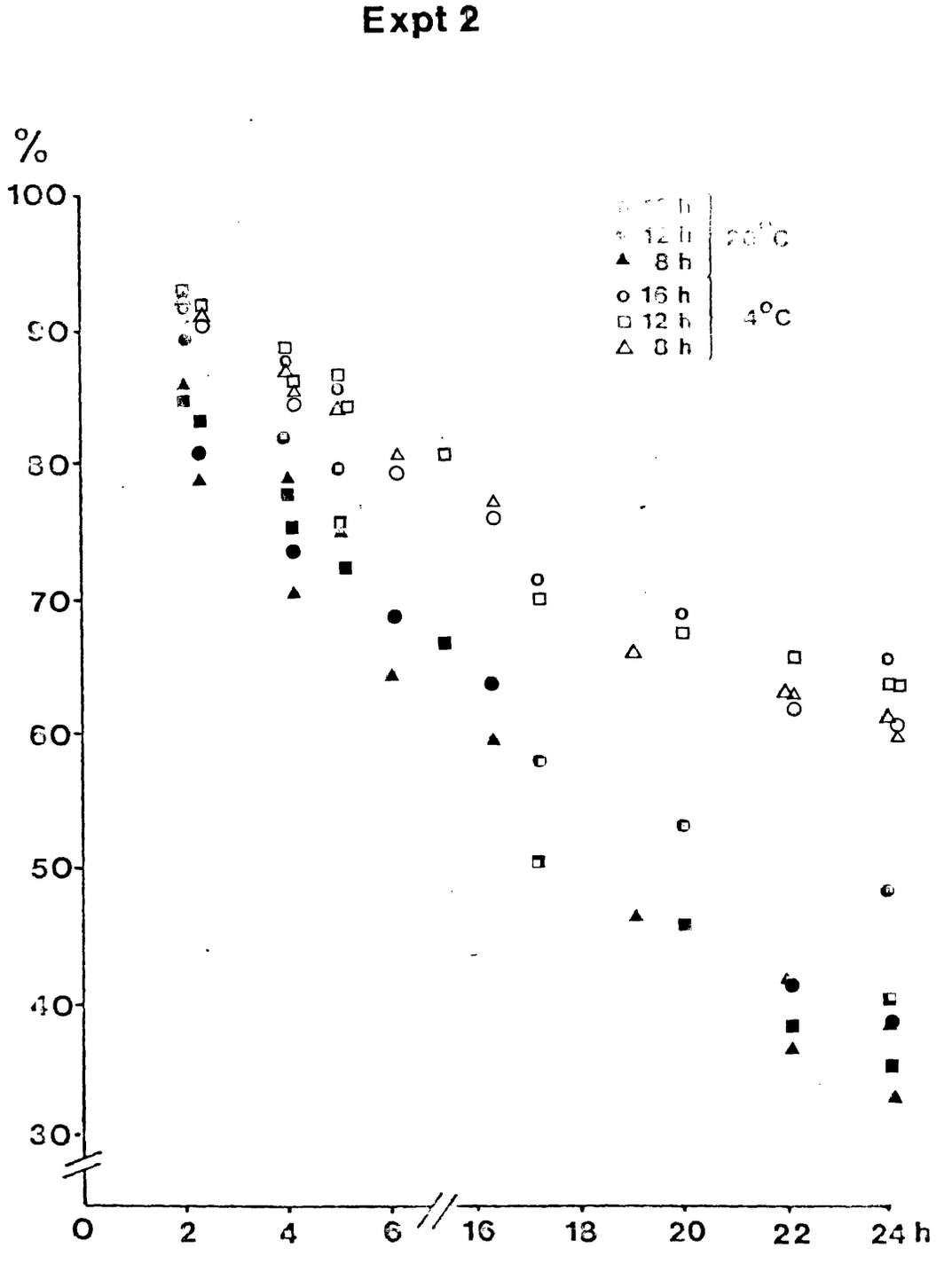


Fig. 1.2 Percentage fresh weight losses for bunches of watercress grown under three different daylengths and stored at 4° and 20°C over a 24 h period.

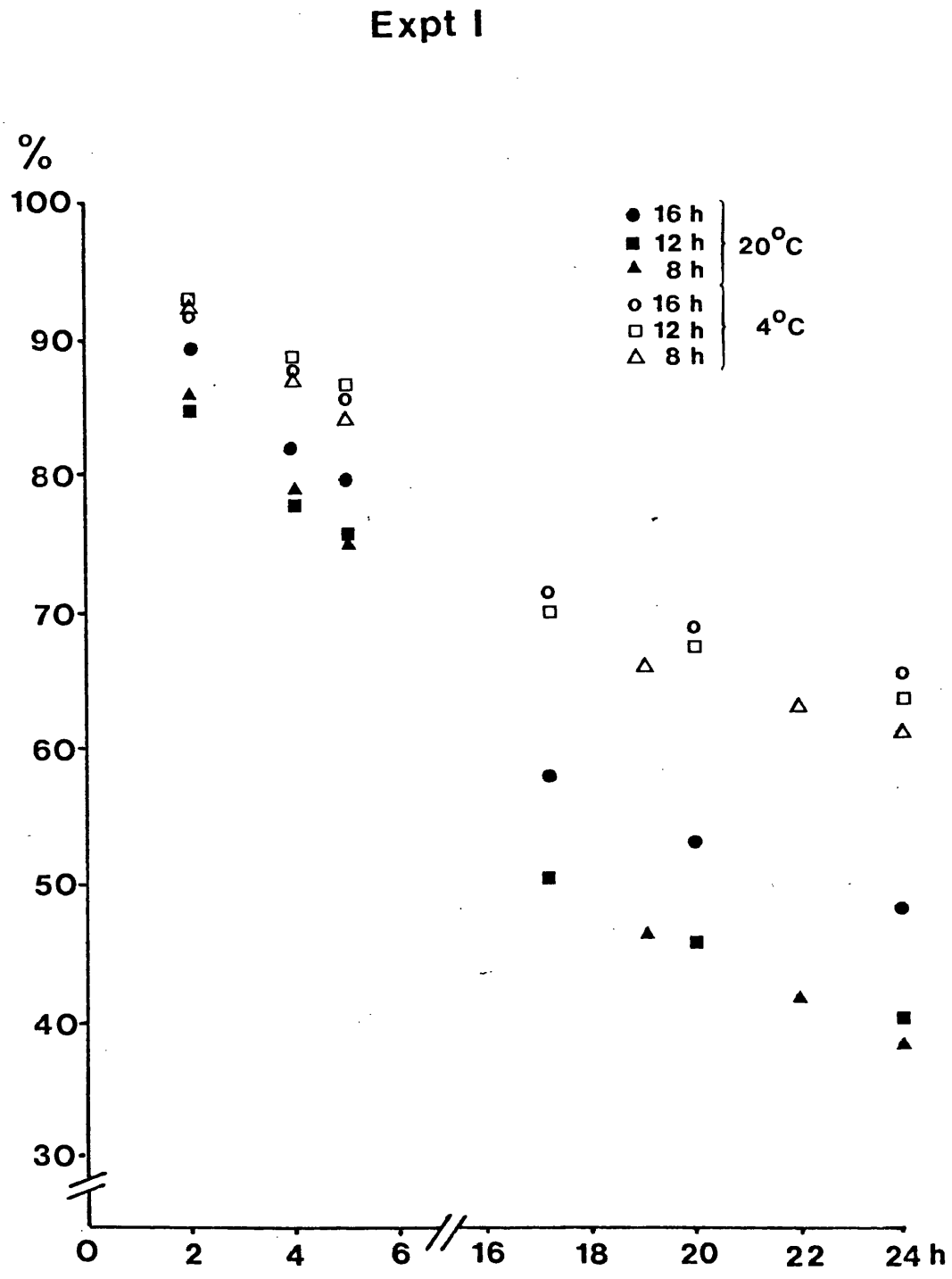


Table 1.11 Initial (Oh) and final (24h) percentage fresh weight losses of bunches of watercress plants grown under three different daylengths and stored at 4° and 20°C.

Storage temperature	Time (h)	Expt.	Daylength (h)		
			8	12	16
4°C	0	1	100	100	100
	24		60.86	63.90	65.57
	0	2	100	100	100
	24		61.15	64.91	61.18
20°C	0	1	100	100	100
	24		38.38	40.14	48.40
	0	2	100	100	100
	24		33.93	36.44	39.61

Table 1.12 Mean values for chlorophyll content (mg cm^{-2}) of plants grown under three different daylengths. Leaf discs were exposed to 72 h light at 20°C and compared with initial (Oh) values as an indication of shelf-life.

Time (h)	Expt.	Daylength (h)		
		8	12	16
0	1	0.02431	0.03536	0.03968
72		0.01686	0.02292	0.02505
0	2	0.02795	0.03131	0.03163
72		0.02629	0.02829	0.02846

However, no significant differences were found in chlorophyll content, either between or within treatments, indicating equal shelf-life for the cress grown under 8, 12 and 16 h daylengths (Table 1.13). Since a weight basis for measuring chlorophyll content is considered more reliable than an area basis used in this study, the method should be modified in future work.

Table 1.13 F ratios of analysis of variance for post-harvest chlorophyll breakdown after 72 h as direct loss (DL) and proportional loss (PL) from initial chlorophyll value, between treatments (BT) and within treatments (WT).

Chlorophyll	Expt 1		Expt 2	
	BT	WT	BT	WT
DL	1.085	2.677	1.906	0.425
PL	1.031	1.362	1.596	0.658

1.5 Summary

Differences in daylength produce variation in the growth and development of watercress plants. Long daylengths (16 h), as experienced naturally in the summer months, produced taller plants, but of more variable height, with a greater number of leaves, but less sturdy stems than shorter daylengths. There does not appear to be any difference in the plants produced under 12 and 8 h daylengths except that the 8 h day plants had a greater percentage dry weight and less

chlorophyll than 12 h day plants. The plants were more uniform when growth was slower in the shorter daylengths. In the natural situation shorter daylengths are also associated with lower temperatures which contribute to the decrease in growth rate. Ambient temperatures may increase the effect of daylength on the rate of growth and development of the plants. Most of the observations by growers regarding the effects of seasonal daylength on watercress were confirmed by these results. An unexpected result was the apparent similarity in shelf-life between cress grown under different seasonal daylengths. However, the methods used to measure shelf-life require modification to increase their sensitivity. An extension of this work would be to include the effects of different seasonal temperatures with different daylengths, thus relating more to the natural watercress production situation.

SECTION 2

A Study of the Commercial Distribution Chains of Watercress

2.1 Introduction

During the last ten years there has been great interest, particularly in the U.S.A., in refrigerated storage and transport for fruits and vegetables. The deterioration of the produce which occurs during transport over long distances between producer and consumer in countries with warm climates is reduced by refrigeration. The beneficial effects on plant produce of lower temperatures are slowing down respiration rate, the production of ethylene gas and the spread of microorganisms which cause rotting.

The storage life of the produce can also be prolonged by controlling the atmospheric composition of the storage areas. A high humidity atmosphere, coupled with low temperature, will reduce water loss and prevent wilting of leafy produce (Mitchell and Kasmire, 1974). However, with certain fruits, high humidity can increase the incidence of certain physiological injuries, such as the superficial scald of apples. Controlled atmosphere storage is widely used for apples and pears which are kept in refrigerated stores in controlled concentrations of oxygen and carbon dioxide. Low pressure storage has also been suggested for horticultural crops, especially where a low oxygen atmosphere may be advantageous and where a rapid rate of air exchange avoids the accumulation of ethylene and other harmful volatiles (Lougheed, Murr and Berard, 1978). Ethylene increases the rate of respiration under certain conditions; low concentrations stimulate ripening in many fruits and can cause yellowing of green leafy vegetables and shedding of flower petals. Although ethylene is potentially harmful, it can also be useful in, for example, the "degreening" of oranges and ripening green bananas. Storage in a modified atmosphere, especially if this is inadvertent, can lead to problems. Such conditions could be

produced in supermarket prepacks. The films used to wrap produce are often highly impermeable to gases, and trouble has arisen from excess humidity, or rather the presence of condensed water, which increases the risk of bacterial rotting.

Dehydration has been reported to be the most important cause of post-harvest deterioration in several British root and leafy vegetables (Derbyshire, 1973). Cool storage has proved successful for many vegetables, providing fungicides are used to prevent certain fungal infections occurring at high relative humidities (Bartlett, 1972). Dips or sprays of antibiotics have been used on vegetables in attempts to prolong storage life by reducing losses from microorganisms. Some success has been reported with salad vegetables and spinach, but there is very little information on the retention of an antibiotic or its fate when it is described as 'destroyed' on cooking; spinach is known to retain 70% of the original quantity of streptomycin after boiling for three minutes (Fidler, 1962).

Apart from the debatable advantages of such adjuncts to cold storage, refrigeration also has some disadvantages. Low storage temperatures can cause chilling injury to produce (Lyons, 1973), and pre-harvest field temperatures can also influence the post-harvest responses of vegetables under different storage temperatures (Kader, Lyons and Morris, 1974).

It is apparent that many inter-related factors contribute to the overall success of particular storage conditions on the post-harvest life of a particular crop. Therefore, the watercress industry must be cautious before introducing 1^{o}- 2^{o}C cold chains as suggested by Robinson, Browne and Burton (1975), since damage might occur which is not evident until later. The large financial cost in buying cooling

and refrigeration equipment may be wasted, or unnecessary, if small growers were to improve their current practices.

The purpose of this study was to examine the post-harvest conditions to which watercress is exposed during long and short-haul distribution chains, using refrigerated and unrefrigerated transport, and packed in two modern prepacks, over a five month period. Physiological and sensory changes occurring in watercress during post-harvest processing, storage and distribution were studied using laboratory techniques and equipment adapted for use in the field. This work should aid the understanding of quality deterioration and lead to recommendations for the improvement of current practices.

The following table indicates the routes examined during studies on bunched/boxed and pre-packed cress in refrigerated and unrefrigerated transport, through short and long distribution chains, from March to July 1978. Total distribution chain times from harvest to return at Bath University are in hours (h).

Short Distance, unrefrigerated transport, watercress bunched/boxed

Waddock (Dorset) to Bristol - March 23.15 h, April 23.35 h,
May 26.00 h, July 21.45 h.

Long Distance, unrefrigerated transport, watercress bunched/boxed

Waddock to Blackburn - March 51.00 h, April 51.15 h, May 48.45 h,
June 48.45 h.

Refrigerated Transport, long distance, watercress bunched/boxed

St Mary Bourne (Hants) to Manchester - May 52.15 h, June 52.45 h.

Unrefrigerated Transport, long distance, watercress bunched/boxed

Waddock to Blackburn - May 48.45 h, June 48.30 h.

Prepack A, partially refrigerated chain, long distance

Abbotts Ann (Hants) via Birmingham to Nottingham - June 29.15 h.
July 29.30 h.

Prepack B, totally refrigerated chain, long distance

Abbotts Ann via Barnsley to Nottingham June 31.00 h
July 27.45 h.

2.2 Materials and Methods

From March to July 1978 watercress distribution chains were followed from harvesting, through packing and storage, to transportation to wholesale markets and shops. This work was carried out at monthly intervals following 3 day long and 1 day short distribution chains, using refrigerated and unrefrigerated transport, and prepacked and traditionally boxed watercress.

2.2.1 Temperature

At suitable points in the transport chain, air and cress temperatures were measured with mercury bulb thermometers (small scale 0 - 50°C).

2.2.2 Relative Humidity (RH)

Measurements were made using a portable, battery powered lithium chloride sensor (SINA-MIK 5307).

2.2.3 Water Vapour and Carbon Dioxide Concentrations

A Dräger multi-gas detector (Drägerwerk AG Lübeck) was used with Dräger tubes for measuring carbon dioxide (0.1 - 6.0 vol.%) and water vapour (0.1 -40 mg l^{-1}). A flap (c.4 x 4 cm) was cut in the centre on one side of a watercress box c.5 cm from the top edge for inserting the Dräger tubes. After measurements were taken the flap was closed

with a piece of tape.

2.2.4 Relative Turgidity (RT)

Changes in the turgidity of leaves were measured using the Barrs and Weatherley (1962) revised technique.

One disc (1 cm diameter) was cut with a cork borer from the first fully expanded leaf below the apex from each of 50 sprigs in the bunches of watercress. Five groups of 10 discs were weighed on a White Precision Balance (Model P) and placed in each of five 15 ml glass specimen bottles with snap-on plastic lids. The bottles were stored in a Dilvac thermos flask, containing crushed ice, to the end of the distribution chain. The discs were then floated, adaxial surface downwards, on 10 ml deionised water in petri dishes placed under a fluorescent lighting bank (21.0 watts m^{-2}) for 4 h. After removing excess moisture the discs were reweighed, dried at $80^{o}C$ for 24 h and reweighed. Relative turgidity was calculated using the following equation : -

$$RT = \frac{\text{fresh weight - dry weight}}{\text{weight when turgid - dry weight}} \times 100$$

2.2.5 Greenness

The first fully expanded leaf greater than 2 cm diameter was picked from each of 5 sprigs in the watercress bunch, placed in a glass specimen bottle, and stored in crushed ice in a thermos container to the end of the distribution chain. The leaves were then placed on a Gardner digital colour difference meter (XL-10 Tristimulus Colorimeter, Gardner Instruments) under a piece of clear glass and their colour measured against a dark green standard tile (CG 6802 GCS-11DG). The hue angle was calculated as described by Little (1975) using the Hunter a,b diagram.

2.2.6 Chlorophyll Content

One disc (1 cm diameter) was cut with a cork borer from the first fully expanded leaf below the apex of 50 sprigs in the watercress bunches. Ten discs were placed in each of five glass specimen bottles containing 10 ml 80% (v/v) aqueous acetone. The bottles were kept in black polythene bags and at the end of the distribution chain placed in a refrigerator at $4^{o}C$ for 7 days before measuring chlorophyll levels at 663 and 645 nm on a spectrophotometer. Chlorophyll content was calculated using the following equation (Arnon, 1949) : -

$$\frac{E_{663} \times 8.02 + E_{645} \times 20.2}{7.85 \text{ (area of 10 discs)} \times 100} = \text{total chlorophyll } (\text{mg cm}^{-2})$$

2.2.7 Sensory Assessment

A sensory assessment was made of three bunches of watercress at each sampling point using part of the sensory assessment sheet developed during the course of this work (Section 3).

2.2.8 Fresh Weight Loss

At each sampling point three boxes, or one crate of watercress (depending on the packaging used) were weighed using a spring balance (Salter Ltd.) to determine fresh weight loss during storage and transport.

2.2.9 Shelf-Life

At the end of each distribution chain six bunches of watercress were each put into a polythene bag, sealed with a piece of wire and kept in a refrigerator at $4^{o}C$. The bunches were assessed daily and judged to be just unsaleable when 3 - 5 leaves in the head were yellowing and the stalks were becoming soft.

2.3 Results and Discussion

Statistical analysis was carried out on the data using the Minitab II statistics package (Penn State University) available on the Multics computer, Avon Universities. Both one- and two-sided two sample t-tests were performed on the refrigerated and unrefrigerated transport chain data for May and June, collected at different stages from cutting to marketing. Measurements were taken at the following stages : -

1) cutting/pulling of watercress
2) arrival at packhouse
3) after bunching
4) after hydrocooling
5) after cold storage
6) lorry change
7) arrival at market
8) selling at market
9) arrival at Bath

Overall the results of the analyses were inconclusive with no apparent trends. Table 2.1 gives a detailed breakdown of the t-test results of the different measurements at the various stages.

The results showed that cress temperatures were initially higher in the unrefrigerated chain. This is probably because it was cut in the afternoon when air temperatures are higher than in the morning, when the refrigerated chain watercress was cut. At stages 4 and 5 the $2^{o}C$ hydrocooling and $6^{o}C$ cold storage proved more efficient in the unrefrigerated chain. This may be due to the denser packing, 20 bunches/box (30 x 17.5 x 16.5 cm) for refrigerated transport compared with looser packing, 15 bunches/box (26.5 x 18 x 16.5 cm) for the

Table 2.1 Results of t-test for May v June, unrefrigerated v refrigerated transport chains.

$\bar{u}$ = mean of unrefrigerated results

$\bar{r}$ = mean of refrigerated results

nd = no difference

Month and Stage	Cress Temperature	Relative Turgidity	Chlorophyll Content	Carbon Dioxide	Water Vapour	Weight Loss
May 1	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	$\bar{u} > \bar{r}$	-	-	-
3	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	nd	-	-	nd
4	$\bar{r} > \bar{u}$	$\bar{r} > \bar{u}$	$\bar{u} > \bar{r}$	nd	nd	-
5	$\bar{r} > \bar{u}$	nd	nd	$\bar{u} > \bar{r}$	nd	nd
8	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	nd	$\bar{u} > \bar{r}$	nd	nd
9	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	nd	$\bar{u} > \bar{r}$	nd	nd
June 1	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	nd	-	-	-
3	$\bar{u} > \bar{r}$	nd	nd	-	-	nd
4	$\bar{r} > \bar{u}$	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	nd	nd	-
5	$\bar{r} > \bar{u}$	nd	nd	$\bar{u} > \bar{r}$	nd	nd
8	$\bar{u} > \bar{r}$	nd	$\bar{r} > \bar{u}$	$\bar{u} > \bar{r}$	$\bar{u} > \bar{r}$	nd
9	$\bar{u} > \bar{r}$	$\bar{r} > \bar{u}$	$\bar{r} > \bar{u}$	nd	$\bar{u} > \bar{r}$	nd

unrefrigerated chain; water during hydrocooling may not have penetrated the more tightly packed boxes. However, high ambient temperatures ($20^{o}C$) during transport increased the unrefrigerated cress temperature to 12^{o}- $14^{o}C$ so that at market it was higher (14^{o}- $15^{o}C$) than the refrigerated cress temperature (10^{o}- $12^{o}C$) (Table 2.1 stage 8).

Although the night temperatures dropped while the watercress was at the market, the respiratory heat of the bunched plant material maintained its temperature.

Results with the other measurements taken showed that in refrigerated transport RT was slightly higher, and carbon dioxide levels in the boxes were lower, compared with unrefrigerated transport. Chlorophyll content, water vapour levels in boxes and fresh weight losses over the distribution chains showed no significant differences between refrigerated and unrefrigerated transport.

To determine whether relationships existed between environmental factors (RH and air temperature) and physiological data (cress temperature, chlorophyll content etc.) various sets of data were plotted against each other. However, no obvious correlations were found, even though carbon dioxide and water vapour measurements were expected to be related to air temperature. The rate of respiration increases as the temperature is raised until temperatures detrimental to enzyme activity are reached (Devlin, 1975). It has been reported that an increase in carbon dioxide concentration (above 0.3%) will retard respiration (Haard, 1976), and a build up of carbon dioxide in the boxes of watercress may explain why no direct temperature/respiration correlation was found.

The plots of mean values for cress and air temperatures against carbon dioxide levels, water vapour levels and RT for watercress along unrefrigerated and refrigerated transport chains in June 1978 are shown in Fig. 2.1. The plots confirm the results of the t-tests, that carbon dioxide levels were lower, and RT higher in the refrigerated chain, and also show no correlation between cress or air temperature when plotted against carbon dioxide levels, water vapour levels and RT.

Fig. 2.1 Plots of air temperature (a) and cress temperature (b) against carbon dioxide and water vapour levels and relative turgidity (RT) for data from unrefrigerated v refrigerated transport chains in June 1978. 2 = a and b at same point on graph

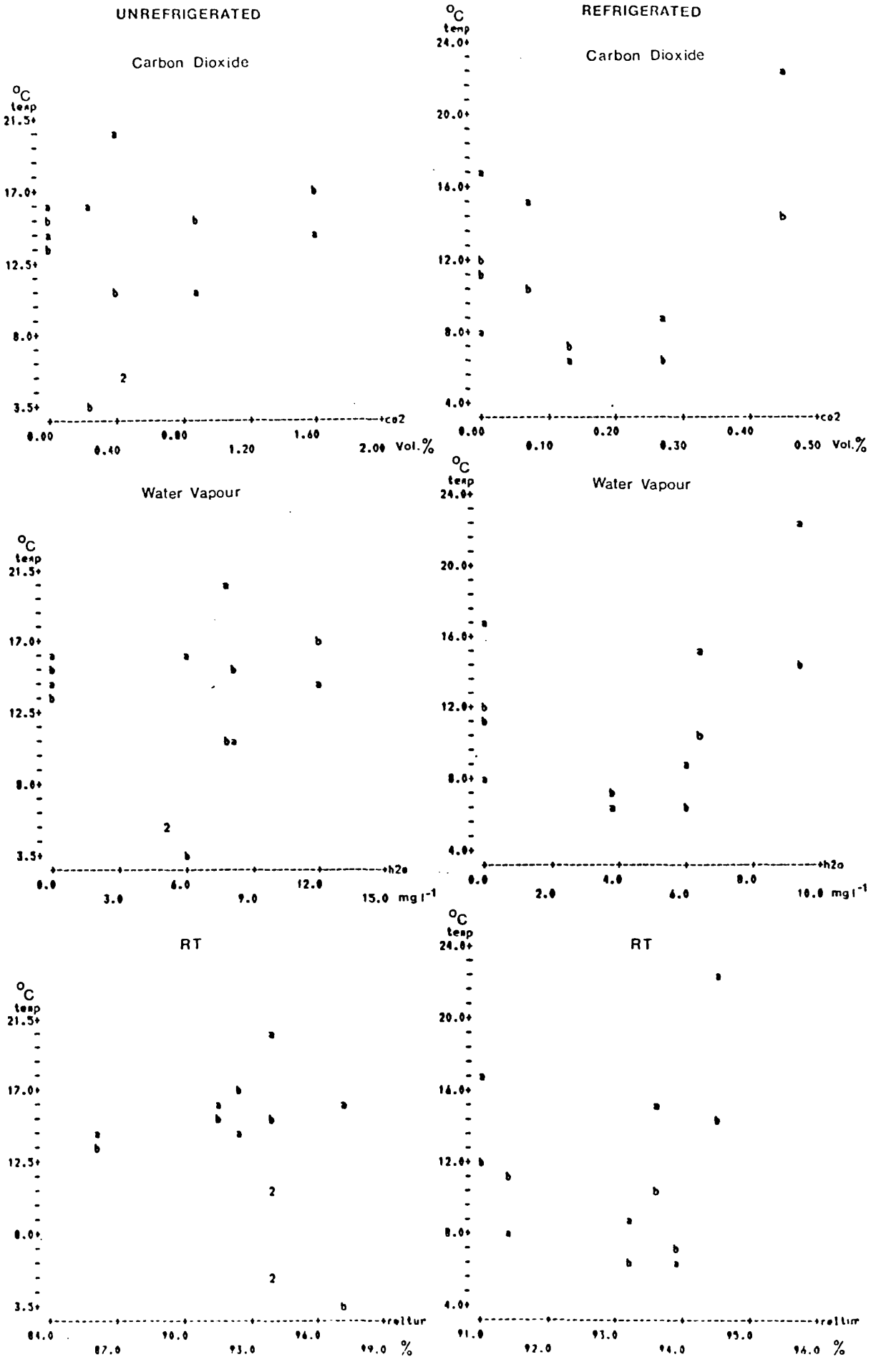
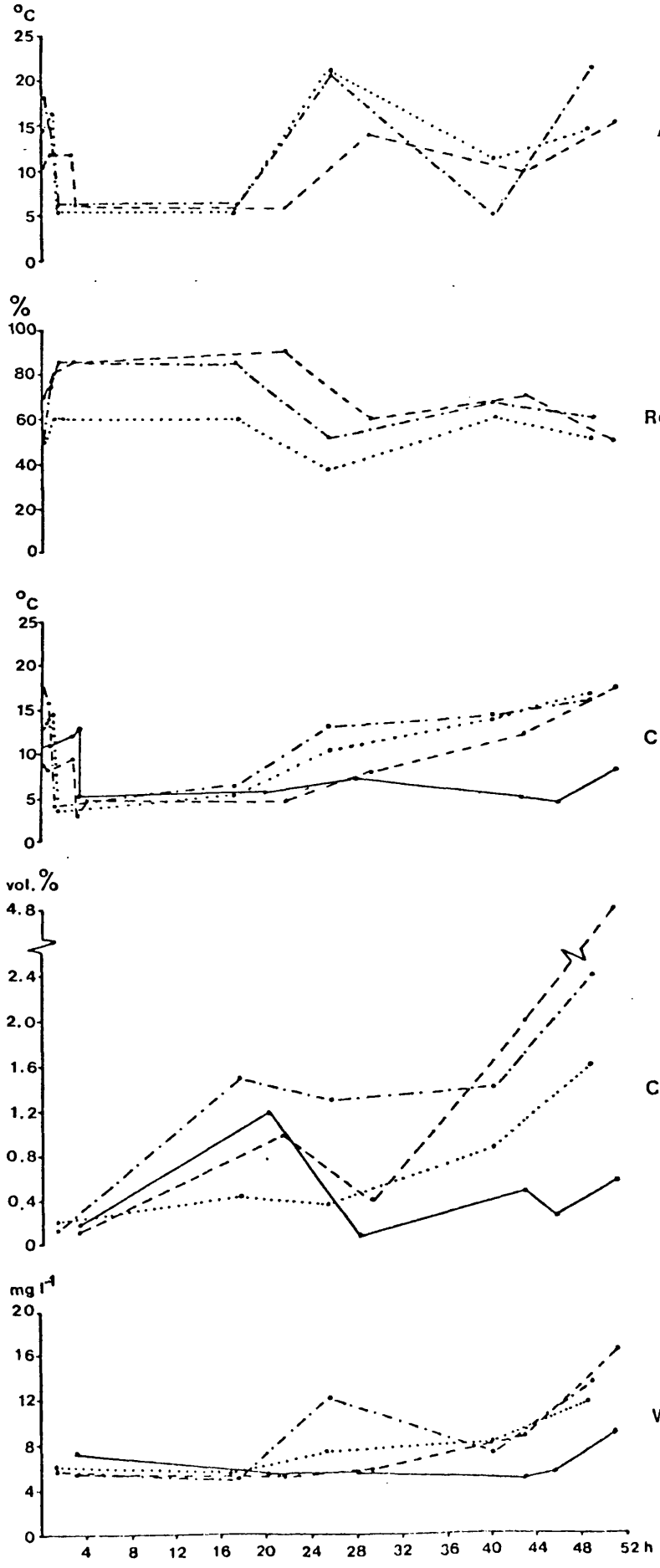


Fig. 2.2 Long Distribution Chain

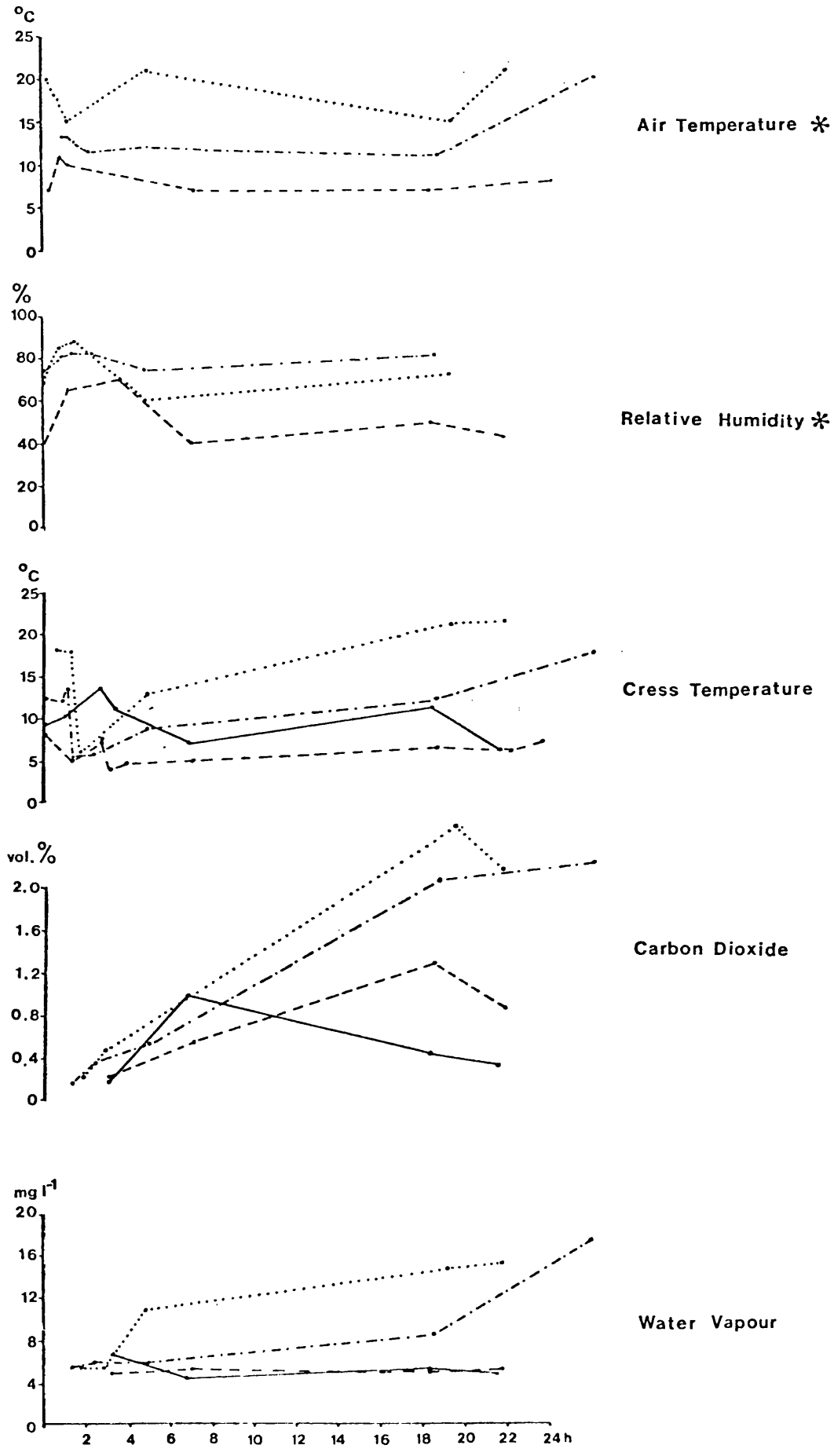
Plots of mean values for air and cress temperatures, relative humidity, carbon dioxide and water vapour levels against time (h) in March (——), April (– – –), May (– · –), June (· · · ·).



* measurements not taken in March

Fig. 2.3 Short Distribution Chain

Plots of mean values for air and cress temperatures, relative humidity, carbon dioxide and water vapour levels against time (h) in March (——), April (– – –), May (–·–), July (····).



* measurements not taken in March

Other measurements were also plotted, for example RH v chlorophyll content, RH v RT, but they are not shown because they demonstrated a similar lack of correlation between environmental and physiological data.

Since the exploratory data analysis provided such disappointing results, a 3-way analysis of variance was carried out between treatments, months and stages along the distribution chains for the physiological data only. Plots were also made of all the data to aid analysis of the results. The following discussion is based on the analysis of variance results (when relevant) and the plots.

1. Long v Short Runs (March, April, May, June, July)

	Cutting/ Pulling	→ Bunching	→ Hydrocooling	→ Cold Storage	→ Loaded on Lorry	→ Lorry Change	→ Selling at Market	→ Arrival at Bath
Long:	0h	0.5h	1h	1.5h	17h	25h	40h	48h
Short:	0h	1h	1.5h - - -	no storage - - -	2h	5h Arrival at Market	18h	24h

Figures 2.2 and 2.3 show curves of mean values of the data for air temperature, RH, cress temperature, carbon dioxide and water vapour levels over these runs.

a) Air Temperature and RH

During the long distribution chain, from Waddock to Blackburn, air temperatures followed the same pattern over all months (Fig. 2.2); decreasing in the cold store to 5^{o}- 6^{o}C, increasing to the mid-afternoon lorry change, decreasing during the night at market, and increasing from the market to Bath. RH remained fairly constant, decreasing slightly when temperatures increased at the lorry change. In July the RH was lower than the previous months as expected with higher air temperatures.

The temperature during the short chain (Fig. 2.3) from Waddock to Bristol, increased during the mid-afternoon journey to market in July, but remained fairly constant in the other months. The temperature increased from selling at the market in the morning to Bath. RH was fairly constant over the 24 h period after a decrease on arrival at the market in the afternoon.

b) Cress Temperature

The 3-way analysis showed no difference in cress temperature between the long and short runs. The highest temperature on long runs was 19°C, and on short runs 22°C, reached after a steady increase from loading on the lorry to arrival at Bath. The difference between months was not statistically significant although the range of temperatures was smaller in March compared with the other months, and there was a slight increase over the months to July. This is shown in Fig. 2.4 where data for both runs are plotted against months and the temperature values occurring with the highest frequency are circled for each month.

Temperature data plotted against stages along the chain are shown in Fig. 2.5. There is an initial increase in temperature followed by a rapid decrease on hydrocooling, and then a steady increase for the rest of the run. A similar pattern is also evident in Figs. 2.2 and 2.3 but statistical analysis did not indicate these differences to be significant.

c) RT and Fresh Weight Loss

All measurements of RT were greater than 78% and after statistical analysis there were significant differences between distribution chains, months and stages. The short run values (82 - 99.6%) were slightly higher than the long run values (78 - 99.6%).

Fig. 2.4 Cress temperature data for long and short distribution chains plotted against months. The figures give the number of values with the same cress temperature. Circles highlight the largest values (c = 1).

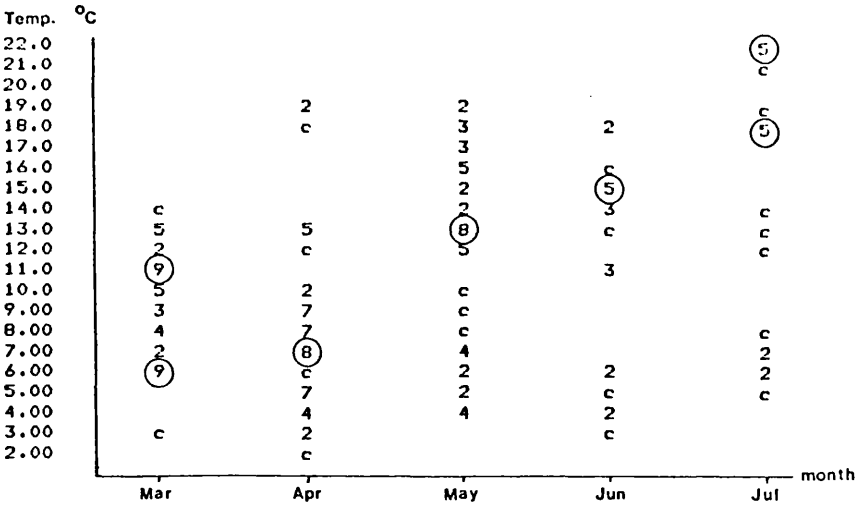


Fig. 2.5 Cress temperature data for long and short distribution chains plotted against stages along the chain. The figures give the number of values with the same cress temperature. Circles highlight the largest values (c = 1).

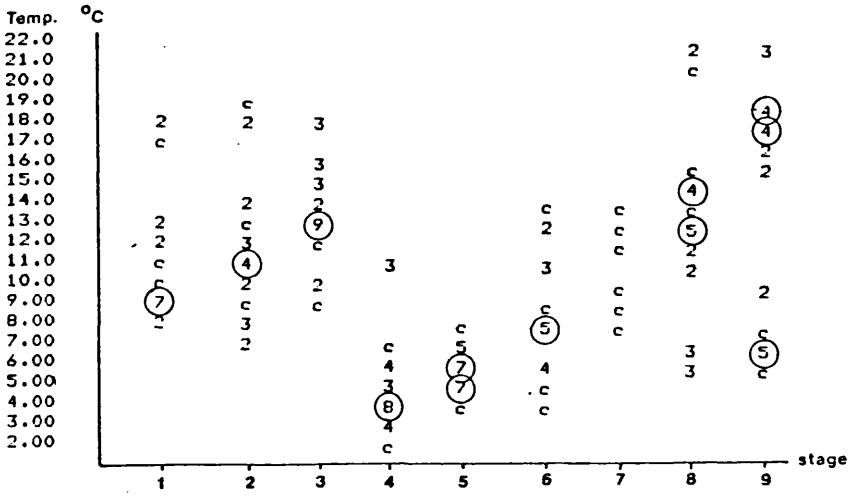
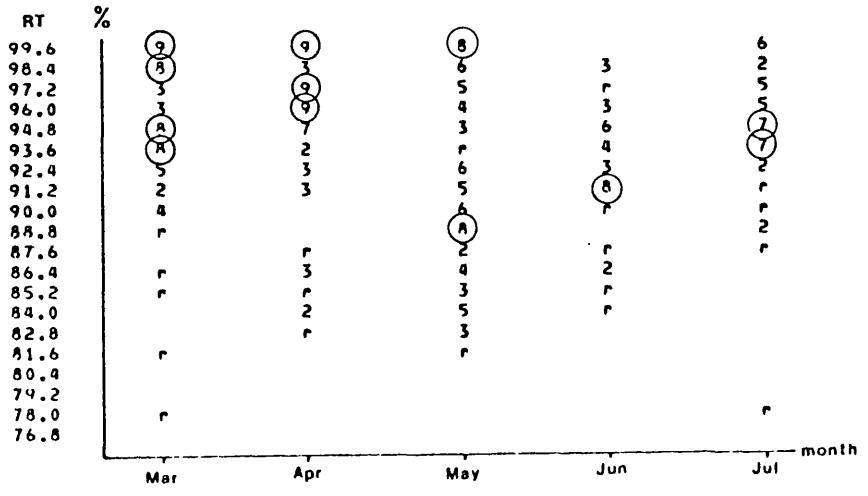


Fig. 2.6 Relative turgidity data for long and short distribution chains plotted against months. The figures give the number of values with the same RT. Circles highlight the largest values (r = 1).



The plot of the data against months (Fig. 2.6) shows the results for March and April were similar, with most values greater than 93%; values for May had a wider variation, and in June and July they decreased as expected with higher air temperatures in these warmer months. The differences between stages were apparent after the watercress was loaded on to the lorries, when average values decreased from 99.6% to 97% during the journey to market, and to 93% at Bath. However, the overall loss of turgor was small, and this is reflected in the sensory assessment of texture (Table 2.2) where the watercress was still crisp or only slightly wilting when on sale at the market.

The weight loss for boxes of cress was 3% on the short runs and 5% on the long runs of the maximum weight after hydrocooling.

d) Carbon Dioxide and Water Vapour

No significant differences were found in the levels of carbon dioxide between long and short runs, months or stages. After hydrocooling, carbon dioxide levels generally increased along the distribution chain to market, but on the journey from market to Bath short run levels decreased and long run levels increased. There was a wider range of values at Bath than at any of the other stages.

Measurements of water vapour levels were significantly different for months and stages, but not between the long and short runs. July values were higher than for the other months on the short run; the reason for this may be related to increased respiration rates with higher air temperatures. Water vapour levels along the distribution chain were fairly constant for March and April (4 - 6 mg l^{-1}), but increased in the warmer months to higher values at the market and at Bath (12 - 17 mg l^{-1}).

Table 2.2 Sensory assessment of watercress bunches in the long and short distribution chains, at bunching (stage 3), selling at market (stage 8) and at Bath (stage 9).

Long Distribution Chain Assessment

Month and Stage		Sheen of Leaves	Texture to Fingers	Aroma	Yellow Leaves on Stalks	Yellow Leaves in Head
March	3	v. shiny	v. crisp	no smell	1	0
	8	shiny	crisp	weak seaweed	1-2	0
	9	dull shine	crisp	medium seaweed	1-2	0
April	3	v. shiny	crisp	wet vegetation	0	0
	8	dull shine	crisp	medium watercress	0	3-5
	9	matt	wilting	medium seaweed	0	3-5
May	3	dull shine	crisp	weak watercress	0	0
	8	dull shine	slightly wilting	medium watercress	0	1-2
	9	dull shine	wilting	medium watercress	0	1-2
June	3	dull shine	fresh but soft	weak primrose	0	0
	8	matt	crisp	medium watercress	2-3	1
	9	matt	crisp	medium watercress	2-3	1

Short Distribution Chain Assessment

March	3	v. shiny	crisp	wet vegetation	1-2	0
	8	v. shiny	crisp	no smell	3	3
	9	shiny	wilting	weak watercress	3	3
April	3	shiny	crisp	wet vegetation	0	0
	8	shiny	crisp	medium watercress	0	1
	9	dull shine	crisp	medium watercress	0	1
May	3	shiny	crisp	no smell	0	0
	8	dull shine	crisp	weak watercress	0	1
	9	dull shine	wilting	medium watercress	0	1
July	3	dull shine	fresh but soft	wet vegetation	0	0
	8	matt	wilting	medium primrose	0	0
	9	matt	wilting	medium primrose	0	0

e) Chlorophyll Content and Greenness

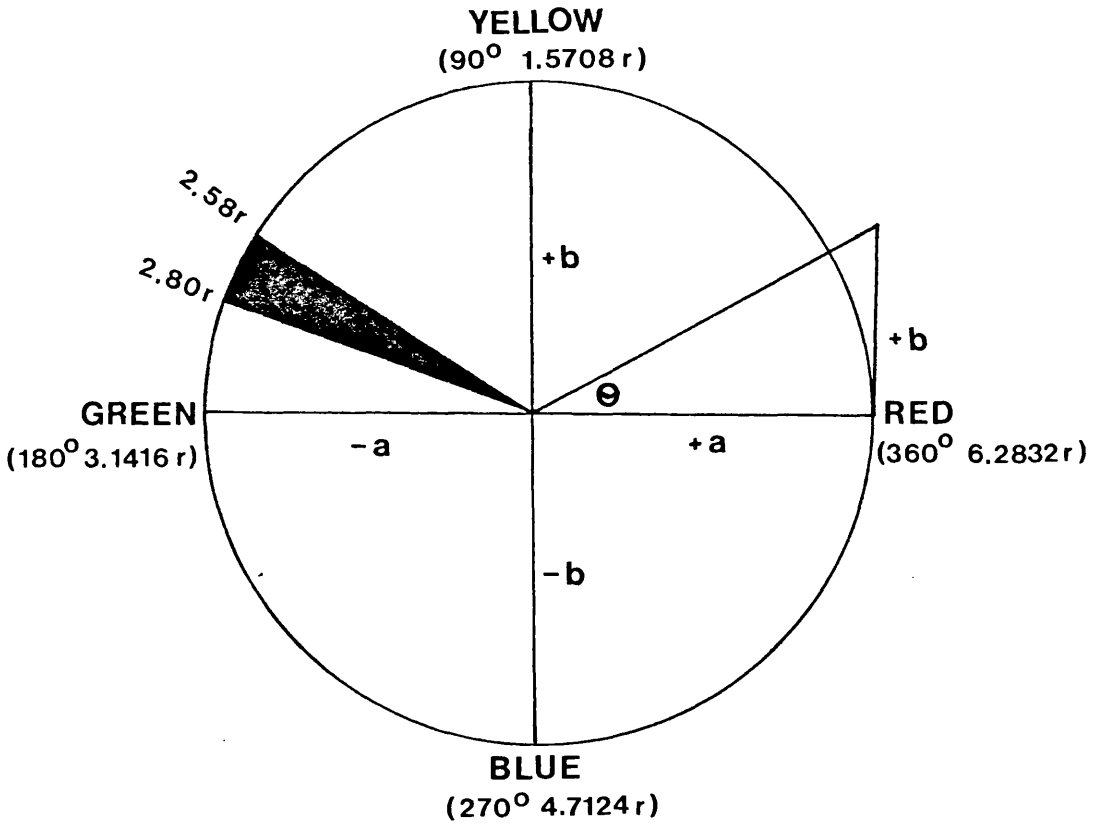
The methods used to measure slight changes in colour over the 24 and 48 h periods of the distribution chains were probably insufficiently sensitive. Rates of chlorophyll breakdown as the plant deteriorates, which may be affected by storage and transport conditions, are possibly not detectable until some time after the consumer has bought the watercress. The differences in chlorophyll content between months and stages were statistically significant, but not between runs. Watercress samples in May contained more chlorophyll than the other months and the smaller amounts in June and July samples may account for the lighter colour of summer cress. However, the process of chlorophyll degradation is complex and many previously accepted ideas concerning breakdown products are being reviewed (Simpson, Lee, Rodriguez and Chichester, 1976). Evidence also suggests that chlorophyllase, the hydrolytic enzyme which converts chlorophyll a and b to their respective chlorophyllides, does not catalyse the initial step of chlorophyll degradation in senescing plant tissue (Walker, 1964). Chlorophyll degradation may be catalysed more effectively by oxidative enzymes, such as lipoxygenase, which has been observed to increase during plant senescence and is known to contribute to loss of chlorophyll in frozen vegetables (Haard, 1976).

The level of chlorophyll in samples at different stages on the distribution chain decreased slightly, but these measurements were calculated on an area basis which is less reliable than on a weight basis. This may account for fluctuations in chlorophyll content and future work should use weight as the basis for calculations.

In the sensory assessment, no change in colour was detected and there were no significant differences in the hue angle measurements.

Figure 2.7 shows the narrow range of hue angle values obtained over all the distribution chains studied.

Fig. 2.7 Depiction of hue angle on the Hunter a,b diagram. Hue angle = $\Theta = \tan^{-1} \frac{b}{a}$. Range of values for watercress is shown by the shaded segment in the yellow to green quarter.

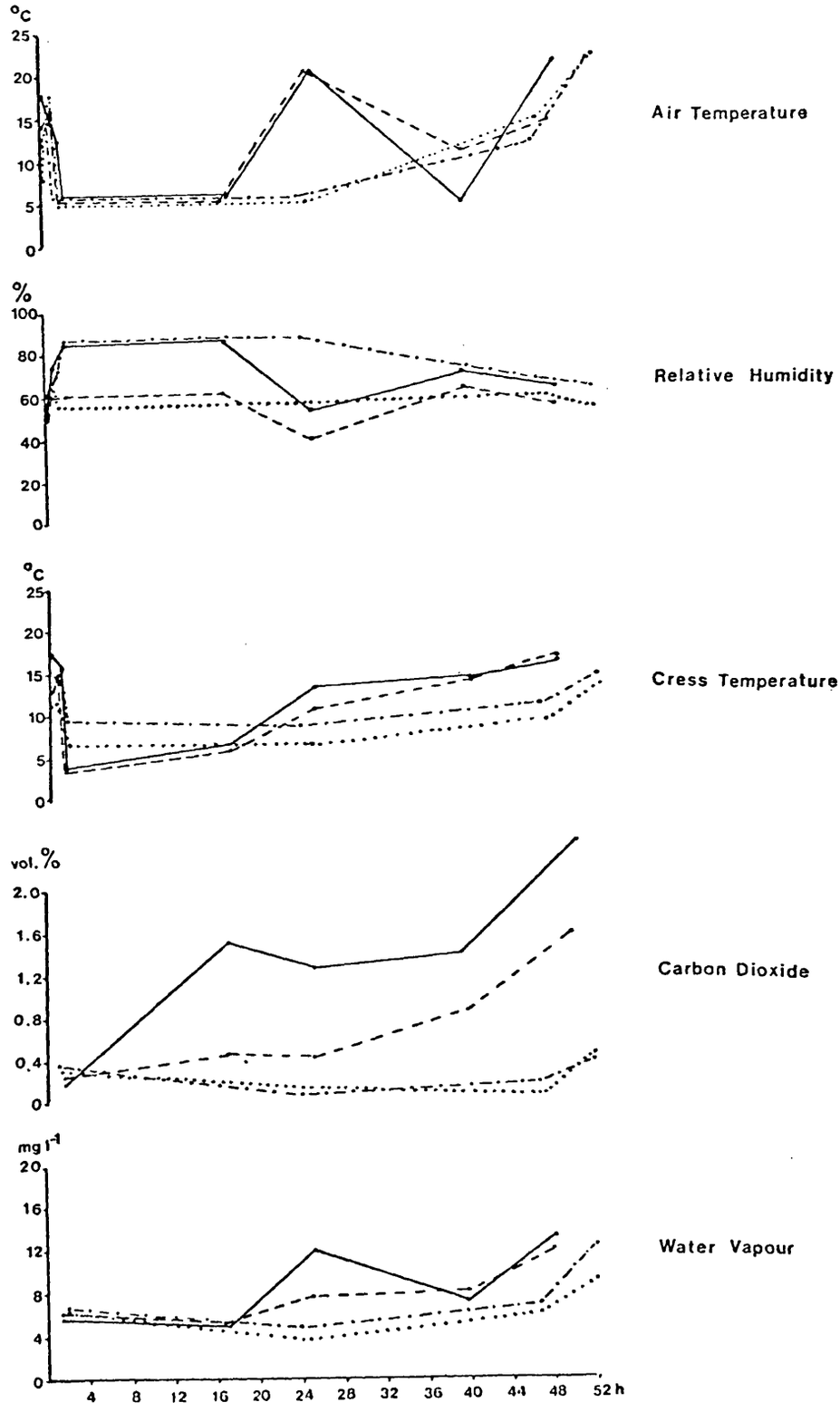


f) Sensory Assessment and Shelf-Life

Table 2.2 gives the results of sensory assessments carried out after bunching, at market and at Bath. There was no change in colour on either long or short runs using the Munsell standards. During the warmer months of May, June and July the leaves had become matt and were slightly wilting when sold at the market. The aroma changes were similar, apart from the short run in March when the cress was still fresh with no odour at the market, and in July when a primrose aroma was evident. Very few yellow leaves were present either on the stalks

Fig. 2.8 Unrefrigerated v Refrigerated Distribution Chains

Plots of mean values for air and cress temperatures, relative humidity, carbon dioxide and water vapour levels against time (h) of unrefrigerated data in May (——) and June (– – –) and refrigerated data in May (–·–) and June (····).



or in the head of the bunch. The outside sprigs of bunches were slightly bruised, but the amount of bruising was variable and probably depended on the skill of the packer. Bunches kept for 7 days after the short distribution chains, and 6 - 7 days after the long chain before they were judged unsaleable.

The statistical analysis of data from the long and short runs showed there was a greater variation in measurements taken at different stages within the distribution chains than between months and the long and short runs. Transporting after overnight storage at 6°C appears to counteract the effect of the longer time before selling at the market. The shelf-life of watercress when bought by the consumer, depends not only on the conditions to which it is exposed en route to the market, but also on correct handling by wholesaler and retailer who should be advised on suitable storage methods.

2. Unrefrigerated v Refrigerated Runs (May, June)

	Cutting/ Pulling	→ Bunching	→ Hydrocooling	→ Cold Storage	→ Loaded on Lorry	→ Lorry Change	→ Selling at Market	→ Arrival at Bath
Unrefrigerated:	0h	0.5h	1h	1.5h	17h	25h	40h	48h
Refrigerated:	0h	0.5h	0.75h	1h	24h	no lorry change	47h	52h

The mean values for air temperature, RH, cress temperature, carbon dioxide and water vapour levels are shown in Fig. 2.8. The unrefrigerated chain was from Waddock to Blackburn and the refrigerated chain from St Mary Bourne to Manchester.

a) Air Temperature and RH

The air temperature in the cold stores in both chains was 6°C and after refrigerated transport this rose to 12°C at the market. In the unrefrigerated chain the temperature rose sharply after cold storage to 20°C at the mid-afternoon lorry change, decreased during

the night and increased again on the journey to Bath. RH was fairly constant for both transport chains (May 80%, June 60%) except for a decrease at the lorry change in the unrefrigerated chain due to the higher afternoon temperatures.

b) Cress Temperature

The 3-way analysis of cress temperatures showed significant differences in temperatures between refrigeration treatments, months and stages along the chains. However, the variation in temperatures was far greater between stages than between months and treatments. In the unrefrigerated chain the watercress was hydrocooled to $4^{o}C$ before storage, and during transport the temperatures rose to 12^{o}- $14^{o}C$, reaching 14^{o}- $15^{o}C$ at market. Although the cress for the refrigerated chain was only hydrocooled to 7^{o} and $9^{o}C$, it remained at these temperatures until it arrived at the market where cress temperature increased to 10^{o}- $12^{o}C$.

The range of temperatures to which the watercress is exposed along the unrefrigerated chain, from $4^{o}C$ at hydrocooling to $15^{o}C$ at market, may cause more stress in the plant material than a more constant, intermediate temperature. The relationship between ambient temperature variation, physiological stressing in the plant and senescence, may be a profitable area of future research.

c) RT and Fresh Weight Loss

There were significant differences in the RT measurements between runs, months and stages. Most RT values were higher in the refrigerated transport chain (91 - 100%) than in the unrefrigerated chain (91 - 94%). The measurements between months showed wide variation, with values ranging from 81 - 96% in May and 85 - 98% in June. RT was lower at harvesting (87%), increasing after hydrocooling to 100%,

decreasing to 94% after cold storage, and then remaining constant.

The fresh weight loss for boxes of cress was very similar along both chains, 6% refrigerated, 5% unrefrigerated, from the maximum value after hydrocooling.

d) Carbon Dioxide and Water Vapour

In all comparisons the differences were statistically significant, but the variation from cutting the cress to selling at market was much greater than between months or refrigeration treatments. The carbon dioxide levels were initially the same for both chains (0.2 vol.%) and remained constant for the cress in the refrigerated transport. However, the level along the unrefrigerated chain increased at the lorry change, decreased slightly overnight to selling at the market, and increased on the journey to Bath.

The water vapour levels were constant along the refrigerated chain at 4 - 6 mg l^{-1}. There was an increase in the level in the unrefrigerated chain at the lorry change, decreasing overnight to a similar level at the market as in the refrigerated chain. Water vapour levels increased in boxes of cress from both chains on the journey to Bath.

e) Chlorophyll Content and Greenness

Chlorophyll content values were only significantly different between months, and not between runs or stages. The values were higher in May than in June. There were no differences in the colour of watercress between months using the Munsell standards or the hue angle measurements, and the reason for the difference in chlorophyll content between months cannot be explained.

f) Sensory Assessment and Shelf-Life

The results of sensory assessment (Table 2.3) show little difference in the appearance and texture of watercress between the distribution chains. However, the aromas of the cress at market (stage 8) suggest that the watercress from the refrigerated chain was very slightly fresher than cress from the unrefrigerated chain.

Watercress was packed more tightly at 20 bunches/box for refrigerated transport, whereas looser packing with 15 bunches/box was used in unrefrigerated transport. Also, many bunches in the refrigerated chain had elastic bands placed partially over the head of the bunch, bruising the leaves. The advantage of refrigerated transport, despite the detrimental effects of tight packing and bruising, is evident in the difference in shelf-life of the watercress; 8 - 9 days, compared with 6 - 7 days after unrefrigerated transport.

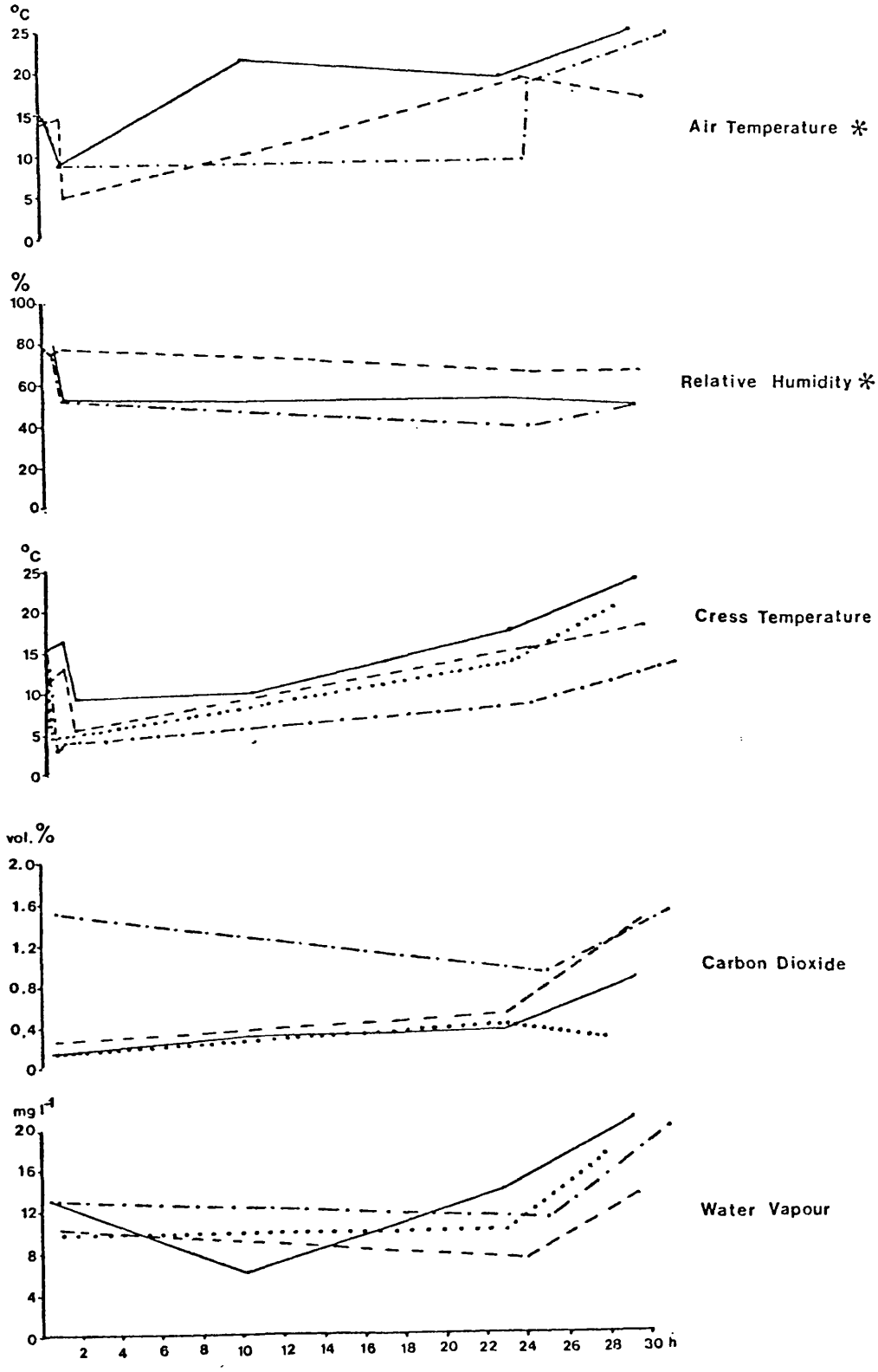
Refrigeration appears to maintain high RT and avoids fluctuations in cress temperature, carbon dioxide levels and water vapour levels, which result from varying ambient temperatures. Sensory assessment of both sets of watercress at market showed little difference in appearance, and the most apparent difference was in the number of days which the cress could remain saleable at $4^{o}C$. However, growers must decide whether the expense of refrigeration is worth the extra shelf-life, bearing in mind that the 6 - 7 days life of the unrefrigerated cress after it is sold at market should allow ample time for the consumer to buy and eat the watercress. To obtain the number of days of shelf-life achieved in this study, cress must be carefully handled and stored correctly after leaving the market.

Table 2.3 Sensory assessment of watercress bunches in the unrefrigerated and refrigerated distribution chains, at bunching (stage 3), selling at market (stage 8) and at Bath (stage 9).

Month and Stage		Sheen of Leaves	Texture to Fingers	Aroma	Yellow Leaves on Stalks	Yellow Leaves in Head
			Unrefrigerated Transport Assessment			
May	3	dull shine	crisp	weak watercress	0	0
	8	dull shine	slightly wilting	medium watercress	0	1-2
	9	dull shine	wilting	medium watercress	0	1-2
June	3	dull shine	fresh but soft	weak primrose	0	0
	8	matt	crisp	medium watercress	2-3	1
	9	matt	crisp	medium watercress	2-3	1
			Refrigerated Transport Assessment			
May	3	shiny	crisp	no smell	1	0
	8	dull shine	crisp	weak watercress	1	1
	9	dull shine	crisp	weak watercress	1	1
June	3	dull shine	crisp	wet vegetation	0	0
	8	matt	wilting	weak watercress	2-3	0
	9	matt	wilting	medium watercress	>5	0

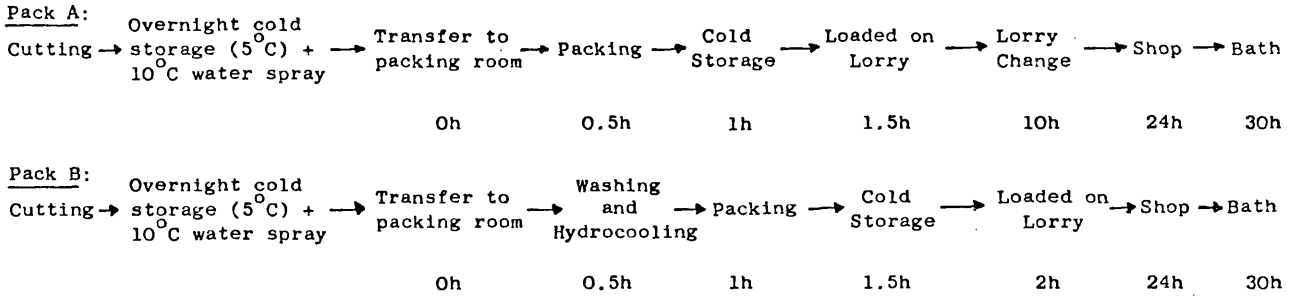
Fig. 2.9 Prepacks A and B Distribution Chains

Plots of mean values for air and cress temperatures, relative humidity, carbon dioxide and water vapour levels against time for pack A data in June (——) and July (– – –), and pack B data in June (–·–) and July (····).



* Due to lorry breakdown Pack B was taken along Pack A chain in July, therefore air temperature and RH were the same for both prepacks.

3. Partially Refrigerated Prepack A Chain v Totally Refrigerated Prepack B Chain (June, July)



Pack A was a shallow, plastic container for stems of watercress (c. 18 cm long), covered with shrink film (a dew pack), and distributed in refrigerated transport until the lorry change. Pack B was an acetate film bag containing small sprigs of watercress which was distributed along a totally refrigerated chain. Both chains were from Abbotts Ann to Nottingham. Graphs of mean values for air temperature, RH, cress temperature, carbon dioxide and water vapour levels against time are shown in Fig.2.9.

a) Air Temperature and RH

The air temperature for Pack A cress increased from 5°C in cold storage to 15°C during packing (time 0 h), and then decreased again when the packs were transferred to the cold store before loading on to refrigerated lorries. The air temperature for Pack A was high at the lorry change (15°- 20°C) and in the shop (19°C). The watercress for Pack B was also stored overnight at 5°C. Air temperatures rose to 14°C during washing, hydrocooling and packing, and decreased during storage before refrigerated transportation. On reaching the shop the packs of cress were kept in a cold store at 8°C until displayed on refrigerated shop shelves.

RH was above 75% during storage and packing, but then decreased to 60% in June, and remained at this value during transport and at the shop. In July the RH decreased gradually from 80 - 66% over the

entire distribution chain.

b) Cress Temperature

The results of the 3-way analysis showed that the temperatures for cress in Pack B were significantly lower than in Pack A. In June the pack B cress temperature remained below 8°C throughout the transport chain to the shop-shelf. The temperature of cress in Pack A was 10°C at the lorry change, and rose to 15°- 17°C in the shop. All cress temperatures increased on the journey to Bath, but the June pack B temperatures were lower than the others because this was the only totally refrigerated chain.

c) RT and Fresh Weight Loss

There were significant differences in RT between runs, months and stages although all measurements were higher than 88%. The average values for pack B were higher than for pack A and this is probably due to the wetter cress in pack B after packing. The RT of pack B cress was constant from packing to the shop-shelf, but the values for pack A cress decreased slightly at the lorry change and in the shop. Both decreased on the journey to Bath, to 92 - 94% for pack A and 94 - 97% for pack B. The measurements taken in July were lower than in June and the reason for this is unknown.

The packing weight in pack A remained constant whereas in pack B weight loss was 2.5% at the shop and 13% at Bath.

d) Carbon Dioxide and Water Vapour

Statistical analysis showed no significant differences between carbon dioxide levels from the two types of prepack chains although levels were higher in June for pack B. However, the values between months and stages were significantly different. The range of values was

greater in June than in July and levels were higher before loading on to the lorries and after selling in the shops because these stages were outside the cold chains.

The results of water vapour levels were significantly different between runs, months and stages. The levels in pack A decreased at the lorry change in June and then increased in the shop and at Bath. In July there was a gradual decrease in the level to the shop and an increase on the journey to Bath in pack A. The level in pack B remained constant until an increase occurred on the journey to Bath. The values at Bath were higher than at any other stage on the distribution chains and overall, the June levels were slightly higher than in July.

e) Chlorophyll Content and Greenness

The measurements of chlorophyll content were found to be significantly different between runs, months and stages. Pack A cress contained less chlorophyll than pack B cress, and the values for June were higher than July. The lighter coloured summer cress may account for the lower chlorophyll content in July. There was also an apparent increase in chlorophyll content during the distribution chains, but this may be due to calculating measurements on an area and not a weight basis.

The analysis of hue angle results showed no significant differences, and the time span over these distribution chains is probably too short for colour changes to be detected by either chlorophyll content or hue angle measurements.

f) Sensory Assessment and Shelf-Life

The results of sensory assessment of cress in the two types of

prepacks are shown in Table 2.4. Both sets of cress were very similar when assessed at the end of the transport chains except for the aroma. The watercress in pack A smelt of "medium watercress" and "weak seaweed" compared with "weak primrose" and "weak watercress" in pack B. This suggests that the cress in pack B had not deteriorated as much as that in pack A. It was noticed, however, that in pack B 50 - 75% of the leaves were bruised compared with only 25 - 50% in pack A, and this may be due to the washing methods used for the pack B cress. Also, the pack B bags contained quite a lot of water and several bruised leaves were softening in the moist conditions.

The prepacks were stored at 4°C on arrival at Bath for shelf-life assessment; pack A kept for 7 days and pack B for 9 days.

This study showed that total refrigeration along the distribution chain for cress in pack B maintained a lower cress temperature and higher RT than for pack A cress in a partially refrigerated chain. However, further measurements are required before the merit of a totally refrigerated prepack chain can be judged, as the results of this work are based on a limited number of measurements. However, it is clear that more importance should be placed on storage conditions after distribution and more care is required to reduce physical injury during mechanical washing and packing.

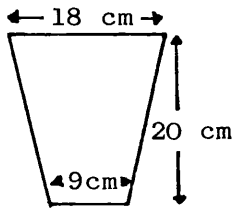
Further research is needed into the types of prepacks suitable for watercress. The materials currently used for prepacks may not be the most suitable as it has been observed that sprigs in acetate bags become dehydrated after several days on the shop-shelf.

In the light of previous results, and in response to a suggestion made by one of the growers, a simple covering for watercress bunches

Table 2.4 Sensory assessment of watercress from prepacks after packaging (stage 3), at the shop (stage 8) and at Bath (stage 9).

Prepack	Month and Stage		Sheen of Leaves	Texture to Fingers	Aroma
A	June	3	dull shine	crisp	wet vegetation
		8	dull shine	wilting	medium watercress
		9	matt	wilting	medium watercress
	July	3	dull shine	crisp	primrose
		8	matt	wilting	weak watercress
		9	matt	wilting	weak seaweed
B	June	3	dull shine	crisp	wet vegetation
		8	dull shine	crisp	weak watercress
		9	matt	crisp	weak primrose
	July	3	shiny	crisp	medium primrose
		8	dull shine	wilting	medium primrose
		9	matt	wilting	weak watercress

was tried out. Polythene cones were made by heat-sealing together two pieces of polythene. Bunches were placed in the cones (one bunch/cone) and packed into two boxes on the June unrefrigerated run for comparison with normal boxed bunches.



The results of measurements taken showed no differences in cress temperature, carbon dioxide levels, fresh weight loss or shelf-life between the bunches in polythene cones and those without. However, the water vapour levels were consistently higher during transport in boxes with polythene cones due to the presence of water droplets on the polythene. Further work using open-ended and closed polythene cones may reduce wilting in bunches of cress by maintaining a humid micro-environment and retaining water in the cones. This type of packing may make the bunch of watercress more attractive to the consumer and be less expensive than the other types of prepacks currently used. Consumer surveys would be valuable in determining public demand for prepacked watercress and in assessing the value of different packaging.

2.4 Summary

During distribution, watercress is subjected to large fluctuations in environmental conditions which overshadow the effects of distance and refrigeration. The time required for distribution (one day or three) had little effect on the appearance and shelf-life of the watercress provided it was stored correctly after leaving the market. Conditions during post-harvest processing and distribution should avoid large fluctuations in temperature and humidity to minimise the physiological stressing of living plant material.

Refrigerated transport avoids large fluctuations in cress temperature, carbon dioxide and water vapour levels during distribution, and as a result the produce had a slightly longer shelf-life. Hydrocooling bunches lowers the cress temperature considerably and immediate packing into insulated containers for transport in the unrefrigerated chain would maintain lower temperatures and reduce fluctuations. This method may be less expensive than using refrigeration but the absence of air movement may be a disadvantage and would need investigation.

Total refrigeration of the prepack distribution chain compared with partial refrigeration does maintain lower cress temperatures and higher RT. Very low temperatures of 2^{o}- 5^{o}C followed by increases to 15^{o}- 20^{o}C may cause more damage to the plant material than constant intermediate temperatures (10^{o}C).

The number of measurements taken during this study was limited and several of the methods were found to be insufficiently sensitive to detect differences during the short time span covered. Although some differences were apparent, they were not large enough to be statistically significant. Difficulties were also experienced in adapting laboratory techniques to the distribution chain situation and sensory assessment may prove more useful than physiological measurements in detecting deterioration in watercress. The results may have been more useful if measurements had been continued at the end of the distribution chain when differences between treatments may have been more apparent.

Further research should examine the different materials available for watercress prepacks and their micro-environmental effects on the

plant material. To determine the effects of different factors on watercress from harvesting to marketing, distribution chains should be studied over a complete year and over the same period for several years. A single type of distribution chain may not be suitable all the year round and this will need investigation. However, work on this scale was outside the scope of this study, and hence only limited results were obtained.

(Raw data given in appendix)

SECTION 3

Development of a Sensory Profile for Assessing Watercress Quality

3.1 Introduction

The quality of a horticultural product is assessed from the relative values of several characteristics which considered together will determine the acceptability of the product by the retailer. The consumer attempts to assess the general quality of a product at the time of purchase, but since quality implies the contribution of several different attributes, general quality cannot be accurately assessed without the separation and measurement of these properties. Kramer and Twigg (1970) classify quality characteristics into three groups : -

1) quantitative, such as yield and net weight
2) hidden, including nutritive value and toxic substances
3) sensory, which is divided into colour, size, flavour, aroma and texture.

Each property does not contribute equally to the general picture of quality, and the relative importance of each differs from one product to another. Consumer preference can only be discovered by consumer research, but the consumer can be educated to select horticultural products on the basis of factors which are not always apparent, but have been assessed and measured by trained sensory panels.

Today the grower must be just as aware of the quality of his product as the consumer. In the past a grower could produce a crop and then decide which market to send it to. However, wholesalers and supermarkets are now introducing their own specifications for the quality and packaging of produce, and many countries have statutory regulations covering labelling of products of which the grower has to be aware.

In setting standards of quality it must be remembered that fruit and

vegetables are biological materials, affected by environmental conditions which cannot be controlled by the grower. Three levels of quality have to be recognised by grower and buyer : -

1) maximum quality, disregarding production costs
2) optimum quality, the best quality within economically acceptable costs
3) acceptable quality, the deviation from optimum quality without falling to substandard or unacceptable.

Foodstuffs are submitted to sensory examination to obtain information which can lead to product improvement, quality maintenance, development of new products, or analysis of the market. Amerine, Pangborn and Roessler (1965) describe the different types of sensory tests and the factors which influence the efficiency of laboratory sensory panels. For quality evaluations, a panel of trained people with a complete framework of reference, derived from experience and prolonged exposure to the specific commodity, must be used for consistent and reproducible results. The Flavour Profile Method, developed by Cairncross and Sjöström (1950), is the basis of much sensory work, and was successfully used by Jellinek (1962) to obtain a profile of aroma and flavour characteristics of certain sea-fish species at different stages of freshness and degree of quality. The profile method concentrates on the entire flavour of a product and puts the flavour components in perspective. The panel is asked to describe the different components and intensities of the flavour, and also the overall impression. Open discussion follows in which descriptive terms are defined and discussed so that each member understands the terms being used, and a tentative profile with standard vocabulary is prepared for the next session. The number of sessions required to achieve the final profile depends on the kind of food

to be examined and the experience and training of the panel.

Different systems of scoring and analysing results have been developed and many specialised statistical treatments used in particular situations. The great variability displayed by trained and untrained participants in sensory testing questions the need for elaborate and highly refined statistical procedures (Amerine et al, 1965; Stone et al, 1974; Williams, 1978). Byer (1964) points out possible flaws in sensory testing situations and cautions against over-complicating the statistical procedures. A major problem associated with numerical scoring systems is the fact that different people score at different levels. An odour which is strong to one person may be weak to another. The introduction of reference samples during assessment of an unknown, for comparison, helps to reduce between-assessor error.

In addition to the fish profile, other profiles have been developed for many fruits and vegetables and are particularly used in the canning and freezing industries to accept or reject produce for processing (Arthey, 1973). The beverages industry has also developed profiles to assess various products (Clapperton, 1973; Shortreed et al, 1979; Williams, 1975). No such profile nor quality specification has been developed, either in this country or by the United States Department of Agriculture, for watercress. During the study of post-harvest processing, storage and distribution of watercress (Section 2) it was thought that sensory evaluation of changes in quality after harvesting might prove of value equal to or greater than physiological measurements. This work was undertaken to establish simple sensory profiles describing colour, sheen, aroma, flavour, texture and the overall acceptability and appearance of watercress, all of which contribute to its quality. This may have application in consumer

surveys and when establishing quality specifications for use by grower, wholesaler and retailer.

3.2 Materials and Methods

3.2.1 Material

The watercress (R.nasturtium-aquaticum) used for the sensory work was obtained from several different sources; shops in Bath, grown in the watercress bed simulation tanks, and supplied by two growers in Hampshire and Dorset.

3.2.2 Composition of Panel

Over the two year period of this work, a group of ten people from the Plant Sciences Department of Bath University; four female and six male (age range 22 - 50 years), were members of the assessment panel. Six people from the group were selected for each assessment.

3.2.3 Procedure

The assessments were carried out in a quiet, well-lit room with a matt white background to the assessment area. The panelists were given written instructions and asked to carry out the assessment on their own, and in their own time. When each member of the panel had completed the assessment, the results were collected and discussed by the group.

3.2.4 Development of the Profile

The following sequence of sensory assessments was undertaken by the panel :

1) Collection of terms to describe watercress properties

In two initial assessments panelists were asked to describe

five bunches of watercress of different quality, using their own adjectives, with only the following headings as guidelines: colour, sheen, aroma, size of leaves, flavour, texture, overall appearance.

2) Rationalization and reduction of descriptive terms

Discussion amongst panel members, directed by the group leader, resulted in a reduction in the number of adjectives describing each characteristic, but still covering a range from good to bad quality. By testing the use of these reduced numbers of terms on seven different watercress samples in two assessments, adjectives were added or deleted as thought necessary during discussion. The adjectives were selected to cover all possibilities of quality without duplication of terms.

3) Use of terms on different formats of record sheets; profile modification and scoring

Different assessment sheet lay-outs were tested and simple scoring was introduced for some of the characteristics. Alterations were made to the profile as thought necessary by the panelists during four assessments of ten different bunches until the final version was developed.

3.2.5 Odour Reference Samples

Reference samples were made for the five aroma categories developed in the sensory profile. The compounds were added to molten paraffin wax (BDH paraffin wax with ceresin for embedding) in small 15 ml glass stoppered bottles. The concentrations of compounds used were decided by smelling for the correct odour.

Dimethyl disulphide	(Fluka A.G.)	25 ppm (v/w)
Phenethyl isothiocyanate	(Fluka A.G.)	325 ppm (v/w)
β-ionone	(Aldrich Chemical Co. Ltd.)	12.5 ppm (v/w)

Cis-3-hexen-1-ol (Aldrich Chemical Co. Ltd.) 25 ppm (v/w)

One bottle contained only wax as the blank sample.

The bottles were numbered and presented to the sensory panel for identification from the following list of adjectives, with the last four being classified according to intensity i.e. weak, medium or strong: wet vegetation cut grass primrose/flowery watercress seaweed rotting

3.2.6 Attribute Ranking Test

A ranking test was carried out to determine the order of importance of different attributes on the profile assessment sheet for use by the consumer. Thirty six people were presented with the sheet shown in Fig. 3.1. The list of attributes was initially randomised, then divided into four groups and the order of these groups randomised. Sheets with the attributes listed in twenty four different orders were used to avoid the possibility of bias due to the order of attributes presented to the assessor. Members of the sensory panel also carried out the ranking test. These results were separated from the untrained consumers' results for comparison. Statistical analysis of the results was carried out using 1) rank total tables (Kahan et al, 1973) and 2) Fisher distribution tables (Kendall,1970).

3.3 Results and Discussion

3.3.1 Vocabulary Development

Table 3.1 presents the results during the development of the sensory profile for watercress. Results of the initial two assessments are given on the left, with intermediate changes in the centre, and the final version on the right. The results for each attribute in Table 3.1 are discussed in the following sections :

Fig. 3.1 Sheet used for attribute ranking test

Please rank the following attributes in order of importance when assessing watercress.
If two or more attributes are of equal importance, bracket together.

Physical damage to leaves (bruising, torn edges)
Aroma of stalks
Tightness of bunch
Texture when in the mouth (crispness)
Flavour and hotness
Colour of leaves
Shape of leaves
Sheen of leaves
Texture when handled (firmness)
Colour of stalks
Aroma of leaves
Firmness and appearance of stalk ends
Size of leaves

1
2
3
4
5
6
7
8
9
10
11
12
13

If you consider any of the lower ranked attributes to be of no or little importance, please underline them.
If there are any attributes which you consider to be important, but which are not included, please list below -

Please complete the following details :

Male / Female
Age
Occupation

Frequency of eating watercress -
- once a week or more
- 2 or 3 times a month
- once every 3 months or more
- less frequently

Do you eat watercress in a salad,
or use it as a garnish,
or use it in a cooked dish ?

Table 3.1 Development of sensory profile for watercress - results of initial assessments on the left, intermediate changes in the centre, and final version on the right.

COLOUR

yellow	dirty green	Intensity :	→	Royal Horticultural Society colour fan for green shades	→	Munsell colour standards
yellow/green	dark green					5GY 4/4 7.5GY 4/4
khaki	browny green	fairly				4/6 4/6
yellow/creamy green	not uniform	slightly				4/8 4/8
bright green	red veins					5/4
cooking-apple green	reddish-brown veins					5/6
olive green						5/8
						5/10

SHEEN

dull	Intensity :	→	5 point scale :	→	Categories altered and scores added :	
dull shine						
velvet sheen	slight		GLOSSY		VERY SHINY	
			VERY SHINY		SHINY	
			SHINY		DULL SHINE	
			DULL SHINE		MATT	
			MATT		DRY MATT	

SIZE OF LEAVES

uniform	Intensity:	Measurements : (diameter cm)	→	6 point scale :	→	Measurements added :	→ Cardboard discs used : (diameter cm)
variable							
	very			VERY LARGE		⩾ 4.0	4.5
large	mostly	3 - 4		LARGE		< 4.0 ⩾ 3.0	3.5
medium	fairly	2 - 2.5		MEDIUM		< 3.0 ⩾ 2.0	2.5
small		1.5		SMALL		< 2.0 ⩾ 1.0	1.5
shrivelled		< 1.0		VERY SMALL		< 1.0	0.5
				SHRIVELLED			

Table 3.1 continued

AROMA

fresh
foliage smell
wet vegetation
apple smell
beer smell
primrose smell
perfume smell
mashed rose leaves smell
watercress smell
peppery
sour
aromatic
acrid

brine
chlorine
fishy
seaweedy
sea water smell
river water smell
brackish
dirty water smell
stale river or pond smell
decaying
rotting
stale

Intensity :

strong
very
mild
slight
little
faint

→ Divided into leaf and stalk aroma with same categories and 2 point intensity scale :

No smell

Wet vegetation smell

Faint primrose/flowery smell
Strong " " "

Faint watercress smell
Strong " "

Faint seaweed smell
Strong " "

Faint rotting smell
Strong " "

→ Intensity scale expanded to 5 points :

No smell

Wet vegetation smell

Primrose/flowery smell 1 - weak
5 - strong

Watercress smell 1 - weak
5 - strong

Seaweed smell 1 - weak
5 - strong

Rotting smell 1 - weak
5 - strong

TEXTURE TO FINGERS

turgid
crisp
springy
waxy
silky
cold
wet

smooth
rough
flexible
bendy
rubbery
soft
dry

limp
lifeless
floppy
flaccid
greasy
slimy
clammy

Intensity :

slighty
fairly
moderately
very

→ Divided into leaves and stalks with a 4 point scale and associated "snapping test".

VERY CRISP - snaps 3 out of 3 times
CRISP - snaps 2 out of 3 times
WILTING - snaps 1 out of 3 times
WILTED - rubbery and will not snap

→ 5 point scale for leaves only adopted and scores added.

VERY CRISP	5
CRISP	4
FRESH BUT SOFT	3
WILTING	2
WILTED	1

TEXTURE TO TONGUE

crisp
firm
brittle
edible
waxy
silky

bendy
rubbery
slimy
limp
flacid
floppy
plastic-like

smooth
lumpy
soft
fibrous
leathery
tough
chewy

Intensity :

slightly
quite
fairly
little
rather
very

→ 5 point scale used :

CRISP AND EASY TO CHEW - TENDER
BIT CHEWY
SLIGHTLY THICK AND TOUGH
TOUGH AND HARD TO CHEW
INEDIBLE

→ Reduced to 2 point scale :

FRESH AND CRISP - acceptable
WILTED - unacceptable

Table 3.1 continued

FLAVOUR

pleasant
sweet
apple-like
insipid
dry
sour
acidic
burning
hot
peppery
watercress
mustardy
'bite'
iron-like
metallic

Time of tasting :
- no taste at first
- initial taste
- immediate
- persistent
- after taste
- after effect
- slow to develop
- after swallowing
- when leaf or stem is broken

Place of tasting :
- on tongue
- at back of mouth

Intensity :
- strong
- quite strong
- slightly
- little
- rather
- quite
- medium
- mild
- very mild
- very little
- weak

→ Divided into FLAVOUR and HOTNESS with a 4 point intensity scale and 2 stage time aspect.

No initial flavour
Slight, immediate
Slight, after chewing
Medium, immediate
Medium, after chewing
Strong, immediate
Strong, after chewing
} watercress flavour

No initial hotness
Slight, immediate
Slight, after chewing
Medium, immediate
Medium, after chewing
Strong, immediate
Strong, after chewing
} hotness

→ FLAVOUR and HOTNESS combined and 3 stages of flavour development used with a 4 point intensity scale for each stage with scoring.

Initially
Flavour and hotness - no 0
- slight 1
- medium 2
- strong 3

While chewing
Flavour and hotness - no 0
- slight 1
- medium 2
- strong 3

After chewing
(persistence)
Flavour and hotness - no 0
- slight 1
- medium 2
- strong 3

OVERALL APPEARANCE OF WHOLE BUNCH

too tightly packed
small
tight
stunted
ragged
loose
uniform colour
uniform size of leaves
fresh
high proportion of stalks

→ size of bunch
state of bunch - tight
fairly tight
loose

Table 3.1 continued

OVERALL APPEARANCE OF STALKS

rooty
short
long
thick
slim
straight
green
yellow leaves attached
mushy
rotting
unappetising
untidy

→ Length of stalks
→ Rootiness
→ Colour → Colour of stalks → GREEN, GREEN/YELLOW, GREEN/RED, YELLOW/BROWN, BROWN
→ Attachment of yellow leaves → Colour of stalk ends → GREEN, GREEN/YELLOW, GREEN/BROWN, YELLOW/BROWN, BROWN
→ Presence of rotting ends → State of stalk ends → WET, DRY
→ ACCEPTABLE (not off-putting to buy), NOT ACCEPTABLE
→ Yellow leaves attached to stalks → none, 1 - 2, 3 - 5, > 5

OVERALL APPEARANCE OF HEAD OF BUNCH

tatty	bruised
ragged	dry
torn	withered
wilted	dull
limp	fresh
tired-looking	unacceptable
lifeless	undesirable
floppy	yellowing leaves
shabby	chlorotic leaves
crumpled	dead leaves
shrivelled	

→ Damage to head of bunch → no damage - 0 ... 100% damage - 10
→ Damage to leaves
→ Presence of yellow leaves → Cause of damage → cut/torn, discoloured, wilting, disfigured
→ Veins prominent on leaves
→ Yellow leaves in head of bunch → none, 1 - 2, 3 - 5, > 5
→ % salvageable to eat → 0, 1 - 10, 11 - 25, 26 - 50, 51 - 75, 76 - 100
→ Bunch saleable at recommended price ? yes or no
or at ½ price ? yes or no

Colour

Descriptions of colour were found to be unsuitable and colour standards were used. Munsell colour standards were used in preference to those of the Royal Horticultural Society since Munsell standards are universally recognised (Munsell, 1905). Ten green standards were selected, covering an adequate range of shades for assessing watercress, without confusing the assessor.

Sheen

A five point scale from GLOSSY to MATT was altered to VERY SHINY to DRY MATT and scores added for assessing sheen.

Size of Leaves

Measurements of leaves defined the size categories on a six point scale from VERY LARGE to SHRIVELLED. Standard cardboard discs with diameters of the mean of each size category were made and used in the final assessment sheet so that actual measurement of individual leaves before selecting a size category was no longer necessary.

Aroma

Aroma was found to be an accurate indicator of freshness in watercress. Similar adjectives used during initial descriptions were grouped together and one adjective selected for each characteristic aroma. The aroma of leaves and stalks was assessed separately, using the same categories with two point intensity scales expanded to five in the final version (1 = weak to 5 = strong, with 2 being twice the strength of 1, 3 three times the strength of 1 etc.). When fresh, watercress has either no smell, or a wet vegetation aroma. It then develops a distinct floral odour, and as the plant deteriorates and cell damage occurs, a typical watercress smell due to the liberation of phenethyl isothiocyanate from its glucosinolate, is detected. Further deterioration results in the production of sulphur compounds which produce a seaweed-like and rotting

odour. The range and sensitivity of these aroma changes were unexpected.

Texture to Fingers

Adjectives used in initial descriptions were grouped together if similar and a four point scale from VERY CRISP to WILTED was developed for leaves and stalks assessed separately. The associated 'snapping' test proved to be too destructive and a five point scale with scores was used. The FRESH BUT SOFT category was added to cater for watercress grown in the summer which is more delicate than the winter cress because it grows more quickly, and although fresh, is not VERY CRISP or CRISP. The texture of stalks was deleted because the crispness of the leaves was considered more important.

Texture to Tongue

From initial descriptions a five point scale from CRISP AND EASY TO CHEW to INEDIBLE was tried unsuccessfully. After discussion, panelists decided on a two point scale of either FRESH AND CRISP (acceptable) or WILTED (unacceptable) because texture in the mouth was not considered a major contributing factor to quality.

Flavour

Panelists decided that the characteristic watercress flavour was most important; any other flavours being irrelevant. The "hotness" of the flavour was also considered a valuable attribute; the intensity preferred varying between members. Even in the initial descriptions, the time effect on flavour and hotness development was mentioned, and three time stages (initially, while chewing and after chewing) with four point scales for flavour and hotness were decided upon in the final assessment sheet. The time effect while eating has also been mentioned by Williams and Lewis (1978). They found that with apples, once the fruit cells are broken in the mouth, different volatile components are emitted due to the release of enzymes and possible pH alteration in

the saliva.

Overall Appearance

After assessing individual attributes of the watercress, panel members decided that any other factors contributing to the overall quality of the bunch should be mentioned, and their importance assessed over several tests with modifications to descriptions as necessary, until a final version was developed. Factors contributing to the overall appearance of the whole bunch were its size and tightness. The colour of stalks, the colour and dryness of their cut ends and the number of yellow leaves, were included in the profile as affecting the quality of the stalks. The quality of the bunch head was affected by the number of yellow leaves and its physical condition. Since these characteristics were noted after the other attributes had been described, and because the development of terms for them was more difficult, they were placed at the end of the assessment sheet. Previous completion of other sections was thought to aid in the modification and clarification of these attributes. To assess the importance of these latter attributes with the former ones, the ranking test (3.2.6) was carried out. Fig. 3.2 is the final version of the assessment sheet used in this work.

3.3.2 Odour Reference Samples

The identification of the reference samples by seven panel members is given in Table 3.2. The perception of aroma varies from person to person, and a threshold test was not carried out on panelists before selection for watercress assessment. However, five of the seven members identified dimethyl disulphide as a rotting smell and one as seaweed, with the intensity as either medium or weak, but not strong. Five of the seven members identified phenethyl isothiocyanate as strong watercress, and the other two as medium watercress. β-ionone was

Fig. 3.2 Final version of watercress assessment sheet

COLOUR Select the colour nearest to the overall colour of the bunch and write down the number -

SHEEN Score the overall bunch according to the scale below and write down the appropriate number -

VERY SHINY	5
SHINY	4
DULL SHINE	3
MATT	2
DRY MATT	1

AROMA Tick the category below for leaves and stalks - where there is a range within a category, 2 is twice the strength of 1, 3 is three times the strength of 1, etc. Insert an adjective if needed.

		Leaves	Stalks
No smell			
Wet vegetation smell			
Primrose/flowery smell	1 - weak		
	2		
	3		
	4		
	5 - strong		
Watercress smell	1 - weak		
	2		
	3		
	4		
	5 - strong		
Seaweed smell	1 - weak		
	2		
	3		
	4		
	5 - strong		
Rotting smell	1 - weak		
	2		
	3		
	4		
	5 - strong		

TEXTURE TO FINGERS Score the leaves according to the scale below and write down the appropriate number -

VERY CRISP	5
CRISP	4
FRESH BUT SOFT	3
WILTING	2
WILTED	1

Fig. 3.2 continued

SIZE OF LEAVES Using the size discs, decide which category most of the leaves in the bunch fall into and put a ring round it -

VERY LARGE	> 4 cm
LARGE	3 - 4 cm
MEDIUM	2 - 3 cm
SMALL	1 - 2 cm
VERY SMALL	< 1 cm
SHRIVELLED	

FLAVOUR AND HOTNESS Eat a whole sprig and write down the appropriate number for each of the 3 stages according to the scale below -

Initially	no flavour or hotness	0
	slight flavour and hotness	1
	medium " " "	2
	strong " " "	3
While chewing	no flavour or hotness	0
	slight flavour and hotness	1
	medium " " "	2
	strong " " "	3
After chewing (persistence)	no flavour or hotness	0
	slight flavour and hotness	1
	medium " " "	2
	strong " " "	3

TEXTURE TO TONGUE Tick the appropriate word -

FRESH AND CRISP - acceptable
WILTED - not acceptable

OVERALL APPEARANCE

State of bunch - loose
- fairly tight
- tight

Colour of stalks - green
- green/yellow
- green/red
- yellow/brown
- brown

Fig. 3.2 continued

Colour and state of stalk ends - look at the cut ends of the stalks and write down the percentage of the total stalks which fall into that category e.g. if half the stalks look green and half green/yellow, write 50% by 'green' and 50% by 'green/yellow.

green
green/yellow
green/brown
yellow/brown
brown

Are the stalk ends - wet or dry ?
- acceptable (not off-putting to buy) or not acceptable ?

Yellow leaves - attached to stalks: none; 1 - 2; 3 - 5; > 5
in head of bunch: none; 1 - 2; 3 - 5; > 5

Damage to bunch head - score the bunch according to the scale below and write down the number.

no damage - 0
1
2
3
4
5
6
7
8
9
100% damage - 10

Cause of damage - tick the appropriate word(s) for the major type(s) of damage in the bunch.

cut/torn
discoloured
wilting
disfigured

% salvageable to eat - estimate the percentage and tick the appropriate category -

0%
0 - 10 %
11 - 25 %
26 - 50 %
51 - 75 %
76 - 100%

Bunch saleable at recommended price of ___ ? (yes or no)

If 'no', would you pay ½ price ? (yes or no)

Table 3.2 Identification of reference compounds by seven members of the sensory assessment panel

Compound	Panelist						
	1	2	3	4	5	6	7
Dimethyl disulphide	weak rotting	weak rotting	medium seaweed	--	medium rotting	weak rotting	weak rotting
Phenethyl isothiocyanate	strong watercress	medium watercress	strong watercress	strong watercress	strong watercress	strong watercress	medium watercress
β-ionone	strong primrose	medium primrose	strong primrose	medium primrose	weak primrose	medium primrose	--
Cis-3-hexen-1-ol	wet vegetation	--	cut grass	--	wet vegetation	--	cut grass

identified as the primrose aroma by six of the seven members, but all three degrees of intensity were used. The member of the assessment panel who could not identify β-ionone, could not detect the aroma of a sample double the concentration. It was also noticed that several other people could not detect this aroma, and possibly they have a particularly high concentration threshold value for this compound. The aroma of cis-3-hexen-1-ol, the classic "green cut-grass" and "green leaf" odour compound, was not identified by three panel members and of the other four, two described it as wet vegetation, and two as cut grass. A fairly low concentration was used in the hope that it would be identified as the wet vegetation aroma, but further work is needed to develop this reference sample because the wet vegetation odour is not very strong and cis-3-hexen-1-ol in low concentrations appears to be below the threshold values of many people. For the seaweed odour reference sample, a compound similar to dimethyl disulphide, or a mixture of dimethyl disulphide and another compound will have to be found.

3.3.3 Attribute Ranking Test

Using Fisher distribution tables, both results of sensory panel members and untrained consumers showed no difference in individual assessor's rankings of the attributes i.e. all assessors agreed with respect to the ranking order.

The probability of any observed rank sum of an attribute being outside the range in the rank total tables was tested at the 5% level. The attributes not significantly different were reranked and the rank totals tested again until no more attributes were significantly different from the others. The results are given in Table 3.3.

Table 3.3

Order of Ranking by 7 Assessment Panelists

1	Physical damage to leaves (bruising, torn edges)
2	Colour of leaves
3	Texture when handled (firmness)
4	Sheen of leaves
5	Aroma of stalks Tightness of bunch Texture when in the mouth (crispness) Flavour and hotness Firmness and appearance of stalk ends Colour of stalks Aroma of leaves Size of leaves
13	Shape of leaves

Order of Ranking by 36 Non-Panelists

1	Physical damage to leaves (bruising, torn edges) Colour of leaves Texture when handled (firmness) Texture when in the mouth (crispness) Flavour and hotness
6	Sheen of leaves
7	Firmness and appearance of stalk ends
8	Colour of stalks
9	Aroma of leaves
10	Aroma of stalks Tightness of bunch Size of leaves Shape of leaves

Members of the assessment panel separated four attributes as the most important, with shape of leaves ranked 13th, and the other eight attributes ranked equally as 5th. Three of the four attributes of the panel were ranked equal 1st by the untrained consumers, along with texture when in the mouth (crispness) and flavour and hotness. Sheen of leaves, ranked 4th by the panel, was ranked 6th by the consumers and they also separated firmness and appearance of stalk ends, colour of stalks and aroma of leaves, which were ranked 7th, 8th and 9th. The remaining four attributes were ranked equally 10th by the consumers, and they were also in the lower rankings of the panel members.

Experience gained during training of panelists seems to enable them to separate and rank the four most important attributes from the rest, whereas the consumers were able only to rank equally the top five. Further questioning of the consumers why texture in the mouth (crispness) and flavour and hotness were ranked in the first five attributes might reveal, as with the panel members, that texture in the mouth is not so important because watercress is rarely eaten on its own, and as long as the flavour of watercress is present, the intensity preferred, which varies considerably between individuals, is of secondary importance. Colour is probably more important than flavour and texture, although they were ranked equally, otherwise people would eat brown or yellow cress as long as the flavour was present. With other vegetables and fruits it is colour which is one of the major factors in attracting the consumer's initial attention in the shop.

Panelists picked out the most important attributes and left the rest as equally unimportant,except for shape of leaves which was significantly less important, suggesting that experience in assessing causes the person to concentrate on the few major attributes contributing to

good quality watercress, with the rest of secondary importance. Consumers were also able to recognise the most important attributes chosen by the panel, but they were not able to separate them into order of importance. The four least important attributes were ranked equally 10th by the consumers, and the others ranked in between.

Further ranking tests including the order of magnitude of importance of attributes would determine the size of the sensory difference between attributes, and not only their order of importance. Tests to determine the major attributes when assessing watercress in the shop would provide useful information for the presentation of the product to the consumer, especially since there is an increase in demand for prepacks.

Since shape of leaves was ranked as the least significant attribute by the panelists, it would be interesting to carry out a consumer preference survey between Florida watercress (described in the General Introduction) and the green watercress at present marketed, because Florida leaf shape is very different from the round leaves of the green watercress.

3.4 Summary

The use of sensory assessments and consumer preference information, related to physico-chemical data, is an important tool in the definition of quality of a product.

The development of vocabulary for a sensory profile is a complex procedure with problems of determining the quality attributes and of selecting precise descriptive terms. This work has succeeded in producing a profile for the assessment of watercress which needs to be

used in a wide variety of situations to determine whether further modifications are required. The scoring system needs to be completed, improved and tested for weighting certain attributes before it can be used for statistical analyses. The results of further ranking tests will provide information for appropriate weighting of attributes. Since aroma of watercress is important in indicating its freshness, the use of odour reference samples will aid assessment by panelists, and increase the sensitivity of the results.

SECTION 4

Analysis of the Volatile Components of Watercress

4.1 Introduction

Fruits and vegetables produce complex mixtures of volatile aroma components which contribute to the character of their typical flavour. Having established the identity and concentration of these compounds, they can be used, in conjunction with sensory assessment, to measure quality and also the degree of maturity, fermentation, or other processes producing the typical flavour or aroma.

Gas chromatography is the main analytical technique used for the separation and identification of volatile compounds and it has been used extensively in the study of fruit flavours (Teranishi, 1966). Since the advent of coupled gas chromatography - mass spectrometry, several hundred flavour compounds have been identified. However, it was soon realised that compound identification was of little use on its own, and more recently increasing effort has been devoted to determining their flavour significance.(Williams,1979).

Comparatively little work has been done on vegetable volatiles compared with those of fruit. The main research interests in vegetables have been concerned with the analysis of aroma and flavour constituents, the maintenance of original flavour characteristics during storage (Freeman, 1979; Heatherbell and Wrolstad,1971), and monitoring chemical changes during storage as indicators of quality. Studies on flavour changes during food processing have been carried out by Buttery, Guadagni, Ling, Seifert and Lipton, 1976; MacLeod and MacLeod, 1970a, b; Heatherbell, Wrolstad and Libbey, 1971; Nursten and Sheen, 1974; Maruyama, 1970. Considerable research has been done on the volatiles of fresh, cooked, dehydrated and fermented cabbage (Brassica oleracea L.) (MacLeod and MacLeod, 1970c; Vorbeck, Mattick, Lee and Pederson,

1961). The flavour of onion (Allium cepa L.) and the pathways by which the important aroma components and their precursors are produced, have been determined (Freeman, 1979). The volatiles of other Allium species, including leek (A. porrum L.) (Schreyen, Dirinck, Van Wassenhove and Schamp, 1976), garlic (A. sativum L.) (Oaks, Hartmann and Dimick, 1964), and chive (A. schoenoprasum L.)(Wahlroos and Virtanen, 1965) have been investigated. Research on vegetable volatiles up to 1970 has been reviewed by Johnson, Nursten and Williams (1971 a,b).

Volatile Compound Formation

The formation of volatile flavour compounds has been studied by many workers. Stevens (1970a) proposed three general systems by which volatile flavour compounds are formed : -

1) compounds naturally present in intact tissue,
2) compounds produced enzymically after tissue damage,
3) compounds produced after chemical change e.g. heating.

He suggests there are at least three biosynthetic pathways which result directly in volatile flavour compound formation : -

1) the isoprenoid pathway leading to the production of terpenoid compounds; terpenes have been identified in a number of vegetables.
2) the shikimic pathway leading to the production of aromatic compounds e.g. benzyl alcohol, benzaldehyde, phenyl acetaldehyde, phenyl alcohol and various volatile phenols which have been found in vegetables.
3) β-oxidation leading to the production of lower alcohols and aldehydes.

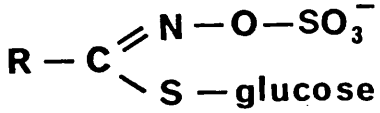
Stevens (1970a) suggested that oxidation is the most important enzymic process in flavour production. The enzymic oxidation of long chain

fatty acids produces many volatile compounds of potential importance in vegetable flavour (Hoffmann, 1962). Several of the major volatile compounds of tomatoes result from the oxidation of polyene-carotenes, including β-ionone (Stevens, 1970b).

Many important flavour compounds are produced enzymically after tissue damage. The most studied precursor system in vegetables is that of amino acids in onion (Schwimmer, Venstrom and Guadagni, 1964). By grinding watercress plants, Gadamer (1899) detected a compound with a strong mustard oil odour. This compound was identified as phenethyl isothiocyanate which is released from its corresponding glucosinolate by action of the enzyme myrosinase when the plant is damaged. In the intact plant enzyme and substrate occur in different cells; myrosinase in idioblasts or myrosin cells (Fahn, 1979) and the glucosinolates in parenchyma cells. Phenethyl isothiocyanate dominates the flavour of watercress and for this reason Nursten (1979) classified watercress under the group of foods whose aroma resides largely in one compound - a "character impact compound" (Teranishi, 1971).

Phenethyl isothiocyanate was identified as the main volatile in watercress by several workers, all of whom used destructive methods to extract the compounds. Ground seeds of watercress were shown to contain phenethyl isothiocyanate using paper chromatography (Kjaer, Conti and Larsen, 1953). This compound was also identified using gas chromatography with an electron capture detector in an extract obtained by soxhlet extraction of a sample of watercress with hexane and ether (Anon.,1964). Freeman and Mossadeghi (1972) carried out a similar soxhlet extraction on fresh watercress tissue as well as head-space analysis of macerated cress. They found that the concentration of phenethyl isothiocyanate increased with increased sulphate concen-

tration of the nutrient medium in which the watercress was grown. MacLeod and Islam (1975) identified 3-phenylpropionitrile, 2-phenethyl isothiocyanate, 8-methylthiooctanonitrile and 9-methylthiononanitrile as the four major compounds in a watercress essence obtained by low temperature-high vacuum distillation of fresh material. Recently, work by Kaoulla, MacLeod and Gil (in press) has shown that the ground seeds contain 2-phenethyl isothiocyanate as the major component (90%), and 3-phenylpropanonitrile, 8-(methylthio)octanonitrile, 7-(methylthio)heptyl isothiocyanate, 9-(methylthio)nonanonitrile, 8-(methylthio)octyl isothiocyanate and phenylacetonitrile as minor components. These compounds are probably derived from the corresponding glucosinolates which have the general structure :



R = different alkyl of aryl groups

Glucosinolates

The glucosinolates are a uniform class of naturally occurring anions which have, so far, been found exclusively in eleven families of dicotyledonous angiosperms (Ettlinger and Kjaer, 1968). By 1960 thirty glucosinolates were known (Kjaer, 1961) and now the number exceeds seventy (Underhill, 1980). The largest family containing glucosinolates is the Cruciferae and of the three hundred species examined, glucosinolates were found in their roots, seeds or leaves (Ettlinger and Kjaer, 1968; Kjaer 1960, 1963, 1966). Glucosinolates are characteristically hydrolysed by the enzyme myrosinase to yield glucose and a labile aglucone which spontaneously rearranges with the loss of sulphate to give an isothiocyanate (mustard oil) as the

major product. These mustard oils have long been known for their pungency and used as condiments, potherbs, preservatives and remedies through the ages (e.g. mustard, horseradish, cress).

Sinalbin, a mustard oil-producing compound in white mustard seeds (Sinapsis alba L.) was isolated in 1831 by Robiquet and Boutron, and Bussy (1840) extracted sinigrin from black mustard seeds (Brassica nigra Koch.). Mustard oils were shown to be isothiocyanates by Hofmann (1868), and the first structural formula for these two classical glucosinolates was proposed by Gadamer (1897). Although a number of reactions of sinigrin, including the formation of nitriles, were not reconcilable with his formula, the Gadamer structure remained essentially unchallenged until Ettlinger and Lundeen (1956) presented the correct formula for sinigrin and sinalbin.

Sinigrin

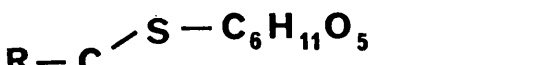
$R = CH_2CHCH_2$

$X = K$

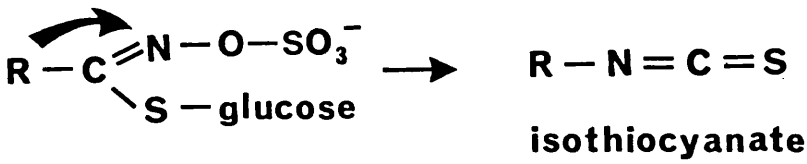
Sinalbin

$R = (p)HOC_6H_4CH_2$

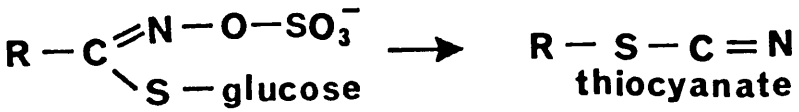
$X =$ sinapine



They also proposed the following reaction, taking place through Lossen rearrangement, for the cleavage of the glucosinolate to isothiocyanate.

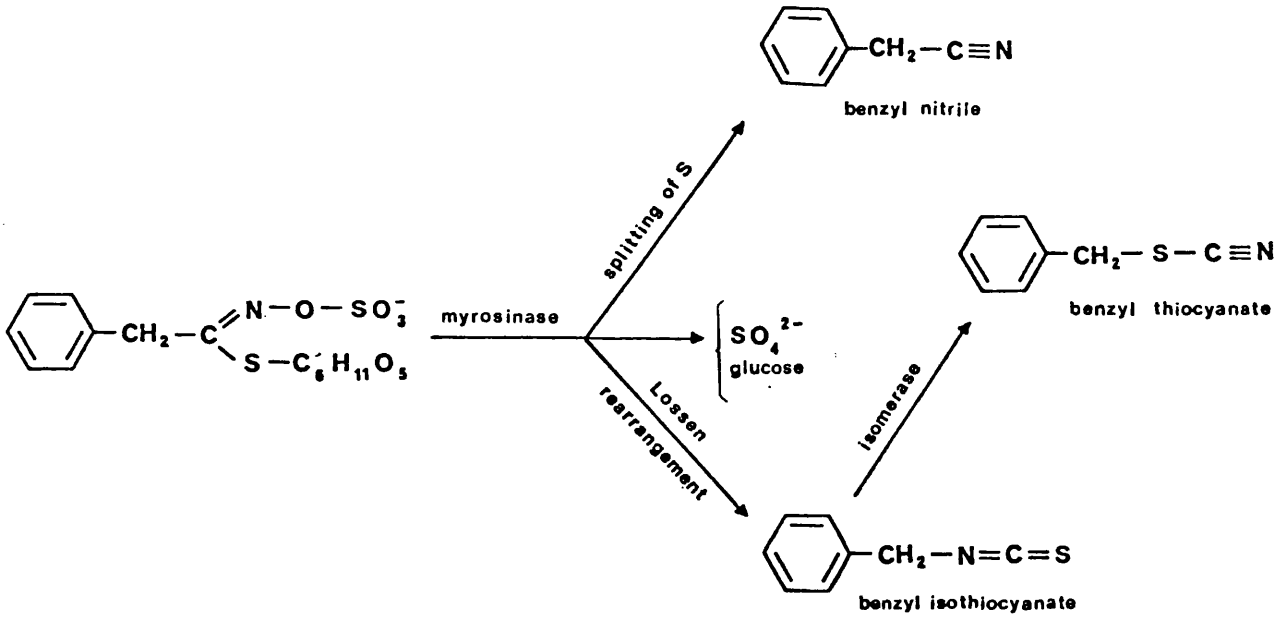


Gmelin and Virtanen (1959) found that the thiocyanate was produced in seeds of Lepidium sativum L., L. ruderales L. and Thlaspi arvense L.



The breakdown of glucosinolates appears to be affected by pH. Ettlinger and Miller (1966) showed that in the pH range 3 - 5 both isothiocyanate and nitrile are formed, and the lower the pH, the higher the proportion of nitrile. Above pH 5 the sole reaction product is isothiocyanate.

Working with Lepidium seeds, Virtanen and Saarvirta (1962) proposed that benzyl glucosinolate is split by myrosinase to isothiocyanate, which is then isomerised to thiocyanate by an unknown enzyme; nitrile was thought to be formed enzymically from the glucoside.



However, further experiments (Saarivirta, 1973; MacLeod and Islam, 1976) showed no evidence for the formation of benzyl thiocyanate from benzyl isothiocyanate, thus questioning the isomerase pathway proposed in 1962. Saarivirta (1973) concludes that the "normal" reaction products appear to be benzyl nitrile and benzyl thiocyanate, resulting from specific enzyme action. Benzyl isothiocyanate is formed only if the formation of benzyl nitrile and benzyl thiocyanate is prevented. However, the established variety in the degradation of benzyl glucosinolate shows that caution is needed in predicting the

fate of a given compound in different plants. The diversity of glucosinolate catabolism is reviewed by Benn (1977). The factors which determine product formation on autolysis of glucosinolates are not clearly understood and further research in this area is required.

Biosynthesis of Glucosinolates

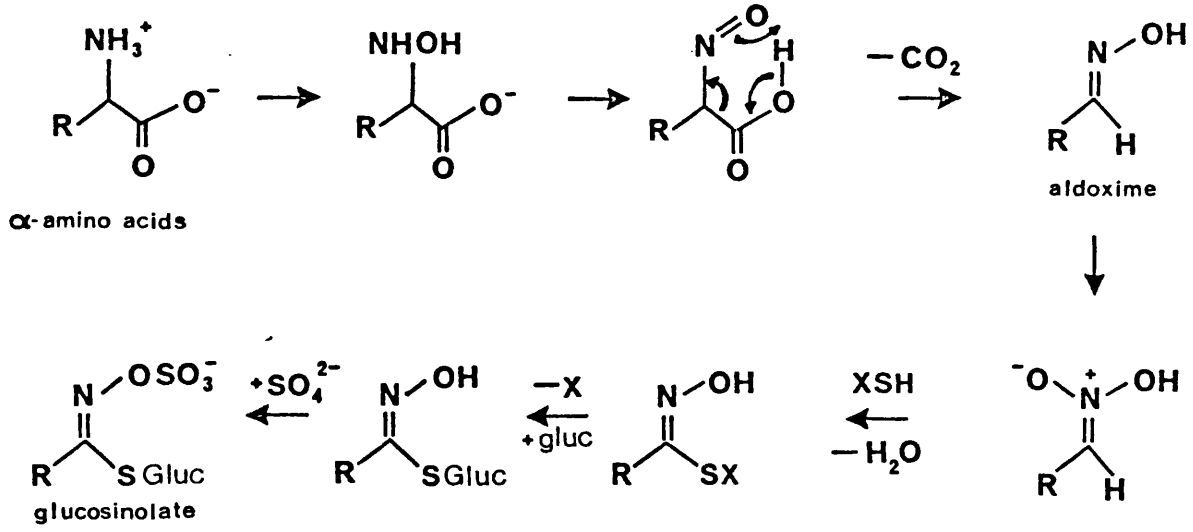
Biosynthetic studies of glucosinolates were initiated in the 1960s. They have revealed that all are derived from amino acids and that most glucosinolates are formed by a common biosynthetic pathway. These studies have involved administration of labelled compounds to plants, isolation of intermediates and, in a few cases, detection of some enzymes involved in the pathway. Glucosinolates which are not related to amino acids are also accepted as being derived from protein amino acids via condensation of their 2-oxo acids with acetate, involving a chain elongation pathway.

Studies on the biosynthesis of 2-phenethyl glucosinolate in watercress have shown that it is formed from phenylalanine and acetate by chain elongation (Underhill, 1965). The natural occurrence of the intermediates 2-benzyl malic acid and 3-benzyl malic acid, and of the chain extended 2-amino-4-phenylbutyric acid, the amino acid precursor of the glucosinolate, was also demonstrated (Underhill, 1968; Dörnemann, Löffelhart and Kindl, 1974). The amino acid content of watercress has been determined and the quantity of phenylalanine given as 62 mg per 100 g edible tissue (Salunkhe, 1974).

The known in vivo synthesis of glucosinolates starts with α-amino acids and proceeds through consecutive oxidative steps, accompanied by decarboxylation, to the aldoximes. Additional oxidation

converts the aldoximes into the dipolar species and then, via dehydrative addition of an unidentified sulphur donor (SX), detachment of the carrier moiety (X), the introduction of the glucose residue, and eventually o-sulphonation, into the final products, the glucosinolates (Kjaer, 1973).

The steps in glucosinolate synthesis



Allowing for biochemical modifications of side chains or rings along the pathway, the biosynthesis of the majority of the known, natural glucosinolates can be explained by this general biosynthetic scheme in combination with the chain-elongating mechanism.

Biological Effects of Glucosinolates

The major source of the increased interest in glucosinolates during the past thirty years has been the antithyroid, goiter-inducing effects that isothiocyanates and their transformation products have in mammals, including man. Since watercress is not consumed in vast quantities, there has been no concern about any antithyroid effect. The main concern has been the extensive use of cruciferous plants as animal feeds. Several workers have gathered information about the occurrence of these latent antithyroid agents in food, liberation in the mammalian body, transfer from cattle feed to milk and general physiological significance (Virtanen, 1961, 1963; Van Etten et al, 1965, 1966; Daxenbichler et al, 1966, 1967, 1968, 1970; Tapper and MacGibbon, 1967).

The glucosinolates in rape and crambe seeds have been examined in some detail as sources of toxic compounds. If these harmful compounds can be removed or inactivated, the by-product meals from these oil seed crops will have more value in animal feeds. Amino acid contents indicate a high nutritional quality for the protein in meals from cruciferous oil seeds (Miller et al, 1962), but at present they can only be fed to livestock in limited amounts. Efficient means of removing the glucosinolates and their hydrolysis products to give a high quality feed are needed. Understanding of the biosynthetic pathways of glucosinolates may lead to plant breeding programmes to eliminate them in those plants used for cattle feed and where flavour is not essential.

The glucosinolates in crucifers are also important to the insect population. Verschaffelt (1910) demonstrated that potassium allyl glucosinolate rendered acceptable the leaves of plants usually

rejected by larvae of Pieris brassica and P. rapae. This work constitutes the first recognition of the role of a secondary metabolite in directing the relationship of an insect and host-plant.

Allyl isothiocyanate has been shown to attract many of the insects whose host plants contain glucosinolates, and stimulate feeding and ovipositing. Feeny, Paauwe and Demong, (1970) showed that adult flea beetles of P. cruciferae and P. striolata locate their food plants, in part, by using the mustard oil allyl isothiocyanate as a "long distance" olfactory attractant. Wallbank and Wheatley (1979) found that the same compound has a role in assisting the female cabbage root fly (Delia brassicae) to locate and select suitable egg-laying sites and it also increased the number of males caught in traps. The experiments of Van Emden and Bashford (1969) and Van Emden (1972) provide some evidence that the variation in glucosinolate concentration may affect fecundity of the crucifer-feeding aphid, Brevicoryne brassicae, when feeding on brussels sprout plants. Work by Cole (1978) found that from turnip seedling emergence until the hypocotyls began to expand, no glucosinolate degradation products were detected. Therefore, insects associated with crucifers, and dependent on these products in host-finding, would not be attracted to the young turnip plants. Differences found in the volatile products of seventy-nine species of crucifers (Cole, 1976) may relate to differences in their status as host plants to cruciferous pests. However, the host-finding behaviour of most cruciferous pests is probably dependent on the interaction of many chemical stimuli provided by its host plant (Wallbank and Wheatley, 1976). On the evidence obtained from experiments with other plants, the attraction of certain aphids to watercress may be due, in part, to compounds resulting from glucosinolate degradation.

Allyl glucosinolate can also act as a deterrent for a number of crucifer insect pests and it is lethal to the aphid Papilio polyxenes (Erickson and Feeny, 1974). The insecticidal properties of 2-phenethyl isothiocyanate were established by Lichtenstein, Strong and Morgan, (1962) and Lichtenstein, Morgan and Müller, (1964), and this compound may affect the insect population which feed on watercress and its microflora, since it is the major glucosinolate product in this plant.

These chemical defence mechanisms in plants are thought to be more effective in reducing the growth and fitness of insect pests which are not specific to that particular plant family or genus, and less effective against insects which exploit the plant as their major or only source of food (Fenny, 1976). It seems reasonable to suppose that although an insect species may possess some general adaptation to tolerate an entire class of chemical compounds, such as the glucosinolates, it may be better adapted to tolerate some of the compounds within this class. This chemical adaptation may explain the maintenance of area separation between six species of crucifer-feeding flea beetles studied by Hicks and Tahvanainen (1974). These observations are important because world-wide damage is caused by host-specific insects to cruciferous crops of economic importance. The establishment of the properties of phenethyl isothiocyanate in watercress in attracting or repelling insects may be valuable in assessing the need for general aphicides currently used by growers.

Mustard oils have long been recognised as possessing antibacterial and fungistatic properties. The bactericidal action of cabbage and other vegetable juices was examined by Pederson and Fisher, (1944), and McKay et al (1959), carried out bacteriostatic assays of a large

series of isothiocyanates. More recent work by Virtanen (1962) showed that the antimicrobial activity of some mustard oils is high with respect to moulds and fungi, and fairly high against many bacteria. Benzyl isothiocyanate has the highest antibiotic activity of all known mustard oils, against fungi and bacteria. The presence of these compounds in certain vegetables, including watercress, may be important in preventing deterioration and rotting during storage and distribution.

In the past only destructive methods have been used to release the volatiles of watercress, giving rise to enzyme action in the damaged tissue with the release of isothiocyanates from their glucosinolates. This resulted in the detection of these degradation products as the main volatile compounds. The purpose of this part of the present study was to collect the volatiles with the least amount of physical damage to the watercress; to identify important volatile compounds which may have previously been masked by the glucosinolate products, and to relate these results to post-harvest deterioration during storage and transportation. Hopefully chemical indicators may be found which can be used to measure product quality.

Collection and Identification of Volatiles

Compounds responsible for the characteristic flavours and aromas of foods may only be present in low concentrations and this creates problems in their isolation, separation, detection and identification.

For isolation of maximum quantities of volatiles and correlation with optimum sensory acceptability, the food product of interest should be examined at the appropriate stage of aroma quality and intensity.

This might be a certain degree of ripeness, or maturity, or a specific time during processing e.g. roasting, boiling. Several different approaches for examining volatiles have been developed. Many start with relatively large amounts of the food, and utilize a variety of distillation and extraction techniques to concentrate and collect all the volatile compounds. However, in these processes, chemical changes often occur in the foodstuff which result in an extract differing from the starting material both in aroma and flavour. Steam distillation, for example, is equivalent to cooking, and the flavours of cooked onion and cabbage, and many other foods, have been shown to differ markedly from the fresh flavours (Boelens et al, 1971; MacLeod and MacLeod, 1970b). The processes of mincing, grinding and chopping may facilitate the removal of the flavour components by breaking up the cell structure. However, these operations also cause the mixing of plant metabolites with the possibility of chemical reactions causing flavour loss and the formation of new compounds. Moreover, by damaging living material, microbial and enzymic activity may occur which produces compounds not present in the original intact tissue. Results obtained using these destructive methods may not represent the true situation in the food as it is consumed, although maceration in the mouth may approach this. Apart from when the aroma from cooked material is to be studied, the mildest conditions, consistent with the recovery of adequate quantities for identification, should be employed.

Most extraction processes also require a concentration step, for example, by evaporation in an inert gas atmosphere. Vacuum sublimation separates the volatile compounds from the non-volatiles with very little physical damage. However, concentration of the extract by distillation must not then cause alteration or loss of any of the

volatile compounds.

Headspace collection of volatiles, in which components in the vapour phase over a food are collected, appears to provide the most authentic representation of aroma and flavour associated with the material. Only a small sample of the foodstuff may be required and very little sample preparation is involved; merely a period for equilibrium to be established in the container. The major limitation with the headspace collection is that the concentrations of some components may be too low for chromatographic detection. This is overcome by careful concentration of a larger vapour sample from the closed system, or by using different trapping methods. These include using cold traps and adsorbents, for example, charcoal and porous polymers where adsorption is selective (Teranishi et al, 1971). Desorption of volatile compounds is achieved either using a stream of inert gas or a suitable solvent.

To avoid introducing contaminants or generating artefacts, the method of volatile isolation for a particular food must be carefully selected to minimize physical damage to the extract. Gas chromatography coupled with mass spectrometry is most frequently used for separation and identification of the components in a mixture. Different columns and various stationary liquid phases, with a range of polarity and selectivity, make this method sufficiently versatile for analysis of both headspace volatiles and concentrated flavour extracts. Sensory assessment during collection and identification ensures that flavour and aroma have not been significantly modified by the isolation technique.

In this study headspace collection of watercress volatiles was used. The plant material was cut and placed in the apparatus (Ismail, Tucknott and Williams, 1980). The volatiles were collected by selective adsorption on Porapak Q from air flowing through the apparatus. Air was used to permit normal respiration of the living plant material. At intervals over a seven day period the Porapak Q traps were removed, eluted with solvent and the samples concentrated. Temperature and illumination of the apparatus were varied to simulate different storage conditions. By identifying those volatiles associated with stages of deterioration, the optimum conditions for post-harvest storage can be determined. Volatiles were also collected by washing plant material in diethyl ether, separating the volatile from non-volatile components by vacuum sublimation, and concentrating the sample by fractionation and evaporation. The volatiles obtained by this destructive method were compared with those from the headspace collection.

4.2 Materials

Watercress - grown in controlled and natural environment watercress bed simulation tanks (1.2.1 and 1.2.2). Green cress, both flowering and non-flowering, and Florida cress were used. Green watercress was cut 12 - 18 weeks from sowing and the Florida type after 14 - 16 weeks growth from stubble. Stems were 18 - 20 cm long when harvested.

Diethyl ether - purified by eluting through a column of aluminium oxide W200 basic, activity grade super 1 (ICN Pharmaceuticals GmbH and Co.) to remove peroxides and water, then redistilled on a 91 x 2.5 cm column packed with glass helices. Analar grade (A.R.), anhydrous, Koch Light Laboratories Ltd.

Sodium sulphate - purified by pyrolysis at 550^{o}C for 5h. A.R. Hopkin and Williams.

Porapak Q - (ethyl vinyl benzene divinyl benzene copolymer) 50 - 80 mesh washed with diethyl ether (2 x 10 ml 1.5 g^{-1}) and dried under vacuum.
Waters Associates Inc.

Acetic acid - chromatography grade BDH.

Acetic anhydride - 98% May and Baker Ltd.

Benzaldehyde - A.R. 99.5% BDH

6-benzyl amino purine - Sigma Chemicals Co.

1,4-dichlorobenzene - BDH

Dimethyl sulphone	-	98% Aldrich Chemicals Co.
Dimethyl sulphoxide	-	BDH
1,2-ethanediol diacetate	-	purum 98% Fluka AG.
1,2-ethanediol monoacetate	-	purum 98% Fluka AG.
Ethyl acetate	-	puriss Koch Light Laboratories Ltd.
Ethyl acetophenone	-	Eastman Kodak Co.
Ethyl benzaldehyde	-	98% Eastman Kodak Co.
Ethyl octanoate	-	99+% Aldrich Chemicals Co.
Hexadecanoic acid	-	GLC pure Field Instruments.
Hexanoic acid	-	99.5% Aldrich Chemicals Co.
β-ionone	-	98% Aldrich Chemicals Co.
Naphthalene	-	May and Baker Ltd.
Pentanoic acid	-	puriss 99% Fluka AG.
Phenethyl isothiocyanate	-	purum 97% Fluka AG.
Phenol	-	May and Baker Ltd.
Tetradecanoic acid	-	GLC pure Field Instruments.

4.3 Methods

4.3.1 Collection of Volatiles

a) Headspace collection

Two types of headspace apparatus were used, both with tubes containing Porapak Q for the adsorption of volatiles. The Porapak Q was eluted with diethyl ether and the samples concentrated by evaporation (4.3.2). The duration of experiments with both types of apparatus was 7 days. Blank collections without plant material were used for reference.

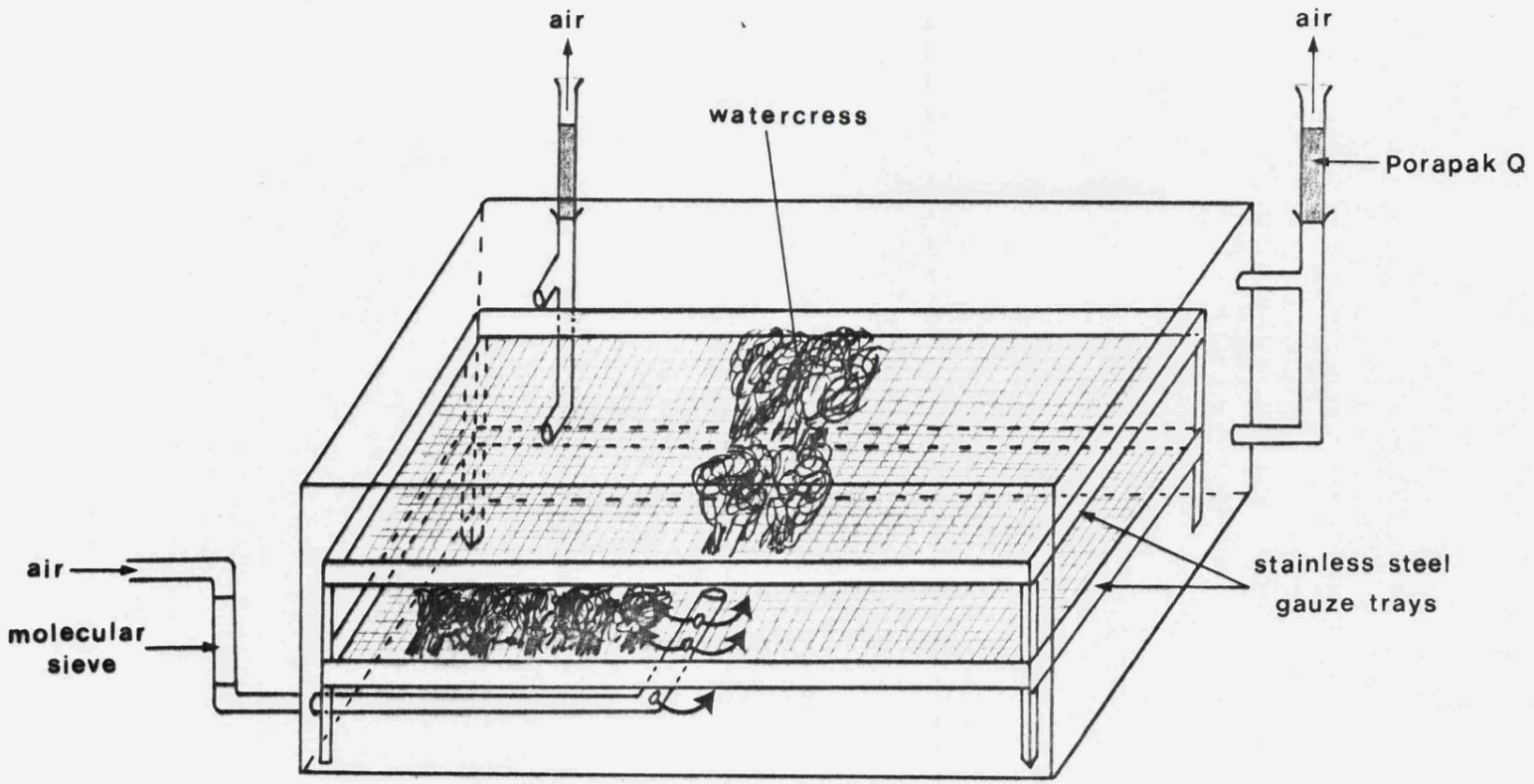
Single Headspace Apparatus

Watercress (1.0 - 1.5 kg), washed in glass distilled water, was placed on two stainless steel gauze trays contained in a rectangular glass vessel (53 x 53 x 30 cm). A glass lid sealed with Apiezon L grease (Apiezon Products Ltd.) closed the front of the vessel. The apparatus was operated at room temperature and air, purified by passage through molecular sieve (BDH type 5A), was passed through it at a rate of 240 ml min^{-1}. A glass tube (10 x 1.0 cm diameter) containing a fluidized bed of 1.5 g Porapak Q (Ismail et al, 1980) was placed at each of the two outlets shown in Fig. 4.1 for adsorption of the volatile components entrained in the air stream.

Dual Headspace Apparatus

Two cylindrical glass vessels (50 x 30 cm diameter) were supported in a cabinet (70 x 140 x 80 cm high) (Fig. 4.2). Each vessel was closed at the bottom with a drainage tap and sealed at the top with a PTFE (Dupont) flange and stainless steel lid. An air flow of 290 ml min^{-1} passed through molecular sieve (BDH type 5A) and into the bottom of each vessel. Two outlets in each stainless steel lid were fitted with glass tubes (10 x 1.0 cm diameter) each containing 1.0 g Porapak Q. The watercress (1.0 - 1.5 kg per vessel) was washed in glass distilled water and placed horizontally in three stainless steel gauze baskets (13 cm high, 28 cm diameter) per vessel (Plate 4.1). The temperature of each cabinet was controlled by a Danfoss thermostatic controller. Each vessel was surrounded by four symmetrically placed vertical 60 cm 20 watt daylight fluorescent tubes. Combined with silver reflective foil on the cabinet sides, this gave illumination within the vessel of 2.6 watts m^{-2}.

Fig. 4.1 Diagram of single headspace apparatus used for collection of watercress volatiles.



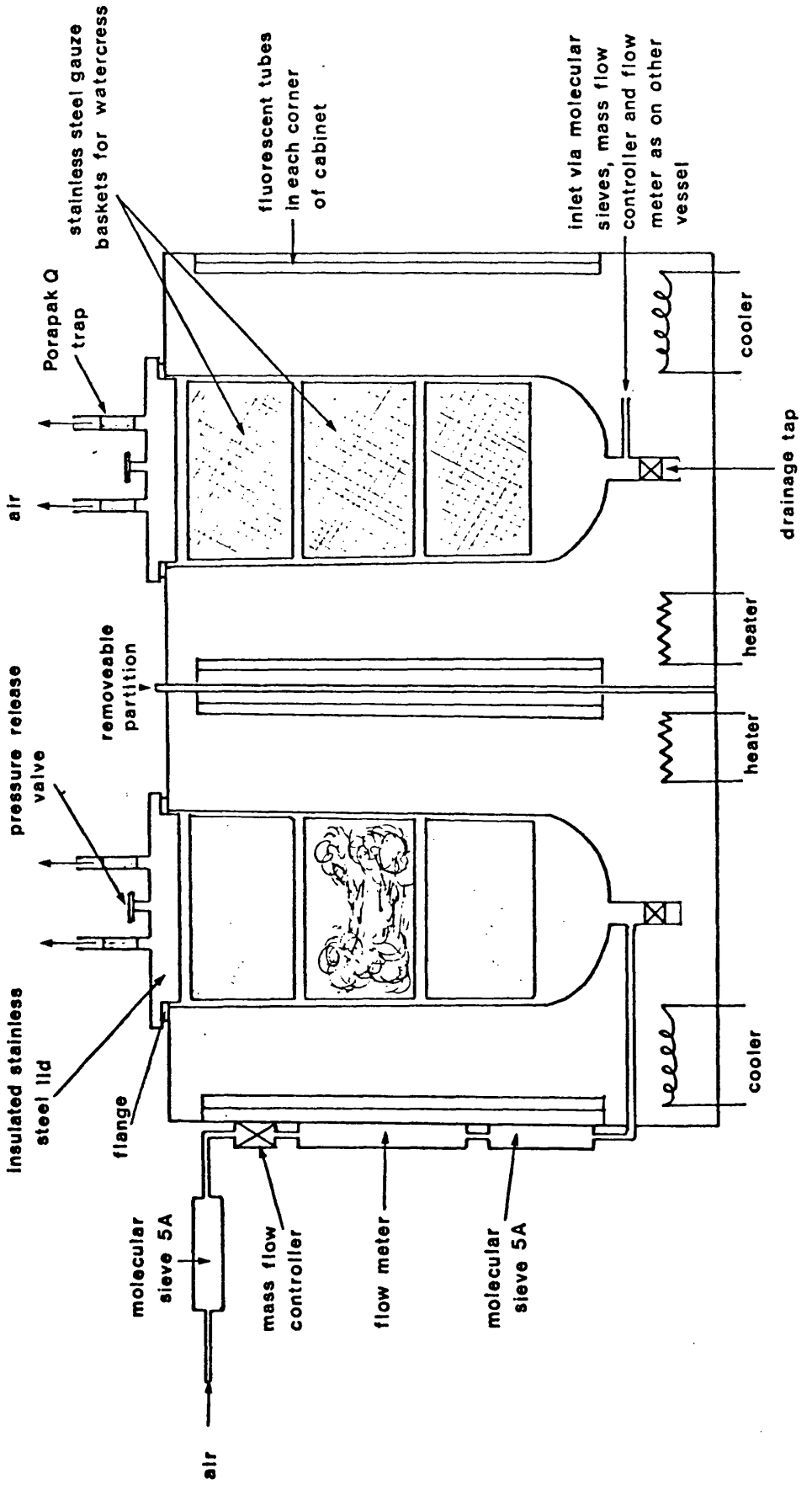


Fig. 4.2 Diagram of dual headspace apparatus used for collection of watercress volatiles.

Plate 4.1 Dual headspace apparatus

a) Front view of apparatus showing :

1 - Porapak Q traps

2 - fluorescent tubes

3 - glass vessels with stainless steel gauze baskets

4 - controlled environment chamber

5 - air filtration and metering unit

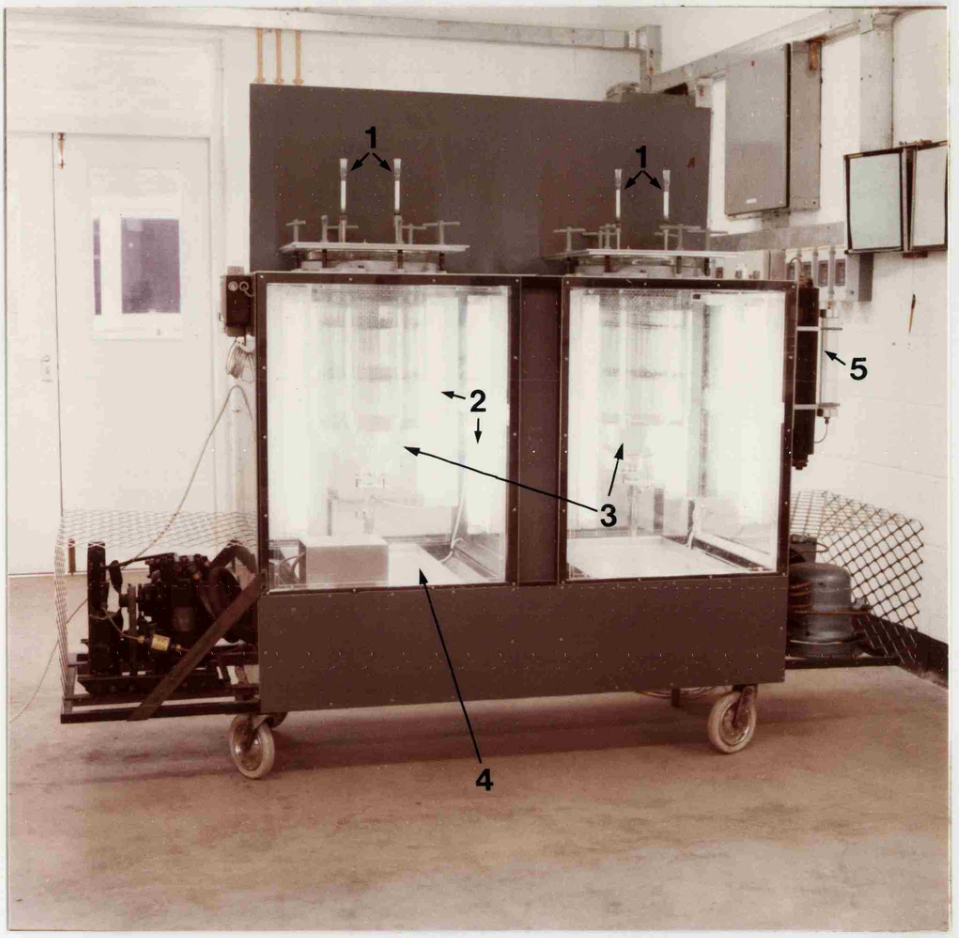
b) Close-up view of one glass vessel showing :

1 - stainless steel gauze basket

2 - drainage tap

3 - inlet pipe for air

a)



b)

The following experiments were carried out :

1. Light and dark storage at 5^{o}, 10^{o} and 15^{o}C.
2. Dark storage at 10^{o}C with one vessel containing watercress rinsed in glass distilled water with 5 mg l^{-1} 6-benzyl amino purine and the other containing watercress rinsed only in glass distilled water.
3. Dark storage at 10^{o}C with one vessel containing Florida, the other green watercress.

The watercress samples were assessed at the beginning and end of each experiment using the profile sheet developed in Section 3.

Solvent Elution of Adsorbed Volatiles

The volatiles were eluted from the Porapak Q in the single headspace apparatus after 2 and 7 days trapping, and from the dual headspace apparatus after 2, 5 and 7 days trapping, with 10 ml diethyl ether per tube. The eluate was concentrated by evaporation in an atmosphere of nitrogen (4.3.2), sealed in ampoules and stored at -20^{o}C until required.

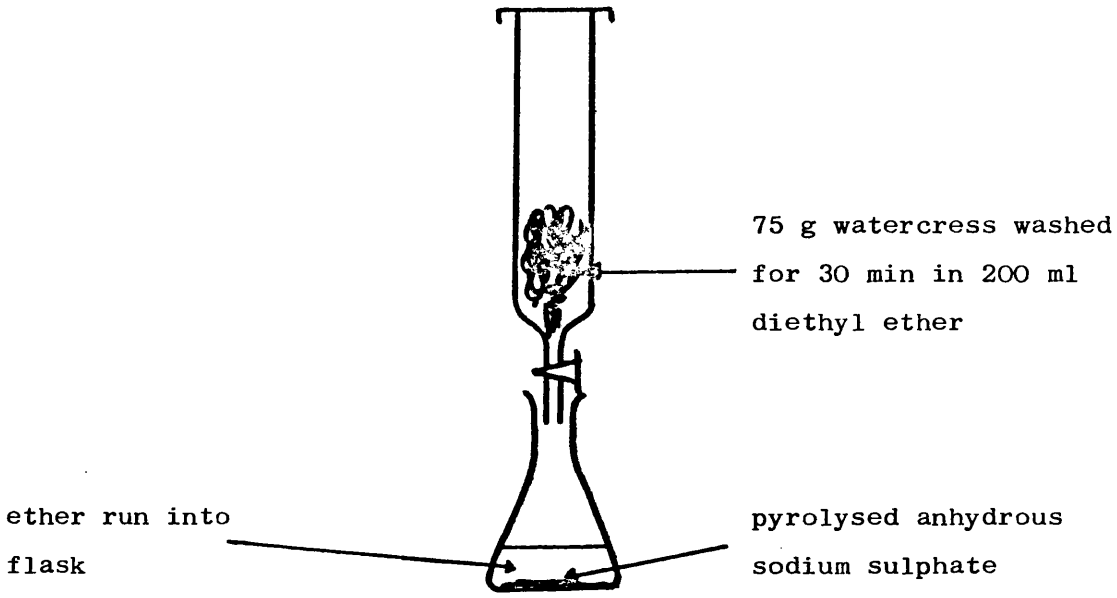
b) Ether Washing

Watercress (75 g), washed in glass distilled water, was placed in a glass funnel (70 x 8 cm diameter) with 200 ml diethyl ether and agitated gently for 30 min to ensure complete immersion of the watercress in the solvent. The ether was then run into a 250 ml conical flask containing 20 - 25 g sodium sulphate (drying agent) and stored at -20^{o}C overnight (Fig. 4.3).

Sublimation of Ether Washings

Volatile compounds were removed from the diethyl ether by vacuum sublimation. The ether washings were placed in a 1 litre round-

Fig. 4.3 Flow diagram illustrating the procedure for extracting and identifying volatile compounds after ether washing.



overnight storage at -20°C

vacuum sublimation

non-volatile compounds

volatile compounds

wax analysis

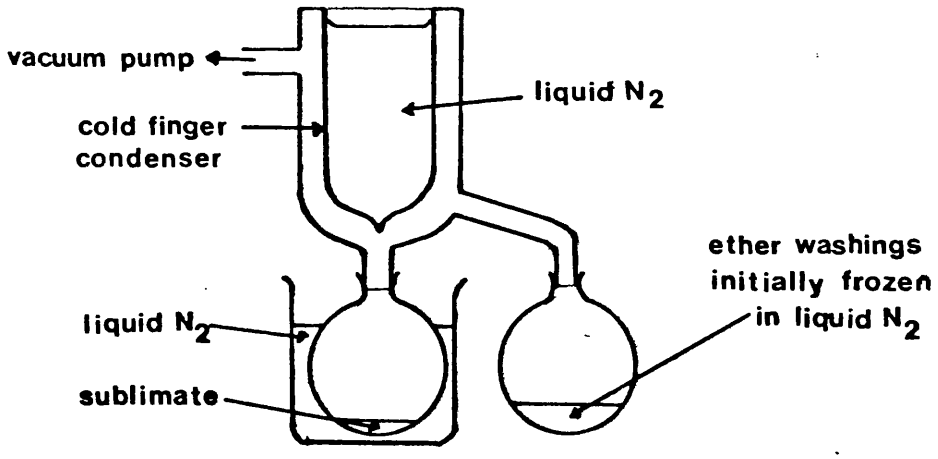
fractionation to remove excess solvent

concentration of sample by evaporation in an atmosphere of nitrogen

analysis by gas chromatography

bottomed flask and frozen in liquid nitrogen before attachment to the apparatus (Fig. 4.4). The volatiles were condensed under vacuum (0.01 mm Hg) on the cold finger and collected in a second round-bottomed flask before concentration by fractionation on a 40 x 1.5 cm diameter column packed with Fenske helices, evaporation in an atmosphere of nitrogen (4.3.2) and storage in an ampoule at -20°C. The non-volatile waxes and lipophilic compounds remaining in the first flask were dissolved in diethyl ether, transferred to an ampoule and stored at -20°C for subsequent use in the epicuticular wax investigation (Section 5).

Fig. 4.4 Diagram of the sublimation apparatus used for the ether washings of watercress

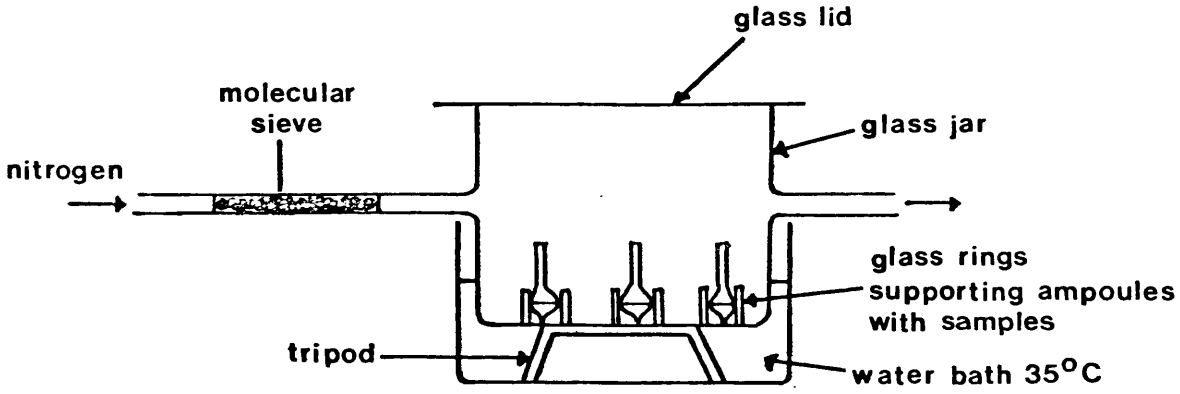


4.3.2 Concentration of Samples

Samples were concentrated by evaporation in a nitrogen atmosphere (Fig. 4.5) to minimise possible oxidative changes. The glass lid was sealed with Apiezon L grease and nitrogen passed through molecular sieve (BDH type 5A) to remove trace impurities before

entering the glass jar.

Fig. 4.5 Diagram of apparatus for concentrating samples of volatiles



4.3.3 Gas Chromatographic and Mass Spectrometric Analyses

Column : (length x internal diameter)

i) 56 m x 0.5 mm glass, SCOT column coated with Carbowax 20M.

Instruments :

A. Hewlett-Packard 5710 gas chromatograph (g.c.) fitted with a flame ionisation detector (F.I.D.).

Temperature programme	:	65°C for 2 min, then 2° min^{-1} to 180°C
Carrier gas	:	nitrogen (oxygen-free)
Flow	:	5 ml min^{-1}
Detector temperature	:	250°C
Injection port temperature	:	250°C

B. Finnigan 4000 coupled gas chromatograph - mass spectrometer (g.c.-m.s.) with a 2100 data system operated at 50 eV; scanning masses 33 - 400 in 2 s cycles.

	Temperature programme	: $65^{o}C$ for 2 min, then 2^{o} min^{-1} to $180^{o}C$
Electron Impact (E.I.)	Carrier gas	: helium (4.67 kg cm^{-2} at cylinder)
	Flow	: 2.0 ml min^{-1}
Chemical Ionisation (C.I.)	Carrier gas	: methane (2.67 kg cm^{-2} at cylinder)
	Flow	: 20 ml min^{-1}
	Source temperature	: $250^{o}C$
	Injector temperature	: $225^{o}C$
	Separator temperature	: $225^{o}C$

All samples were run on both instruments using column i).

4.4 Results and Discussion

Collection of Volatiles by Ether Washing

The concentrates from ether washings of several samples of watercress were bulked and examined by g.c. and g.c.-m.s. (Fig. 4.6 i). The same numbering system was used for the wax (Section 5, Fig. 5.5) and single headspace samples (Fig. 4.7) for easy comparison.

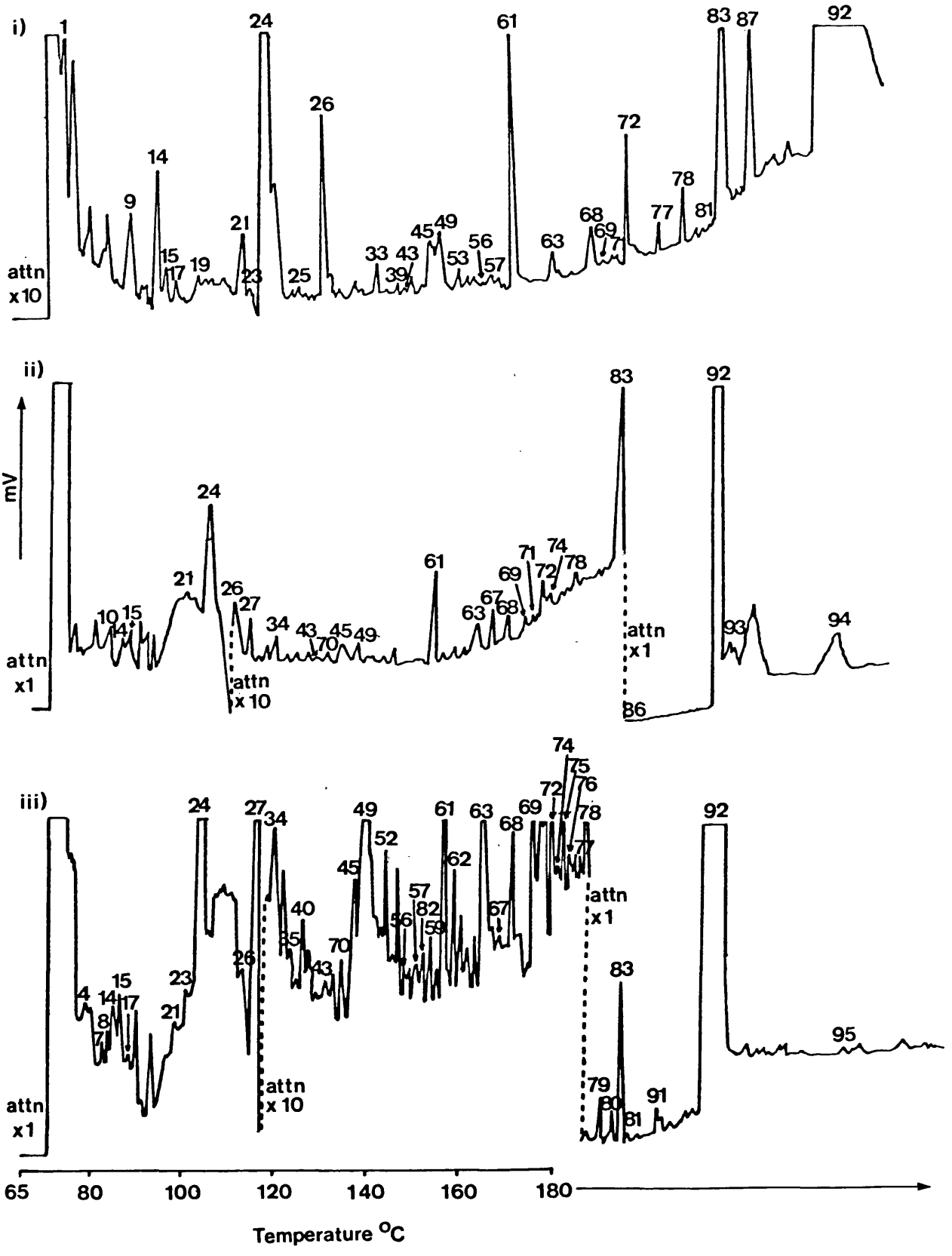
The removal of epicuticular wax by washing in ether resulted in tissue damage and glucosinolate degradation. Most of the non-volatile compounds which comprise the wax layer were then removed by sublimation. However, several compounds also detected in the wax (Section 5) were found in the ether extract; namely, benzaldehyde (peak 26), naphthalene (56), phenyl methanol (68),

Peak identifications : -

1 3-penten-2-one
4 Ethyl benzene
7 Propyl benzene
8 1-ethyl-2-methyl benzene
9 Tetrahydro-2-methyl furan
10 Ethyl acetate
14 Vinyl benzene
15 Acetic anhydride
17 Methyl isothiocyanate
19 3-hydroxy-2-butanone
21 4-hydroxy-4-methyl-2-pentanone
23 1,4-dichlorobenzene
24 Acetic acid
25 Benzofuran
26 Benzaldehyde
27 Unknown, base peak m/e 43, possible M^+ 180
33 2-hydroxy-2-methyl propanoic acid
34 2-methyl propanoic acid
35 1,2-ethanediol diacetate
39 Benzonitrile
40 Pentadecane
43 Butanoic acid
45 Unknown, aromatic, base peak m/e 91
49 1,2-ethanediol monoacetate
52 Unknown, base peak m/e 83
53 5-methyl-3-hexanol
56 Naphthalene
57 Pentanoic acid
59 2-hydroxy-methyl benzoate
61 Possible aromatic aldehyde M^+ 148 (2%), base peak m/e 91 (100%)
62 Octadecane
63 Hexanoic acid
67 α-ionone
68 Phenyl methanol
69 2-methyl butyl propanoate
70 Possible isothiocyanate, M^+ 157 (3%), base peak m/e 43 (100%)*2
71 Phenethyl alcohol
72 Phenethyl nitrile
73 β-ionone
74 Benzothiazole
75 Unknown, base peak m/e 43 possible M^+ 135
76 Heptanoic acid
77 Dimethyl sulphone
78 Phenol
79 Unknown, base peak m/e 43
80 Unknown, base peak m/e 99
81 Octanoic acid
82 Possible isothiocyanate, M^+ 171 base peak m/e 43 *2
83 Phenethyl nitrile
86 Benzyl isothiocyanate
87 Ethyl tetradecanoate
91 Eicosane
92 Phenethyl isothiocyanate
93 Hexadecanoic acid
94 Tetradecanal
95 Diethyl-1,2-benzene dicarboxylate

*2 homologous series increasing by CH_2

Fig. 4.6 Chromatograms of samples from ether washing watercress, i) bulked sample of several washings, ii) young watercress, iii) old watercress. Column: carbowax SCOT capillary. Attenuation (attn) given on chromatograms.



phenethyl alcohol (71), phenol (78), phenethyl nitrile (72, 83) and phenethyl isothiocyanate (92). These are probably glucosinolate degradation products which are both trapped in the wax and present within the plant. Benzyl isothiocyanate, detected in the wax, was not found in the ether extract, but its corresponding nitrile, benzonitrile (39), was only present in the ether extract. The nitrile of phenethyl isothiocyanate, phenethyl nitrile, was identified in the ether extract as two major peaks (72 and 83) and methyl isothiocyanate (17) was also found. These nitriles and isothiocyanates are major contributors to the characteristic flavour and aroma of crushed watercress. They are present in ether washings but not in headspace collections, except for a small amount of the major isothiocyanate (phenethyl), because the tissue is damaged.

Dimethyl sulphone (77) has not been reported in other vegetables reviewed by Johnson et al (1971 a,b) or by Van Straten (1977), but it has been isolated from pasteurised milk, butter and butter oil (Shankaranarayana et al, 1974) and is thought to be an odourless oxidation product of dimethyl sulphide. Dimethyl sulphide has been identified in several brassicas (MacLeod and MacLeod, 1970 a,b,c) and it is possible that it is oxidised to dimethyl sulphoxide, (identified in headspace collection samples, Fig. 4.7), which is further oxidised to the corresponding sulphone.

dimethyl sulphide ⟶ dimethyl sulphoxide ⟶ dimethyl sulphone

In support of this, Ralls and coworkers (1965) have demonstrated the formation of dimethyl sulphoxide from dimethyl sulphide in commercial samples of blanched peas. The enzymic formation of oxidising agents and their interaction with precursors to yield flavour components,

have been studied in several foodstuffs (Richardson, 1976). Lipoxygenase, a ubiquitous enzyme in plants, forms hydroperoxides which are capable of oxidising susceptible food constituents to yield flavour compounds. Therefore, it is suggested that the dimethyl sulphone, detected in ether washing and headspace collection samples, and dimethyl sulphoxide, identified in headspace samples, are products of oxidation occurring in watercress.

In species of Allium the flavour precursors have been identified as amino acid sulphoxide derivatives. Enzymic degradation of the sulphoxide results in formation of disulphides, which are responsible for the characteristic odour and flavour of Alliums (Eskin, 1979). Other flavour compounds have also been linked to the enzymic conversion of precursors, for example, L-phenylalanine serves as a precursor for β-phenyl ethanol and phenolic ethers in bananas, and L-valine is converted into 2-methyl-1-propanol, 2-methyl propyl acetate, 2-methyl propanoic acid and 2-keto isovaleric acid (Tressl and Drawert, 1973). In many unripe fruits short chain fatty acids such as acetic, butyric, hexanoic and decanoic are converted to a wide variety of aromatic esters, alcohols and acids during ripening. As a plant matures, a similar conversion might occur and explain the detection of six acids in the ether washing sample of watercress (acetic peak 24, 2-hydroxy-2-methyl propanoic 33, butanoic 43, pentanoic 57, hexanoic 63, octanoic 81). As many acids are also intermediates in plant metabolism, it is difficult to know the exact source of these compounds. The reason for the large amounts of acetic acid in both ether washing and headspace samples is unknown. The possibility of plant microflora producing acetic acid was investigated and described in Section 4.6.

The benzene derivatives found in the headspace samples (Fig. 4.7, ethyl benzene 4, propyl benzene 7, vinyl benzene 14, 1,2,4-trimethyl benzene 16) were initially assumed to be the breakdown products of Porapak Q since artefacts are readily produced, especially on heating the polymer (Williams and Lewis, 1978). However, as many of these compounds were also identified in wax and ether washing samples where no Porapak Q was used in the collection of the volatiles, and also because the Porapak Q in the headspace apparatus was not heated at all, these compounds may be genuine volatiles of watercress arising from lipids (Forss, 1972). These compounds have also been identified in other foodstuffs (Johnson, Nursten and Self, 1969) and although in several cases they were contaminants, in many they were not. Degradation of hydroperoxides and β-oxidation may account for the presence of the ketones and esters detected, but obviously it is possible for a compound to be formed by several pathways.

The chromatograms of ether washing samples from young and old cress (Fig. 4.6 ii and iii) show an increase in the quantities of compounds as the watercress ages. Dimethyl sulphone, several hydrocarbons (pentadecane, naphthalene, octadecane, eicosane and an unknown) and benzenes (ethyl benzene, propyl benzene and 1-ethyl-2-methyl benzene) were only present in the old watercress sample. Further work is necessary to determine those new compounds produced as watercress ages and those which only increase in quantity.

In the sample from old and young watercress, α-ionone (67) may be responsible for the floral odour observed as watercress deteriorates (Section 3) because its odour is described as "warm-woody, balsamic, floral, violet-like" by Arctander (1969). This compound is reported to be present in carrot oil (Alabran, Moskowitz and Mabrouk, 1975)

and celery oil (Wilson, 1970). It has been suggested by Stevens (1970b) that α and β-ionones are formed by the oxidation of polyene-carotenes. Both α and β-carotenes are precursors for vitamin A and watercress is reported to contain c. 300 μg carotene per 100 g tissue (Paul and Southgate, 1978). It therefore seems feasible that the ionones could be formed by the oxidation of the polyene-carotenes in watercress.

The results from Florida watercress were similar to those from the old watercress sample, both in the compounds identified and quantities detected (Fig. 4.6 iii). Ether washings from the flowers of watercress also gave similar results, but the sample was much less concentrated. Several compounds present in the flower wax sample (Section 5), including possible thiocyanates and sulphides, were found in the ether extracts of flowers. However, many more isothiocyanates and possible thiocyanates and sulphides were identified in the wax samples than in ether washing samples, suggesting that these compounds are produced in the cells, released, and then trapped in the wax. A freeze fractured cross section of a watercress leaf (Plate 4.2) shows numerous air spaces between cells in the loosely structured palisade and mesophyll layers. Volatiles synthesized in cells and released can diffuse into the intercellular water vapour and flow along transpiration gradients to accumulate at the wax layer where they are trapped.

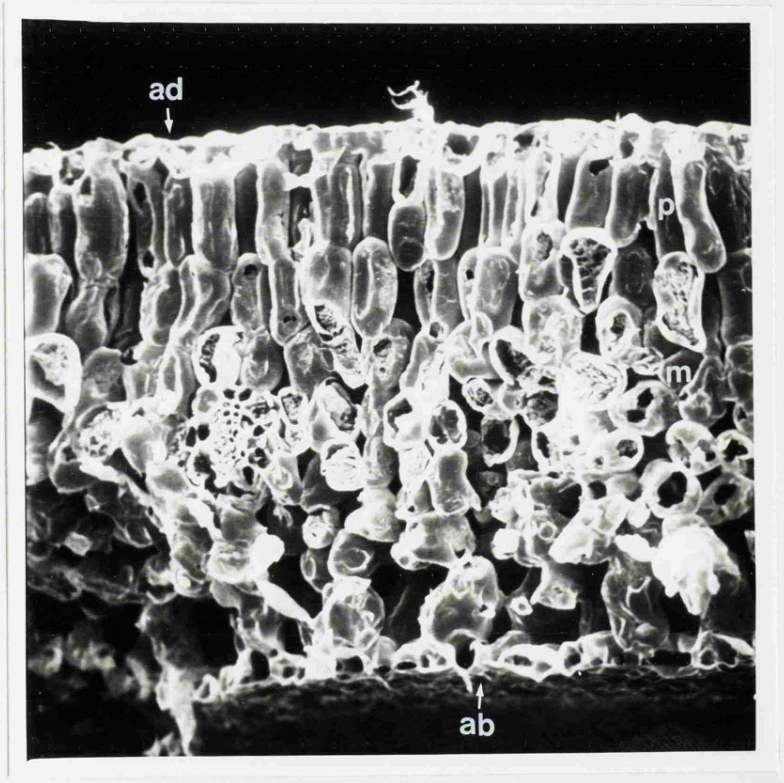
Collection of Volatiles using Single Headspace Apparatus

The chromatograms of samples obtained from the single headspace apparatus are given in Fig. 4.7. Samples were collected after 2 and 7 days trapping (Fig. 4.7 i and ii). Fewer compounds were found in the headspace samples because the plant tissue was not damaged as with the ether washing. Phenethyl isothiocyanate (92) was only a

Plate 4.2

Cross section of watercress leaf obtained by freeze-fracture - leaf plunged in liquid nitrogen, snapped into small pieces, freeze-dried for 6h, mounted on stubs for gold coating and viewed with a Cambridge S4 stereoscan microscope.

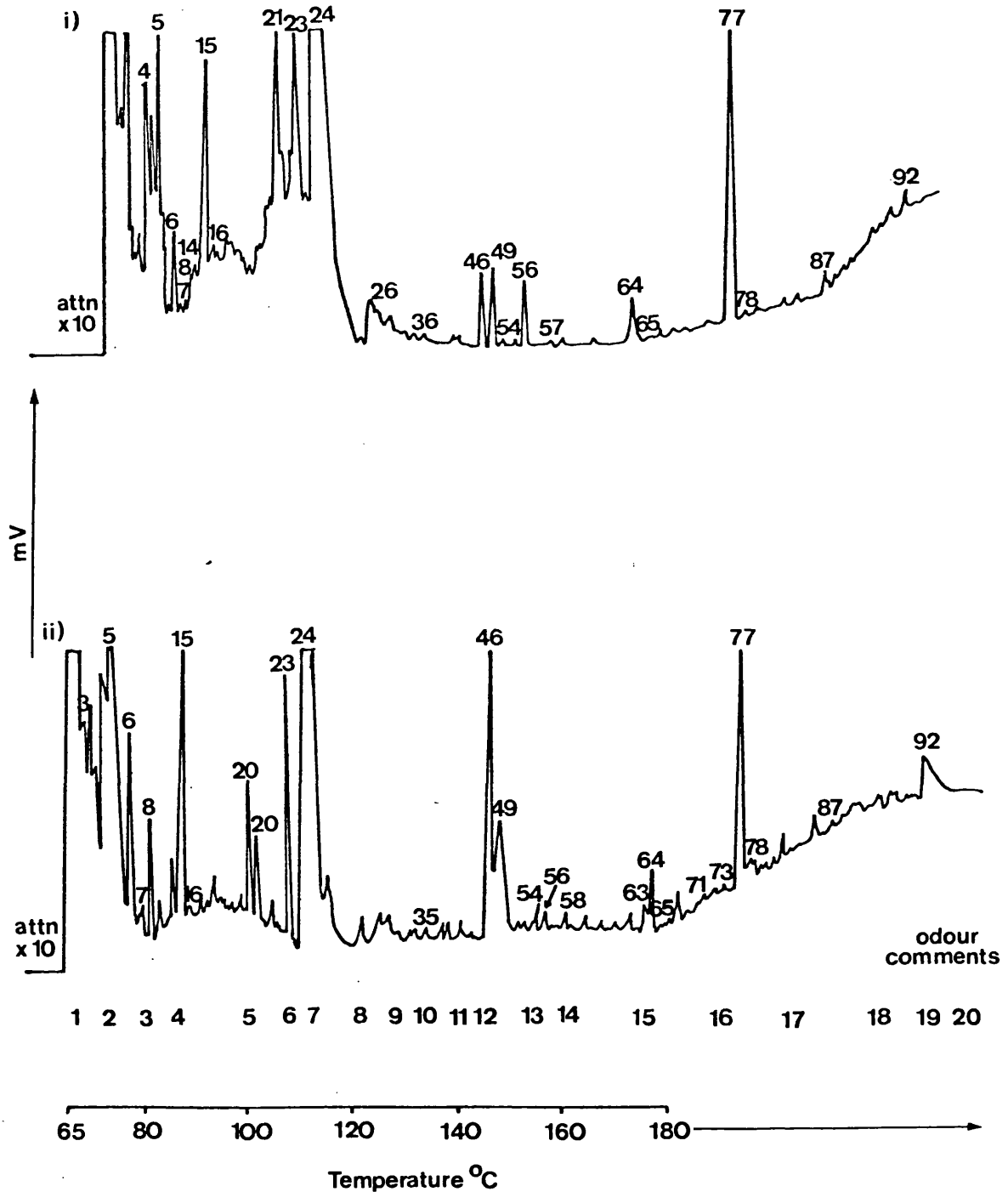
ad = adaxial epidermis
ab = abaxial epidermis
p = palisade layer
m = mesophyll layer



Peak identifications : -

3 Dimethyl disulphide
4 Ethyl benzene
5 1,4-dimethyl benzene
6 1,3-dimethyl benzene
7 Propyl benzene
8 1-ethyl-2-methyl benzene
14 Vinyl benzene
15 Acetic anhydride
16 1,2,4-trimethyl benzene
20 Aliphatic chain (approx. C_6) with large side chain containing oxygen - isomers, M^+ 184 (0.4%), base peak m/e 101 (100%)
21 4-hydroxy-4-methyl-2-pentanone
23 1,4-dichlorobenzene
24 Acetic acid
26 Benzaldehyde
35 1,2-ethanediol diacetate
36 Vinyl acetate
46 Dimethyl sulphoxide
49 1,2-ethanediol monoacetate
54 2,5-dimethyl benzaldehyde
56 Naphthalene
57 Pentanoic acid
58 Ethyl benzaldehyde
63 Hexanoic acid
64 2,4-dimethyl acetophenone
65 2-methyl naphthalene
71 Phenethyl alcohol
73 β-ionone
77 Dimethyl sulphone
78 Phenol
87 Ethyl tetradecanoate
92 Phenethyl isothiocyanate

Fig. 4.7 Chromatograms of samples from single headspace apparatus after 2 (i) and 7 (ii) days trapping. Column: carbowax SCOT capillary. Attenuation (attn) given on chromatograms.



small peak on the chromatograms due to the breakdown of the glucosinolate at the cut ends of the stalks. The amount increased after 7 days trapping as tissue breakdown occurred.

Deterioration of the plant causes an accumulation of sulphur compounds from glucosinolate degradation. Dimethyl disulphide (3) was detected after 7 days trapping, but not after 2 days. Dimethyl sulphoxide (46) increased over the 7 days, while dimethyl sulphone (77) decreased slightly; possibly because the production of sulphoxide exceeded its rate of oxidation to sulphone. Dimethyl disulphide is important in the formation of the typical cabbage flavour and results from the breakdown of 5-methyl-L-cysteine sulphoxide (Dateo et al, 1957). A similar reaction may cause its production in watercress as the plant deteriorates. β-ionone was identified as a small peak (73) after 7, but not 2 days trapping and it is thought that this compound, together with the α-ionone found in ether washing samples, is formed by the oxidation of polyene-carotenes.

The results of an experiment when volatiles were collected after 1, 4 and 7 days trapping show a similar pattern of differences in compounds as after 2 and 7 days trapping, i.e. an increase in dimethyl sulphoxide and phenethyl isothiocyanate, a decrease in dimethyl sulphone, and the appearance of dimethyl disulphide after 7 days trapping.

Odour assessment of the 7 day trapping sample was carried out by sniffing the effluent from the gas chromatograph, with the flame extinguished, and marking the places where different aromas were detected on chart paper for later comparison with a chromatogram of the same sample. The odour comments are numbered at the bottom of the chromatogram (Fig. 4.7 ii) where the odours were detected during

the run. Table 4.1 shows which compounds identified on the chromatogram co-incide with the odour assessments, and it gives descriptions of characteristic odours associated with these compounds (Arctander, 1969).

Many of the compounds identified are associated with the odours detected. Dimethyl disulphide has a characteristic onion-like odour and its production during deterioration is important in contributing to the rotting, seaweed odours noted in stale watercress. Acetic acid was easily identified and detected by its characteristic odour. 1,2-ethanediol diacetate probably contributed to the odour detected at 10, and the compounds identified in the regions of 13, 14, 15 and 16, have odours which agree with those from the effluent. Although there was a time-lag before the odours appeared, ethyl tetradecanoate and phenethyl isothiocyanate were responsible for odours 19 and 20. Phenethyl isothiocyanate may have masked other odours at the end of the chromatogram because it was also detected at 18, and it possibly provided the spicy note in 19.

The fruity odour detected at 2 was probably not due to 1,4-dimethyl benzene, but to ethyl benzene, which was identified in the 2 day trapping sample in this area of the chromatogram. The benzene compounds possess a gassy, kerosene type odour and they were identified in the region with an odour described as waxy and greasy (3). In the region where odours 4 and 5 were detected no compounds were identified which could account for these aromas. In the wax samples (Section 5), heptanal and nonanal were found in this region and they may contribute to the odours detected. For odours 8 and 9 benzaldehyde has been identified in this region from the 2 day trapping sample, 2-ethyl hexanol from 1, 4 and 7 day trapping samples

Table 4.1

Odour comments from 7 day trapping sample with compounds identified in region of odour detection and their descriptions (Arctander, 1969). Bracketed compounds are those identified in other samples with relevant odours.

	Odour Comment	Compounds identified in region of odour detection	Odours of compounds
1.	Ether + cabbage	Ether (solvent) Dimethyl disulphide	onion-like, cabbage
2.	Fruity	1,4-dimethyl benzene (Ethyl benzene)	(sweet, gassy, hyacinth-like)
3.	Waxy, greasy	1,3-dimethyl benzene Propyl benzene 1-ethyl-2-methyl benzene	gassy, kerosene
4.	Spicy	Acetic anhydride (Heptanal)	(fruity and fermented fruit in low concentrations)
5.	Fishy, rotting, cabbage	(Nonanal)	(fatty, floral, waxy odour)
6.	Burnt	1,4-dichlorobenzene	
7.	Acetic acid	Acetic acid	pungent, stinging, sour odour
8.	Green	(2-ethyl hexanol) (Benzaldehyde)	(mild, oily, sweet and slightly floral, rose odour) (fresh crushed bitter almonds)
9.	Very faint watercress	(Nonanol)	(oily-floral, fresh and petal-like)
10.	Woody, resin	1,2-ethanediol diacetate	sweet, oily-wine odour
11.	Sweet, syrupy	(Vinyl acetate)	(fruity, ethereal, rather sharp but sweet)
12.	Floral - persistent	(Acetophenone)	(pungent, sweet, flowery, orange-blossom-like, hawthorn-like)
13.	Musty, old	2,5-dimethyl benzaldehyde Naphthalene	sweet, mildly floral pungent, choking, dry, tarry
14.	Sweet, sugary	Ethyl benzaldehyde	sweet, "bitter almond", slightly gassy, floral greenness
15.	Cut grass	Hexanoic acid 2,4-dimethyl acetophenone	"sweat-like", acrid sweet-floral, woody, mimosa-like
16.	Strong primrose smell	β-ionone	warm-woody, "cedar-wood with raspberry undertones"
17.	Spicy, apple-like		
18.	Faint watercress		
19.	Spicy, floral	Ethyl tetradecanoate	oily, ethereal, violet-like
20.	Peppery	Phenethyl isothiocyanate	pungent, rich and earthy-sweet, radish, horseradish-like

(chromatograms not given), and nonanol from the leaf wax sample. Vinyl acetate in the 2 day trapping sample, and acetophenone in the leaf wax sample, were found in the region of odours 11 and 12, and their odour descriptions are characteristic of the aromas detected. The spicy odour (17) may be due to the persistence of odour 16, combined with a slight odour due to phenethyl isothiocyanate (20).

Further study on the odours from the wax samples and identification of more peaks on the chromatograms may provide more information on those compounds responsible for the aromas. Although olfaction is the most primitive of the senses, it is exceptionally sensitive, and its value in chemical assessment must not be underestimated.

Collection of Volatiles using Dual Headspace Apparatus

The dual headspace apparatus was used to monitor changes in the production of watercress volatiles at different temperatures, in light and dark conditions, and with post-harvest treatment of cytokinin, to determine the optimum conditions of storage with the minimum reduction in quality. The chromatograms of the volatiles are shown in Figs. 4.8 - 4.12. The evolution of volatiles by the quantity of watercress used in this investigation resulted in extracts of low concentration. However, identification of major compounds was possible using g.c.-m.s., and most were confirmed with chemical standards listed in 4.2.

Changes in the quantities of sulphur compounds collected probably reflect the rate of deterioration of watercress, but no general pattern was found. This was made more difficult by the exclusion of an internal standard for quantitative assessment. The results of keeping watercress in light and dark at different temperatures

are shown in Figs. 4.8, 4.9 and 4.10. At both 5° and 15°C (Figs. 4.8 and 4.10) light-treated samples contained more dimethyl sulphoxide than samples kept in the dark. The amount of dimethyl sulphoxide collected in the illuminated samples increased from 2 to 5 day trapping and decreased by the 7th day. This increase may be due to the 5th day sample being taken after a three day interval, whereas the 2 and 7 day samples were collected after two day intervals. However, the amount of dimethyl sulphoxide is more than would be expected from an extra day's trapping. The amount of dimethyl sulphone increased in the samples over 7 days at 5°C in the light (Fig. 4.8 i) and 15°C in the dark (Fig. 4.10 ii), but slightly decreased in the 5°C dark and 15°C light treatments. At 10°C very little dimethyl sulphoxide was collected from either the light or dark treatments compared with the other temperatures. The amount of dimethyl sulphone collected from the 10°C dark treatment increased from the 2 day to the 5 day samples, and declined in the 7 day sample to approximately the 2 day sample level. A similar change occurred in 10°C light treatment samples, but the quantity in the 7 day sample was triple that in the 2 day sample.

In all the temperature treatments, except for the 15°C light, the amount of dimethyl sulphoxide was less than that of dimethyl sulphone in the final samples, which may suggest that the rate of oxidation of dimethyl sulphoxide to the sulphone is exceeding the rate of formation of the sulphoxide.

The amount of phenethyl isothiocyanate was very small in all samples and was most likely due to slight glucosinolate breakdown at the cut stalk ends. Naphthalene increased in the 5° and 15°C dark samples (Figs. 4.8 ii and 4.10 ii) and the 10°C light samples (Fig. 4.9 i)

Figs. 4.8 - 4.12 are chromatograms of samples from the dual headspace apparatus after 2 (a), 5 (b) and 7 (c) days trapping. Column : carbowax SCOT capillary. Attenuation : x 10

Fig. 4.8 i - 5°C Light treatment
ii - 5°C Dark treatment

Fig. 4.9 i - 10°C Light treatment
ii - 10°C Dark treatment

Fig. 4.10 i - 15°C Light treatment
ii - 15°C Dark treatment

Fig. 4.11 i - plus cytokinin (10°C Dark) treatment
ii - minus cytokinin (10°C Dark) treatment

Fig. 4.12 i - Green cress (10°C Dark)
ii - Florida cress (10°C Dark)

Peak identification for Figs. 4.8 - 4.12

1 Ethyl acetate
2 Acetic anhydride
3 1,4-dichlorobenzene
4 Acetic acid
5 Benzaldehyde
6 1,2-ethanediol diacetate
7 Dimethyl sulphoxide
8 1,2-ethanediol monoacetate
9 2,5-dimethyl benzaldehyde
10 Naphthalene
11 Ethyl benzaldehyde
12 2,4-dimethyl acetophenone
13 4-ethyl acetophenone
14 2-butoxy-ethanol
15 β-ionone
16 Dimethyl sulphone
17 Phenol
18 Di(isobutyl)1,2-benzene dicarboxylate
19 Ethyl tetradecanoate
20 Tetradecanoic acid
21 Butyl iso butyl 1,2-benzene dicarboxylate
or Di(n-butyl)1,2-benzene dicarboxylate
22 Phenethyl isothiocyanate

Fig. 4.8 i 5°C LIGHT

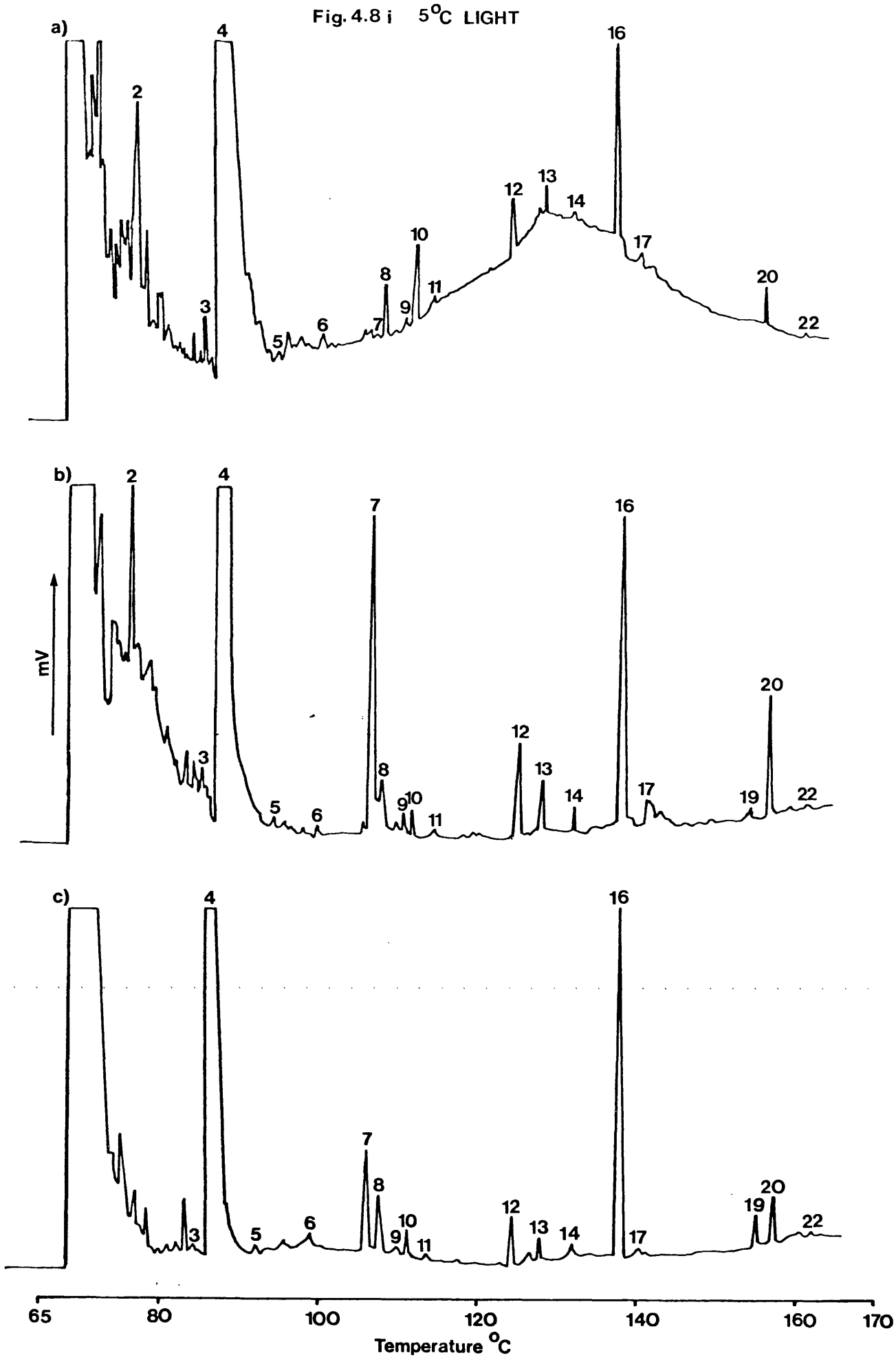


Fig.4.8 ii 5°C DARK

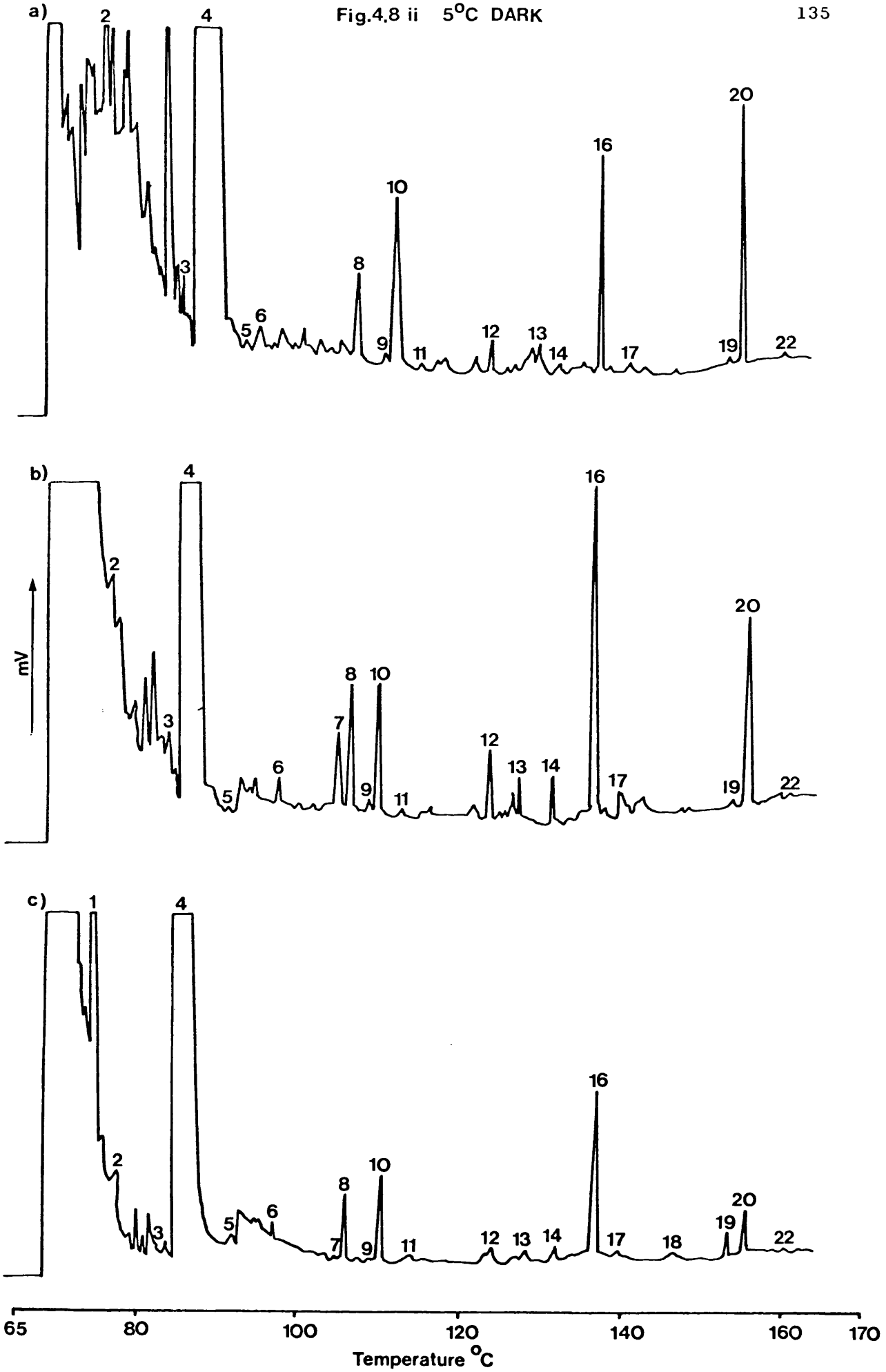


Fig.4.9 i 10°C LIGHT

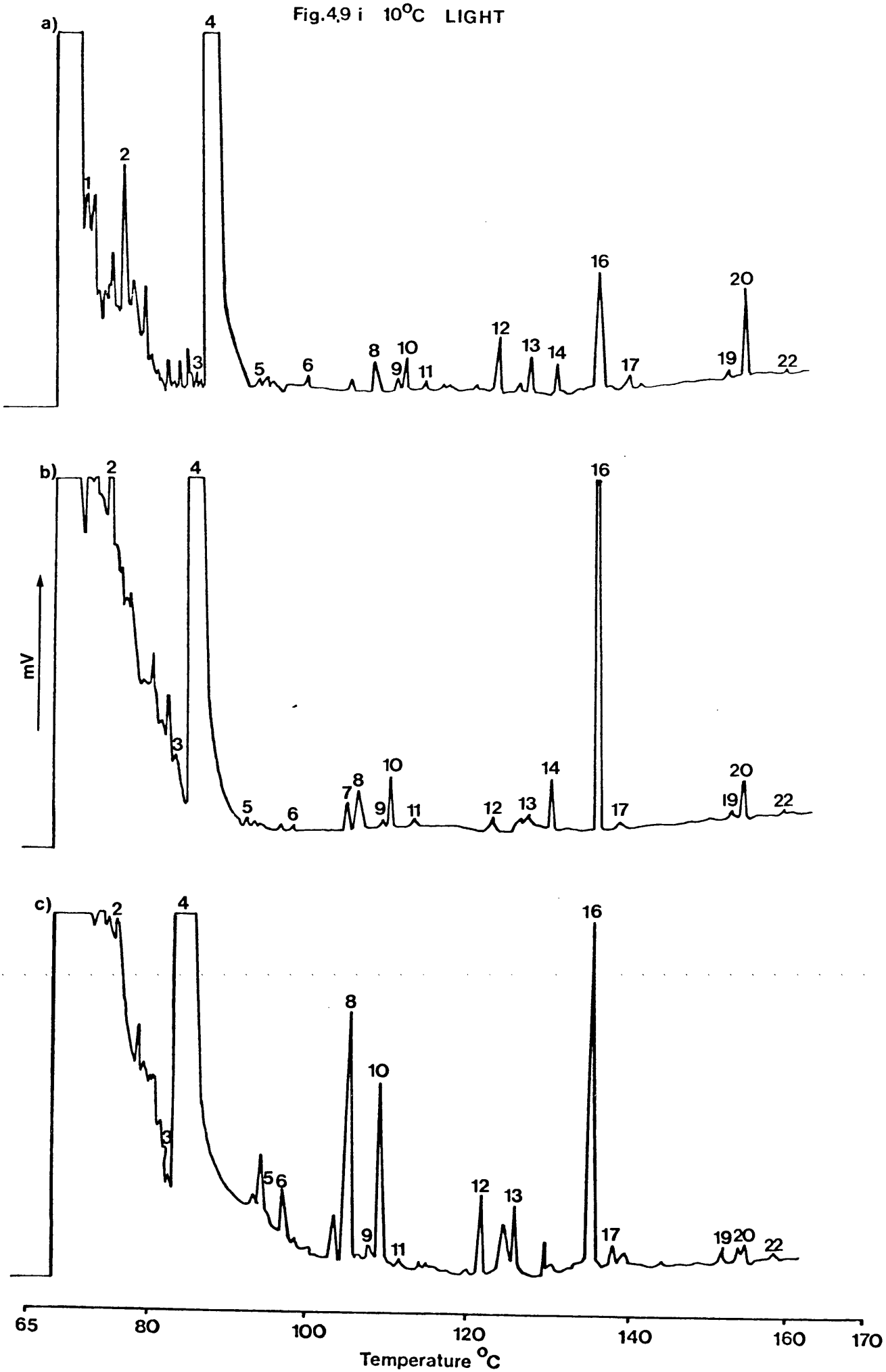


Fig.4.9 ii 10°C DARK

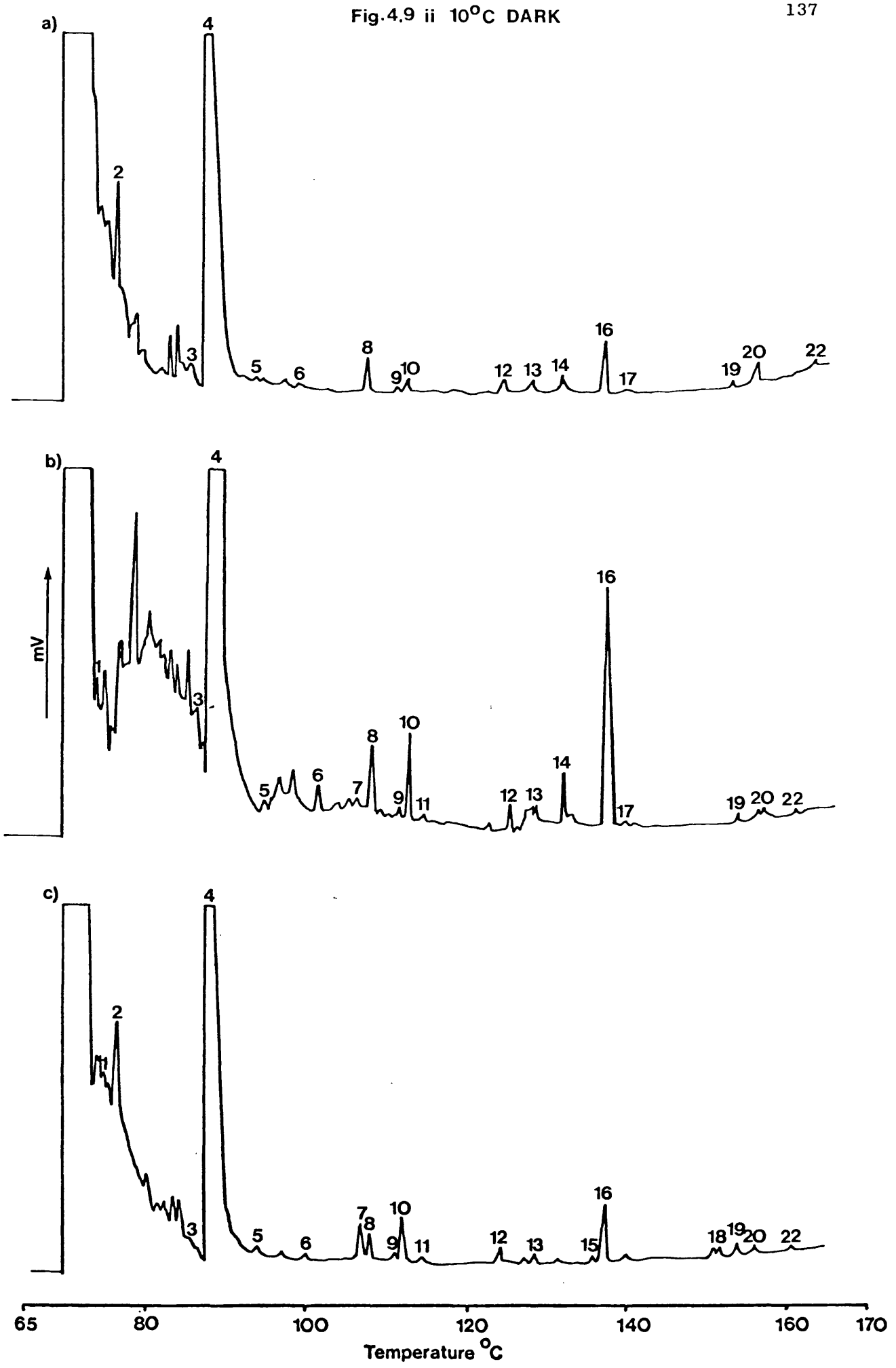


Fig. 4.10 i 15°C LIGHT

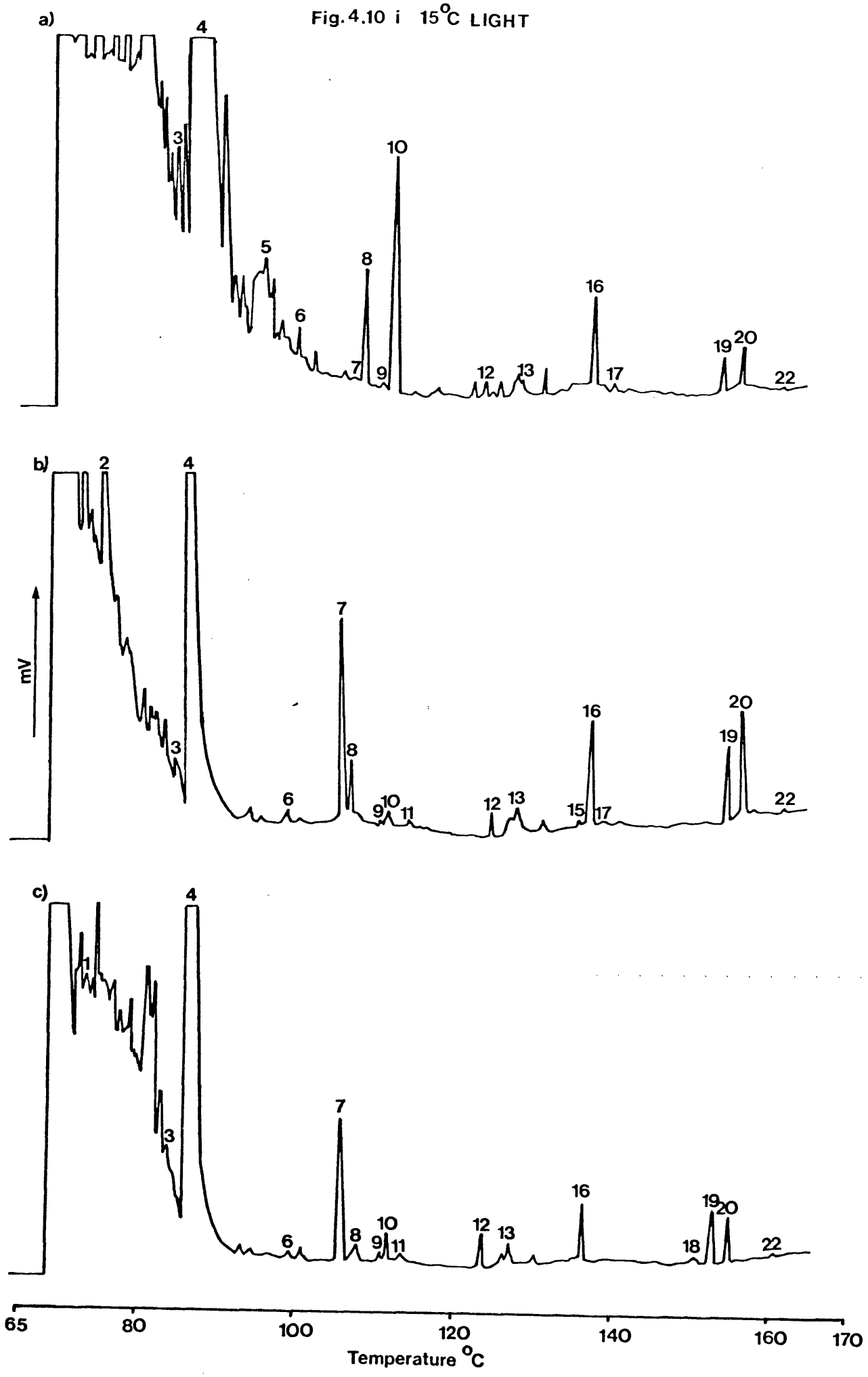


Fig. 4.10 ii 15°C DARK

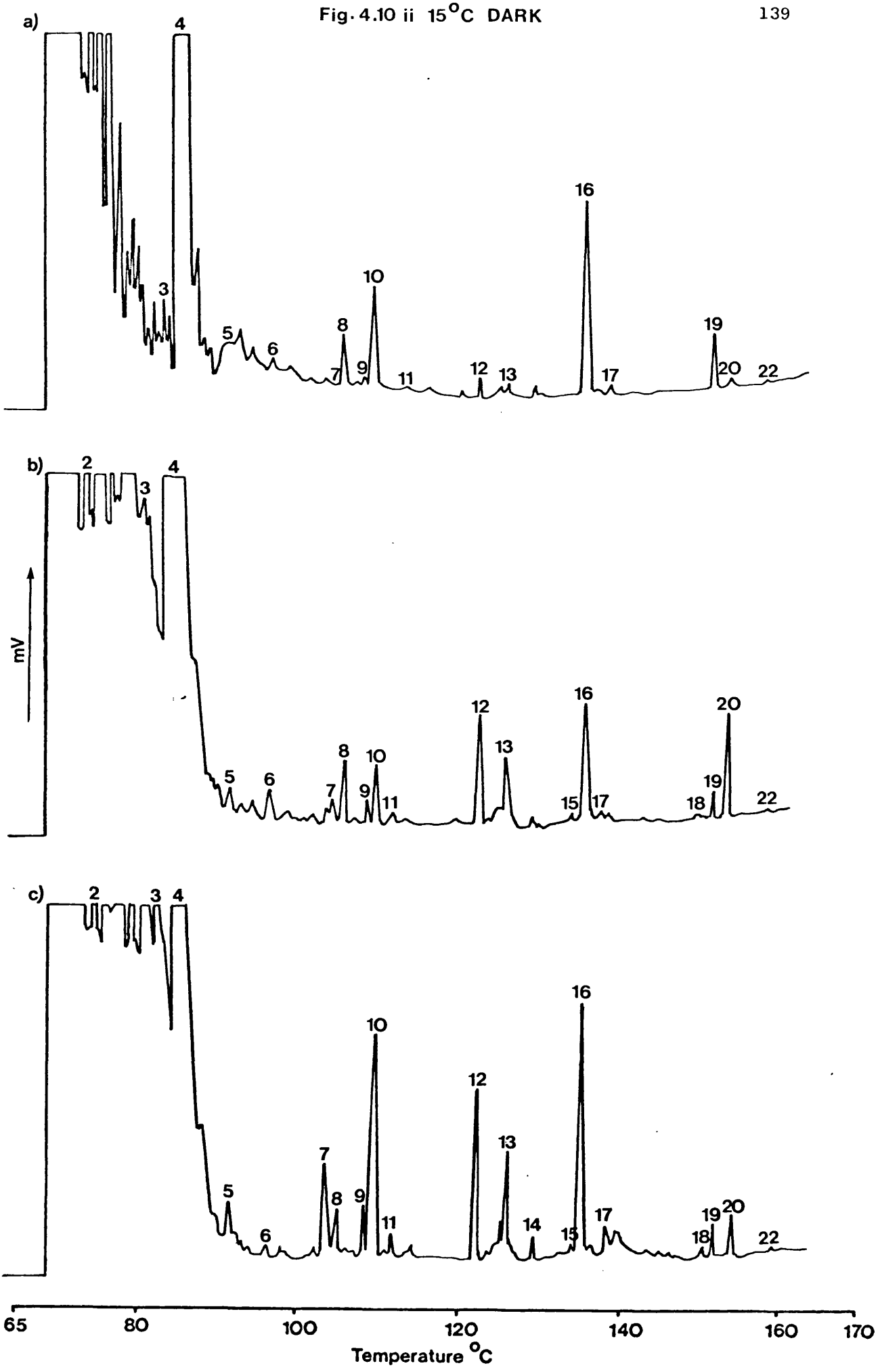


Fig. 4.11 i + CYTOKININ

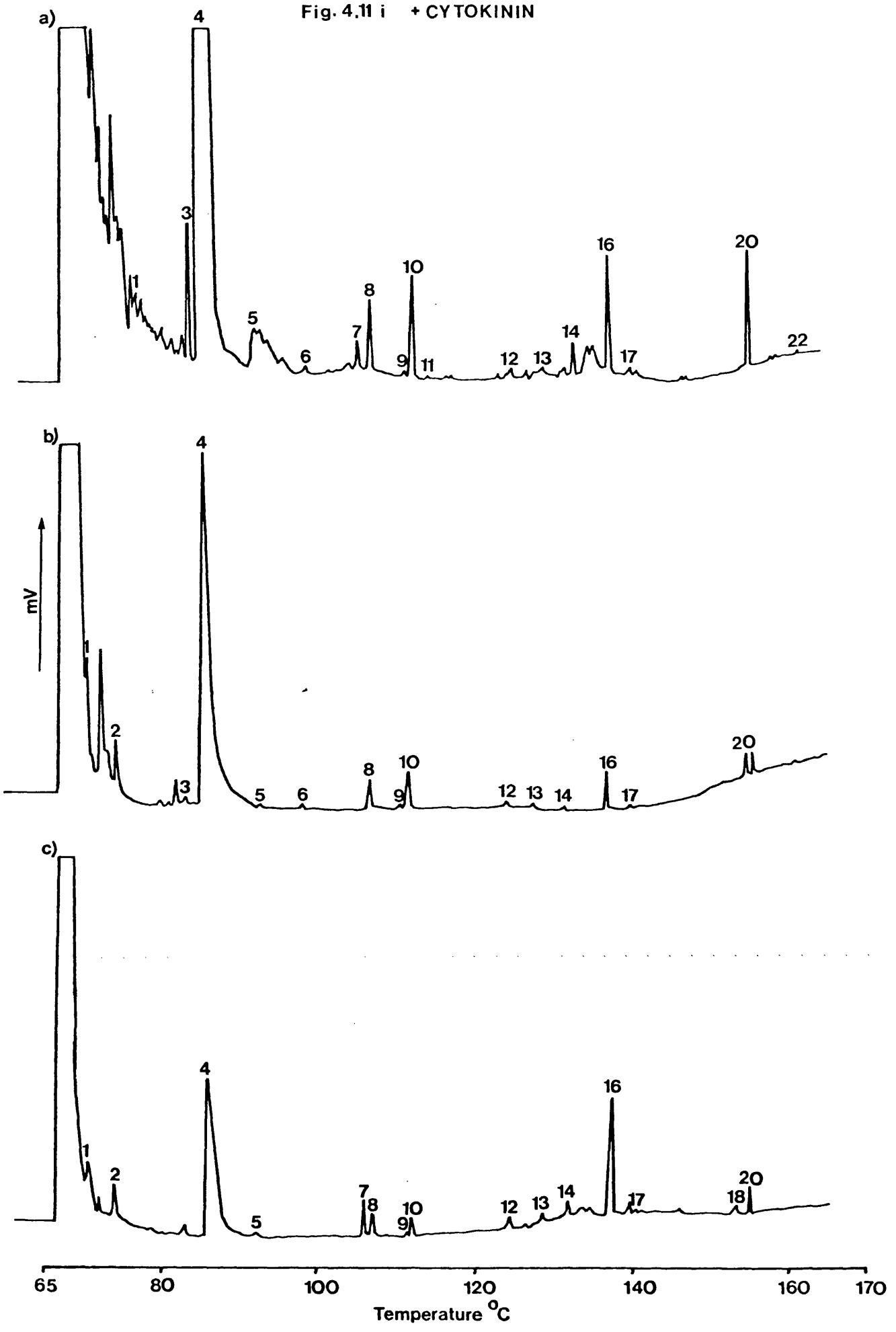


Fig. 4.11 ii –CYTOKININ

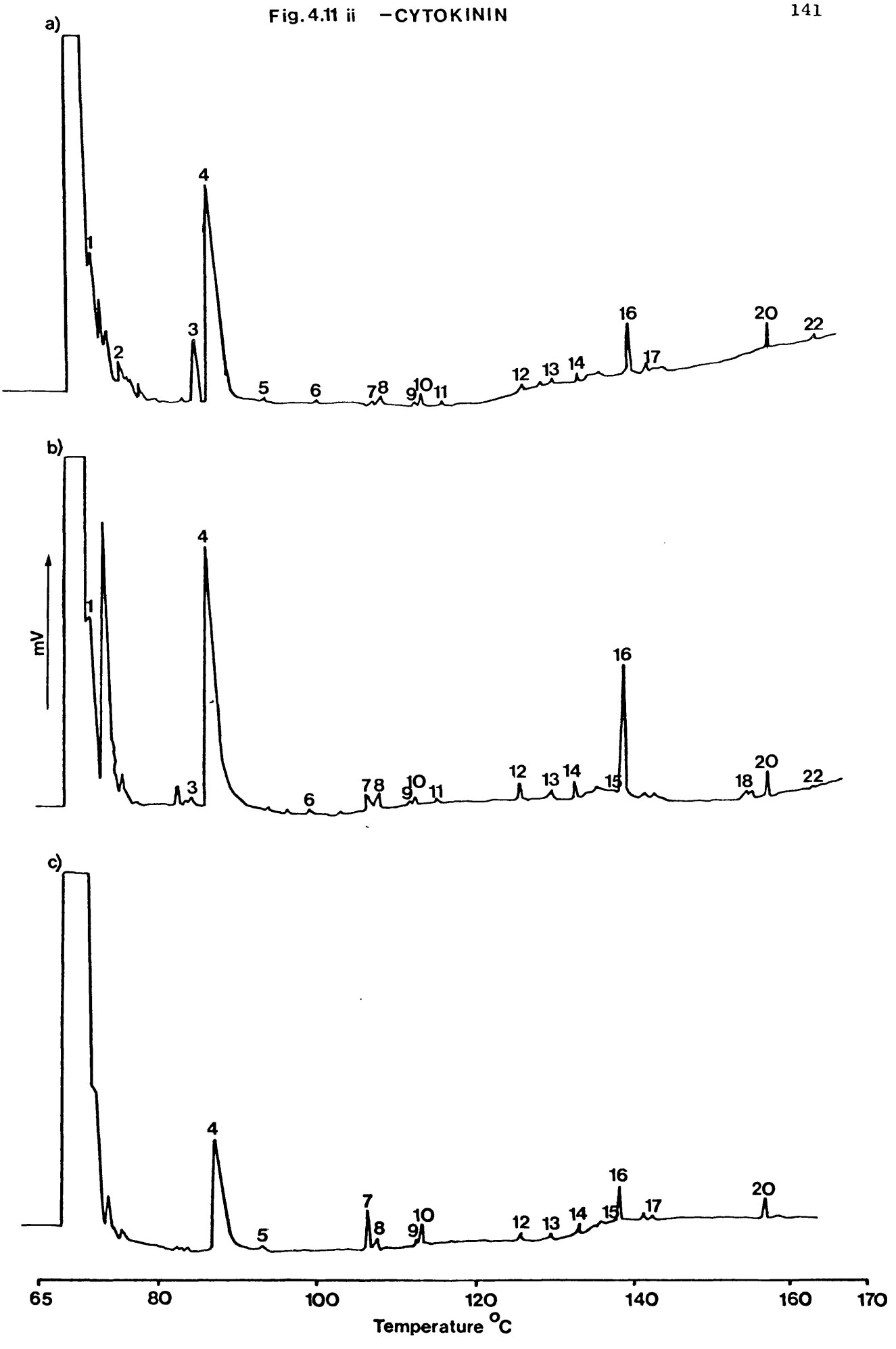


Fig. 4.12 i GREEN CRESS

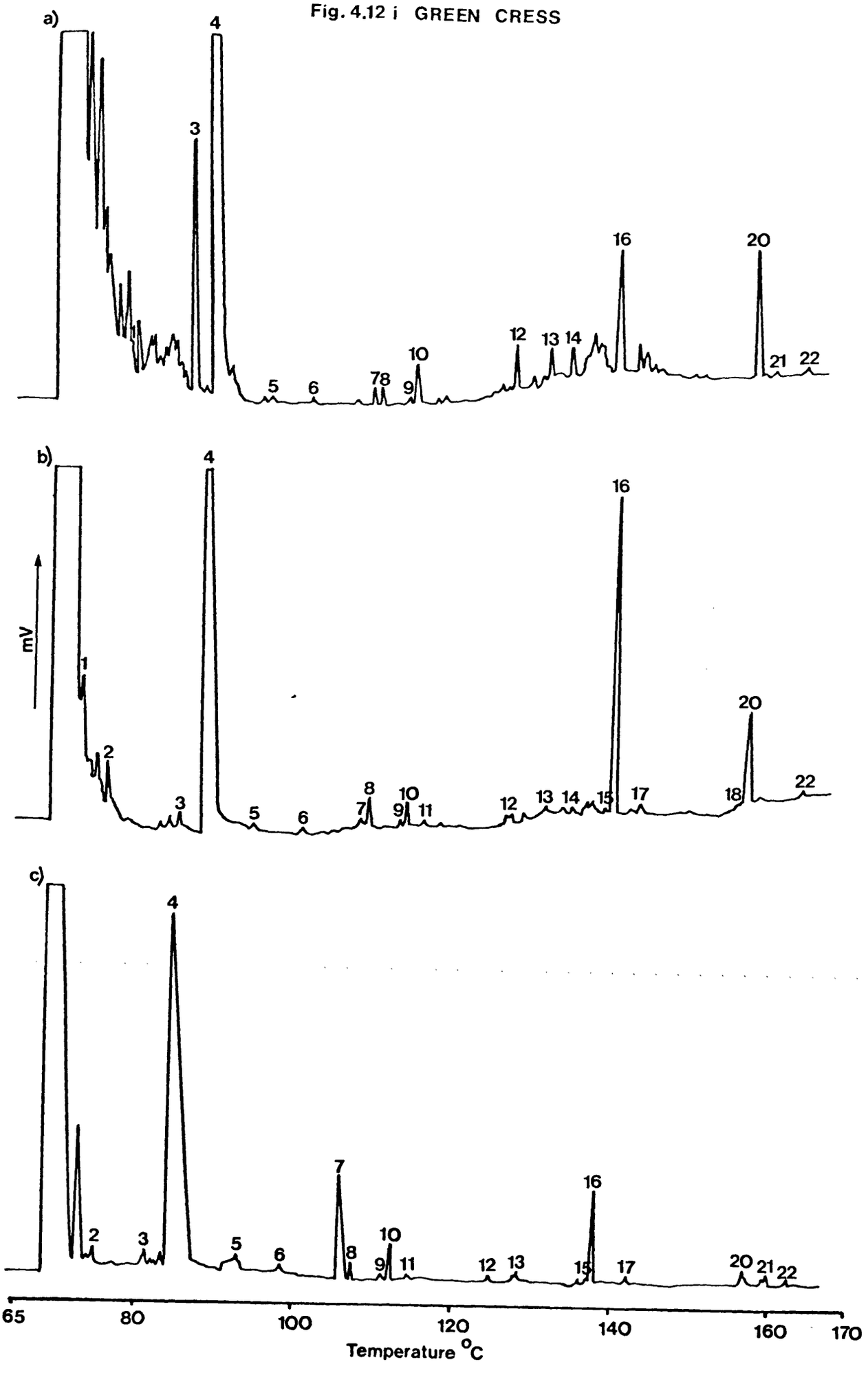
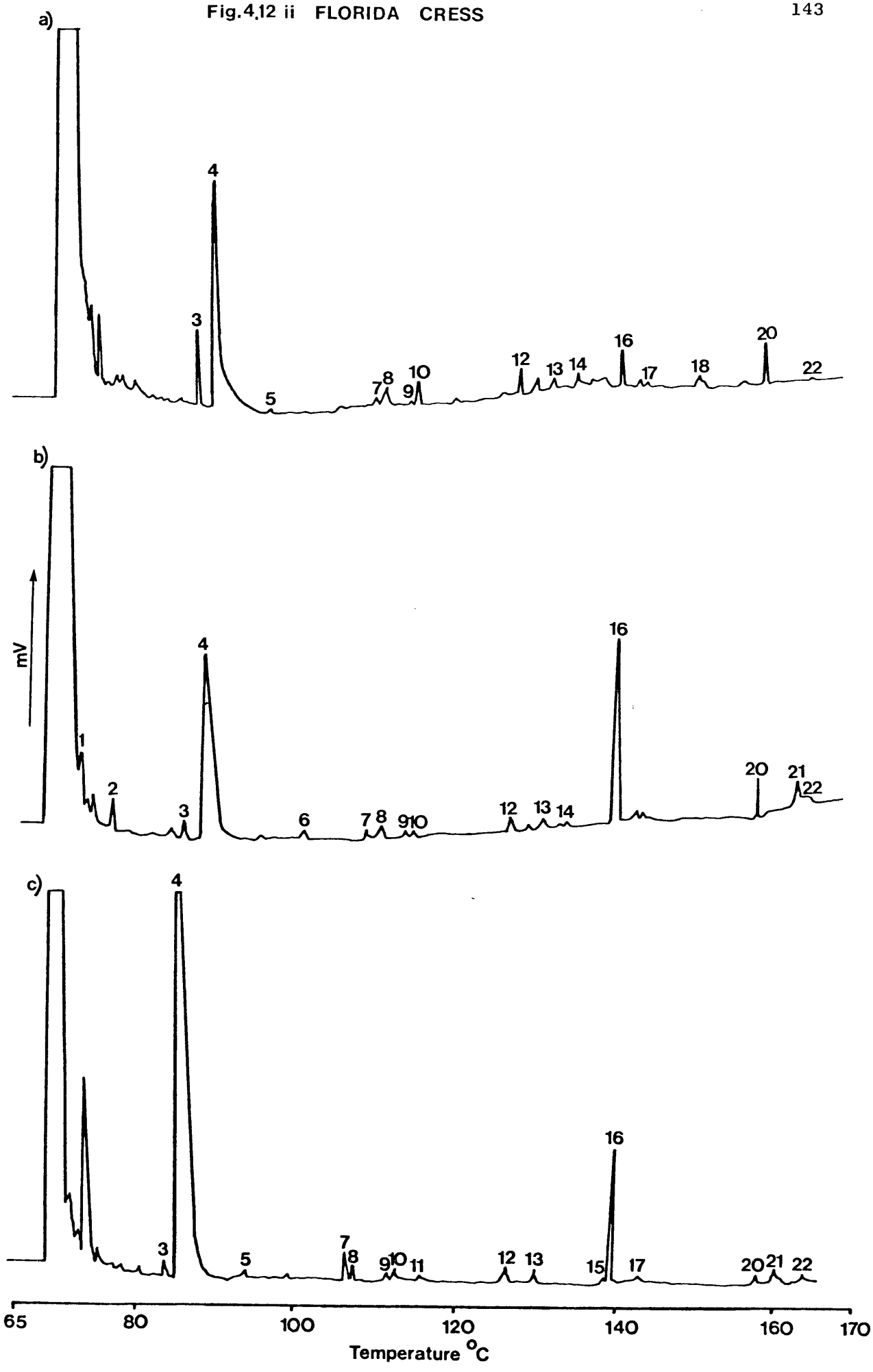


Fig.4.12 ii FLORIDA CRESS



but its source is unknown, although it may result from increased breakdown of Porapak Q.

The quantities of 1,2-ethanediol monoacetate in the 2 day and 7 day samples are similar at all temperatures, except for the 10°C light treatment, where there was a large increase in the amount in the 7 day sample compared with the 2 day sample. The quantity of 1,2-ethanediol diacetate also increased in the 7 day 10°C light sample, but not under the other treatments. This observation may be explained by a possible relationship existing between the production of these two compounds. Only in the 15°C dark treatment (Fig. 4.10 ii) did the amounts of 2,4-dimethyl acetophenone and 4-ethyl acetophenone increase markedly in the samples over the trapping period, but the reason for this requires further investigation. Complex metabolic pathways are involved in the production of many of the compounds identified, and the trends and changes occurring cannot be explained without further work.

Sensory assessment of the watercress was carried out at the end of each experiment. Parts of the assessment sheet developed in Section 3 were used and the results are given in Table 4.2. Watercress kept for 7 days at 5°C in both light and dark with air flowing through the vessels, was still crisp and fresh-looking and with less than 5% of the leaves yellow (Plate 4.3). However, the aromas of both leaves and stalks indicated the presence of sulphur compounds, and the chromatograms show this in the amounts of dimethyl sulphoxide and dimethyl sulphone which were collected. The costs of cold storage and refrigerated transport for keeping watercress at 5°C will require considerable expenditure by the grower. The sensory results for the 10°C treatment show that the plant material was still

Table 4.2 Sensory assessment of watercress after dual headspace experiments.

Treatment	Colour of Leaves (Munsell scale)	Leaf Sheen	Texture to Fingers	Aroma of Leaves	Aroma of Stalks	Colour of Stalks	Colour of Stalk Ends	Stalk Ends Wet or Dry	% Leaves Yellow
5°C Light	5GY 4/6	Dull shine	Very crisp	Weak watercress	Weak seaweed	Green	Green/Yellow	50% wet	< 5%
Dark	5GY 4/6	Dull shine	Very crisp	Weak seaweed	Weak rotting	Green	Green/Yellow	50% wet	< 5%
10°C Light	5GY 4/8	Dull shine	Crisp	Medium watercress	Weak seaweed	Green/Yellow	Green/Yellow	Dry	50%
Dark	5GY 4/8	Dull shine	Crisp	Medium primrose	Wet vegetation	Green/Yellow	Green/Brown	Dry	66%
15°C Light	5GY 4/6	Dull shine	Crisp	Weak rotting	Weak rotting	Green	Green/Brown	Dry	50%
Dark	5GY 4/8	Matt	Crisp	Medium rotting	Strong rotting	Green/Yellow	Green/Brown	Dry	75%
- cytokinin	5GY 4/6	Dull shine	Crisp	Medium watercress	Medium seaweed	Green/Yellow	Green/Yellow	Dry	66%
+ cytokinin	5GY 4/6	Dull shine	Crisp	Medium primrose	Weak rotting	Green	Green/Yellow	Dry	< 10%
Green	5GY 4/6	Matt	Wilting	Weak watercress	Medium watercress	Green/Yellow	Green/Yellow	Dry	66%
Florida	5GY 4/6	Matt	Fresh But Soft	Medium rotting	Medium rotting	Green/Yellow	Green/Yellow	Dry	25%

Plate 4.3 Watercress after 7 days in the dual headspace apparatus.

i) 5°C LIGHT (a) DARK (b)

ii) 10°C LIGHT (a) DARK (b)

iii) 15°C LIGHT (a) DARK (b)

i)

ii)

iii)

crisp, although the stalks were slightly yellow after a week. The aromas of the leaves were quite different after the light and dark treatments; the leaves kept in light smelt of watercress due to the phenethyl isothiocyanate and other sulphur compounds present, whereas those in the dark smelt of primroses. On the chromatograms (Fig. 4.9) it can be seen that dimethyl sulphone was collected in larger quantities from watercress kept at 10°C in the light than in the dark, and this contributed to sulphur compounds responsible for the watercress odour. In the dark-treatment sample of volatiles ß-ionone, which has a floral odour, was detected after 7 days trapping. The main disadvantage of the 10°C treatment compared with the 5°C, was the high percentage of yellow leaves after 7 days (50% in the light and 66% in the dark), and at 15°C yellowing increased further although the leaves were still crisp (Plate 4.3). At 15°C both leaves and stalks had a rotting odour, but this was only shown on the chromatogram by an increase in the amount of dimethyl sulphoxide. From both the sensory assessment and collection of volatiles, it appears that watercress can be stored at 10°C in the dark in a current of air for 7 days with little deterioration in quality apart from yellowing of the leaves.

Cytokinin Treatment

Cytokinins have been shown to delay chlorophyll degradation and senescence of leafy vegetables such as broccoli (Dedolph, Wittwer, Tuli and Gilbart, 1962) and lettuce (El-Mansy, Salunkhe, Hurst and Walker, 1967). After a crop is harvested, general degradation occurs, resulting in the destruction of soluble ribonucleic acid. Protein synthesis, therefore, slows down and as the mechanism of protein formation is disturbed, the pigments and other constituents degrade. Cytokinins are thought to delay the reaction by providing

the necessary adenine to restore soluble ribonucleic acid molecules, thus maintaining protein synthesis. Leaf senescence has been linked with stomatal aperture by Thimann and Satler (1979 a,b) who suggest that the delaying of senescence by light is primarily due to stomatal opening, and that senescence occurring in the dark can be delayed by cytokinin treatment causing stomata to remain partly open. If these chemicals are approved for use on edible foliage, they may have applications in the watercress industry; watercress can then be stored and transported at $10^{o}C$ without the expense of illumination.

The dual headspace experiment using a 5 mg l^{-1} wash of 6-benzyl amino purine and keeping watercress in the dark at $10^{o}C$ has given promising results. The quantities of volatile compounds collected after 7 days trapping from both the control (minus cytokinin) and the treatment (plus cytokinin) were very similar (Fig. 4.11) except for a larger peak of dimethyl sulphone in the treatment sample. Although β-ionone was present in the control samples after 5 and 7 days trapping, its reported threshold levels are very low (7×10^{-5} mg l^{-1} in air, Stahl, 1973), and it was not detected in the aroma of the watercress which was assessed as "medium watercress" (Table 4.2). No ionone was found in the treatment samples although the watercress smelt of primroses after 7 days. The stems of the watercress treated with 6-benzyl amino purine were still green, whereas those of the control were green/yellow. The percentage of yellow leaves in the cytokinin treatment was significantly less than the control (less than 10%, compared with 66%) (Plate 4.4).

Apart from changes in volatiles during storage, the ascorbic acid content should also be monitored. It has been shown, using the method of Horwitz (1975) (Burroughs 1980 - personal communication),

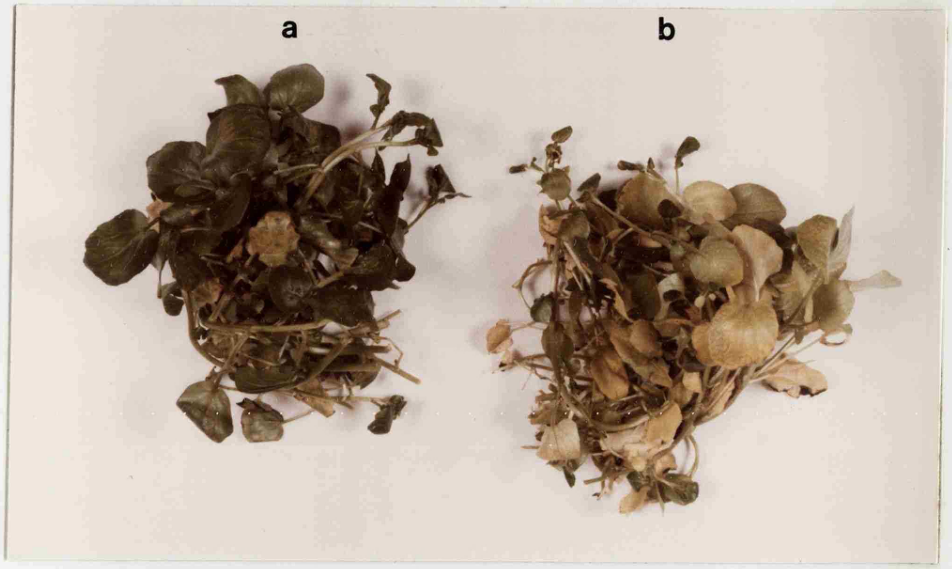
Plate 4.4 Watercress after 7 days in the dual headspace apparatus in the dark at 10°C

(a) plus cytokinin

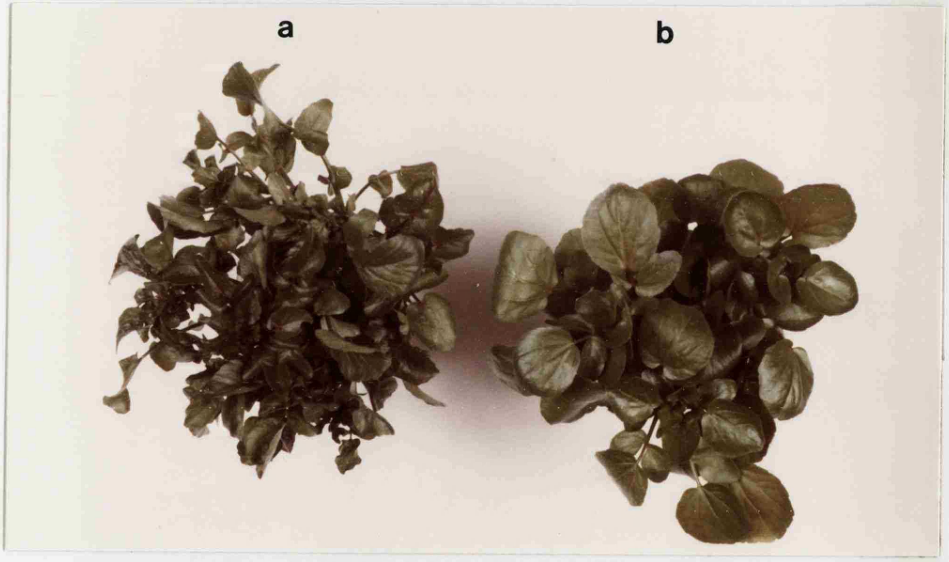
(b) minus cytokinin

Plate 4.5 i) Florida (a) and Green (b) watercress at the beginning of dual headspace experiment, showing differences in leaf shape.

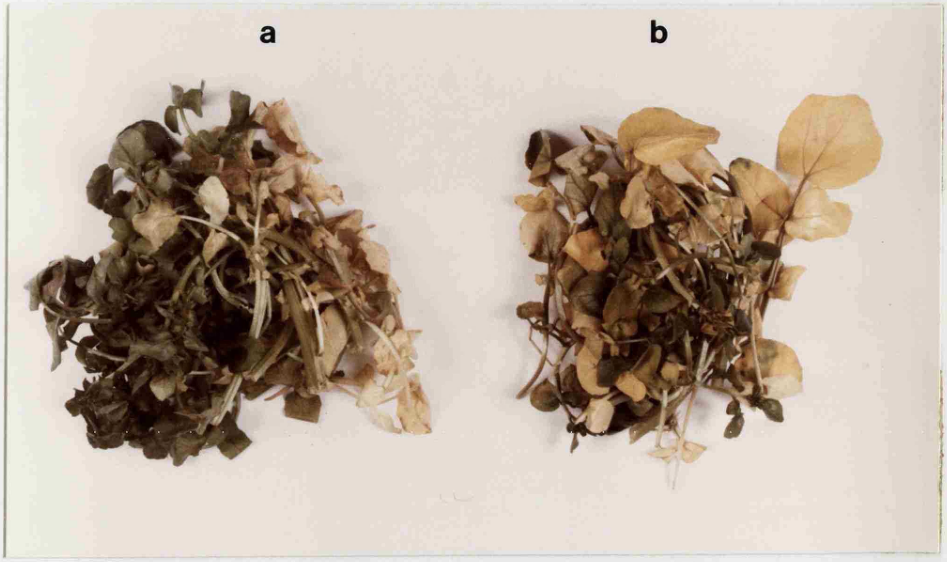
ii) Florida (a) and Green (b) watercress after 7 days in dual headspace apparatus in the dark at 10°C.



i)



ii)



that when watercress is stored at 10°C, ascorbic acid decreased from 68 to 54 mg per 100 g tissue in the light, and from 68 to 39 mg per 100 g tissue in the dark. The effect of 6-benzyl amino purine on the ascorbic acid content of watercress would have to be determined before its use can be recommended because the decrease observed on storing at 10°C in the dark is considerable. Ezell and Wilcox (1959, 1962) have also shown that high temperatures and wilting hasten the loss of carotene and ascorbic acid from several leafy vegetables. A study of the effect of cytokinins on both the ascorbic acid and carotene content in watercress would be valuable in determining whether the disadvantages of storage at 10°C can be overcome , thus omitting the currently used expensive cooling to 6°C and below. These results warrant further investigation into the use of cytokinins as a post-harvest wash for watercress and its effect on the composition of the plant.

During assessment of the watercress samples it was observed that although the flavour of the green leaves did not alter noticeably over the period of each experiment, the leaves which had yellowed no longer possessed the characteristic watercress flavour. Although the glucosinolate seemed to have disappeared during leaf yellowing, there was no corresponding increase in the amount of phenethyl isothiocyanate collected in the headspace. Ether washing of yellow leaves to analyse both the wax and the volatiles collected by this method may explain this anomaly. Further experiments with radio-labelled glucosinolates may suggest the possible catabolic pathways.

Florida and Green Watercress

Florida type watercress has been reported by growers to have a longer shelf-life than green watercress. The results of the headspace collection of volatiles from both types kept in the dark at 10°C

show similar quantities of most of the compounds identified (Fig. 4.12). Samples from 7 day trapping showed green cress produced a larger amount of dimethyl sulphoxide, whereas Florida cress produced slightly more dimethyl sulphone. The major difference between the two types of watercress was in the percentage of yellow leaves (25% Florida, 66% green) at the end of the experiment (Plate 4.5). The green watercress was also more wilted than the Florida. From this single experiment, Florida watercress appears to have a longer shelf-life than green, due mainly to less yellowing of the leaves. Confirmation of these results would be needed for firm conclusions to be drawn. As Florida watercress has a brownish tinge to its leaves, it seems improbable that its apparent longer shelf-life will make it more marketable than the green type.

4.5 Summary

This investigation has identified many volatiles not previously reported in watercress. Ether washing samples contained several glucosinolate degradation products, as expected after cell damage by the solvent, and also other compounds which are probably the products of complex metabolic pathways. Dimethyl sulphoxide and sulphone may result from the oxidation of dimethyl sulphide, which is important in the flavour of several brassicas. The quantities of compounds increased as the watercress aged and further work will determine whether the production of new compounds also occurs.

In ether washing and headspace samples α and β-ionones were detected and these may be responsible for the primrose odour observed in the sensory work (Section 3). These ionones are possibly formed by the

oxidation of polyene-carotenes, and this may be the case in watercress as it contains vitamin A of which the carotenes are precursors.

Fewer volatiles were collected using headspace apparatus because the tissue was not damaged. Over the 7 days of each experiment the amount of dimethyl sulphoxide and phenethyl isothiocyanate increased, dimethyl sulphone decreased and dimethyl disulphide appeared after 7 days. This supports the hypothesis that deterioration of the plant causes sulphur compounds to accumulate as a result of glucosinolate degradation. Results of odour assessment confirmed the identities of several compounds which may be important in the aromas of watercress.

Changes in the production of watercress volatiles in different storage conditions were monitored using dual headspace apparatus. The different ratios of several related compounds over the 7 day period could not be explained and further investigation is needed to understand the anabolic and catabolic processes involved. Storage for 7 days at $5^{o}C$ in a current of air kept the watercress fresh and crisp, and the major effect of raising the temperature to 10^{o} and $15^{o}C$ was to increase leaf yellowing. The quantities of sulphur compounds collected from samples kept in the dark at $10^{o}C$ were less than when illuminated or stored at $15^{o}C$ in light and dark. From both sensory assessment and collection of volatiles it appears that watercress can be kept at $10^{o}C$ in the dark, in a current of air, for 7 days with little deterioration in quality apart from yellowing of leaves. The results from using a cytokinin wash before storage suggest that this treatment may overcome the problem of leaves turning yellow. However, the ascorbic acid content, after cytokinin treatment, should be

monitored because storage in the dark caused a marked decrease in ascorbic acid compared with samples stored in the light.

The reported longer shelf-life of Florida watercress appears to be mainly due to decreased yellowing of leaves and not to differences in the volatiles produced when compared with the green type.

A cross section of a watercress leaf revealed numerous large air spaces between cells into which volatile compounds may diffuse. On reaching the wax layer the volatiles may be trapped or released depending on the state of dynamic equilibrium of these compounds in relation to rates of synthesis, degradation and release into the atmosphere.

4.6 Examination of the Microflora of Watercress

Acetic acid was found to be one of the major compounds in the extracts from the headspace collections and for this reason watercress was examined to determine whether the acid is produced by the plant, or by associated microflora, as in the case with plums (Ismail, 1977).

4.6.1 Materials

Watercress	-	as for the study of epicuticular wax (Section 5)
Media	-	Table 4.3.
Phenethyl isothiocyanate	-	purum 97% Fluka AG.

4.6.2 Methods

1. Direct Examination of Different Parts of the Plant

Different parts of the watercress plant were directly examined under a microscope to determine the distribution of microorganisms.

a) The base of a petiole was pressed on to a slide and examined.
b) A piece of sellotape was pressed on to the adaxial surface of a leaf and then pressed on to an agar plate. The piece of agar was placed on a slide and examined (Beech and Davenport, 1971).
c) A drop of the condensation from a polythene bag containing watercress was examined on a slide.
d) Adventitious roots (rootlets) from the axil of the stem and a leaf petiole were placed on a slide with a drop of water.
e) The axil of the stem and a leaf petiole was swabbed with sterile cotton wool and the swab shaken in a bottle of sterile water. A drop of the water was examined on a slide.

Table 4.3 Details of media used for isolation of microorganisms

Media	Code used	pH	Agar ($g\ 100ml^{-1}$)	Chemical status ($g\ 100ml^{-1}$ dist.H_2O)		Sterilization (^{o}C/min)	Reference
Plate count agar	PCA	7.0	1.5	tryptone	0.5	121/15	London Analytical and Bacteriological Media Ltd. (labm) (1977)
				yeast extract	0.25		
				glucose	0.1		
Malt extract (CM 59)	Malt	5.4	1.5	malt extract	3.0	115/10	Oxoid (1971)
				peptone	0.5		
Glycerol	Glyc	6.0	2.0	yeast extract	3.0	121/15	Carr (1968)
				glycerol	3.0		
Chalk	Chalk	7.0	2.0	yeast extract	3.0	121/15	Carr (1968)
				glucose	10.0		
				$CaCO_3$	3.0		
Bromocresol green	BG	from 3.6 (yellow) to 5.1 (blue)	2.0	yeast extract	3.0	121/15	Carr (1968)
				bromocresol green 0.1ml 2.2% alc.soln.			
				ethanol	2.0		
Apple juice yeast extract	AJYE	4.8	3.0	yeast extract	1.0	115/10	Carr (1952)
				Cox's orange pippin juice			
de Man, Rogosa and Sharpe (CM 361)	MRS	6.2	1.0	peptone	1.0	121/15	Oxoid (1971)
				Lab-lemco	0.8		
				yeast extract	0.4		
				dextrose	2.0		
				'Tween' 80	0.1ml		
				K_2HPO_4	0.2		
				$CH_3COONa3H_2O$	0.5		
				$(NH_4)_3C_6H_5O_7$	0.2		
				$MgSO_4 7H_2O$	0.02		
				$MnSO_4 4H_2O$	0.005		

f) Sterile water was slowly ejected on to the stem, left for 30 s, and then withdrawn into a syringe. A drop from the syringe was placed on a slide for examination.

2. Isolation of Organisms

Seven media (PCA, Malt, Glyc, Chalk, BG, AJYE and MRS) were plated out and used to isolate organisms from different parts of the plant. Since watercress is semi-aquatic, growing in water at pH ≏ 7.0, most of the media used was at this pH. Plates were incubated for 3 days at 10° as well as 28°C as watercress grows in water which is usually at 10°C. The following methods of isolation were used : -

a) Petiole bases were squashed on to the plates.
b) Leaves were attached by the abaxial epidermis to plates with sterile water.
c) Leaves were stuck on to the lids of plates with sterile water, adaxial surface against the lid to detect ballistospore-forming yeast-like organisms.
d) Condensation from watercress kept in a polythene bag overnight was streaked on to plates.
e) Sections of stalks with rootlets were placed on plates.
f) The axil of the stem and a leaf petiole was swabbed with sterile cotton wool, the swab shaken in a bottle of sterile water and drops of the water streaked on to the plates.
g) Sections (2 cm long) of the stem were placed on plates.

3. Action of Phenethyl Isothiocyanate on Isolated Organisms

Phenethyl isothiocyanate was dispersed in PCA at two concentrations (500 and 1000 ppm v/v) before pouring plates. Three of the most common bacteria found during isolation of the organisms from different

parts of the plant (a Bacillus sp. and yellow and cream micrococci) were picked from plates and added to 10 ml sterile water. Aliquots of this suspension (0.1 ml) were placed on the PCA + phenethyl isothiocyanate plates and incubated at 10^{o} and 28^{o}C for 3 days.

4.6.3 Results and Discussion

1. Direct Examination of Different Parts of the Plant

a) Petiole base squashed on slide - many bacteria, some motile and possibly acetic acid bacteria, moulds and yeasts, including Aureobasidium pullulans, were seen. There were distinct micro-colonies of yeasts and bacteria suggesting possible microorganism interactions.

b) Sellotape leaf-press - Aureobasidium mycelium and yeast cells were identified.

c) Condensation droplet - large, small and encysted protozoans were seen.

d) Rootlets - bacteria (rods and cocci) and yeasts were aggregated at the root tips.

e) Swab of stem and leaf petiole axil - many bacteria (some motile) and yeasts were seen amongst wax and mucilaginous debris. Algal clusters and fungal hyphae were also observed.

f) Water from stem - only a few bacteria and yeasts were present. The yeasts were morphologically different from those seen on examing the rootlets (d).

These results suggest an uneven distribution of microorganisms over the watercress plant with possible interactions between organisms. On leaves and stems few organisms were present, with more occurring

in leaf axils and amongst rootlets where they are less easily detached and sheltered from the aqueous environment. Since swabbing the leaf axils several times did not remove all the organisms, confirming observations by Davenport (1970), and very few were seen in the condensation droplets, some mechanism of attachment seems to prevent the microorganisms from being easily dislodged. Most bacteria and red yeasts are probably anchored to the plant by producing polysaccharide-like material. Red yeasts are also able to degrade fatty materials and, following initial colonisation, may grow into the wax layer (Davenport 1980 - personal communication).

2. Isolation of Organisms

No acid production by microorganisms was indicated by the chalk or bromocresol green media. The rate of growth of the organisms was faster, and the number of organisms greater, at 28°C compared with 10°C incubation, except for the yeasts which preferred the lower temperature. Since there is an interaction between time and temperature of incubation, the plates incubated at 10°C would probably have shown the same number of organisms as the 28°C plates had they been incubated for longer. However, this may not reflect the natural situation on the growing plant where temperatures fluctuate daily and seasonally. Further research is necessary to determine the optimum time and temperature of incubation for the isolation of all the microorganisms present on the watercress plant.

Table 4.4 shows the numbers of identified organisms on plates using different isolation techniques. The swab of the stem and leaf petiole axil shaken in sterile water proved to be the most successful method of isolation of organisms. Similar numbers of bacteria, but not so

Table 4.4 Visual assessment of the number of organisms growing on plates of seven different media incubated at 10°C and 28°C. - = absent, + = isolated colony, ++++ = numerous colonies.

Organisms identified (Genera)	Part of plant from which samples were taken						
	Petiole base	Leaf-adaxial epidermis	Leaf-abaxial epidermis	Condensation	Stalk + rootlets	Petiole + stem axil	Stem
Moulds -							
Penicillium		+		+		++	+
Aspergillus		++					
Yeast-like organisms -							
Rhodotorula		+	+			++++	
Aureobasidium		+++				++++	
Cladosporium		+					
Sporobolomyces			+				
Trichosporon				+		++	++
Bacteria -							
Xanth-omonas		+			++	++	
Bacillus	+++	++		+	+	+	+
Proteus		+				++	
Pseudomonas		++				+	
Micrococcus	+++	+	++	+		+++	
(Group)							
Actinomycetes		+					

many yeasts, were isolated from a leaf placed on an agar plate with the adaxial epidermis exposed. Few organisms were isolated using the other methods. This may be due to the fact that not many organisms were present on the parts of the plant examined, for example the stem, or because the method used was unable to dislodge the organisms present on the plant, as with the collection of condensation. Previous bacteriological examinations of watercress, carried out by the Ministry of Health and A.D.A.S., have only been concerned with the numbers of coliforms present and not other organisms (Walters et al, 1957; Tee, 1962, 1966; A.D.A.S. 1973).

This study shows the majority of the microorganisms isolated from different parts of the watercress plant are situated in the axil of the stem and leaf petiole where they are protected from dislodgement. This was also found with grape leaves by Davenport (1970). The bacteria identified are common in soil and water, except for Xanth-omonads which are always found in association with plant material, and many are plant pathogens. Both the identified moulds are found on all types of decomposing vegetation; the yeasts also occur on plant material (Buchanan and Gibbons, 1975; Stevens, 1974). No acetic acid bacteria were isolated with the methods used, even though favourable conditions for the growth of these organisms were selected; AJYE medium is selective for acid tolerant bacteria; glycerol medium is used to isolate acetic acid bacteria; and chalk and bromocresol green media are indicators for acid production. Some yeasts may produce acetic acid as a metabolic product, for example Kloeckera apiculata, but none of these yeasts were identified in this study. Most of the acetic acid detected in the volatiles of watercress is produced by the plant and not by its associated microflora.

3. Action of Phenethyl Isothiocyanate on Isolated Organisms

There was a profuse white shiny growth over plates with both concentrations of phenethyl isothiocyanate and incubated at the two temperatures. There was no other growth on the plates, demonstrating the bacteriostatic effect of phenethyl isothiocyanate on the Bacillus sp. and the yellow and cream micrococci. Microscopic examination identified the organism which had grown as Proteus, a common spoilage organism found in soil and water, but not harmful to man. This organism may be consistently found on watercress because it has evolved a resistance mechanism to isothiocyanate.

4.6.4 Summary

The unusual environment of watercress has produced an uneven distribution of microorganisms on the plant. There appeared to be interactions between organisms on the plant, and mechanisms for their attachment to the plant to avoid dislodgement by water.
Few organisms were present on the stems, with more on the adaxial epidermis of leaves, and the largest numbers in sheltered axils of the stem and leaf petioles. Several moulds, yeasts and bacteria, commonly associated with plants, were identified, none of which could produce the amount of acetic acid detected in watercress volatiles. Phenethyl isothiocyanate added to PCA prevented the growth of the three most common organisms isolated from the watercress plants. However, Proteus grew despite the presence of phenethyl isothiocyanate, but since it does not cause illness in man, it would not be harmful if eaten on raw watercress.

Phenethyl isothiocyanate is released on damaging the plant tissue of watercress. The bacteriostatic effect of this compound when released only from the cut stems in a bunch is negligible, but in the "jumble pack", where the cress is cut into smaller pieces, there may be some bacteriostatic effect. The microflora on watercress within prepacks requires study to determine the effects of the atmosphere in the pack on the microflora population of the plant product.

In the growing situation, the phenethyl isothiocyanate liberated when damage occurs would not be washed away by the water, but it is trapped in the wax layer. It may protect the plant from invasion by those microorganisms which could cause the tissue to rot in water and allow the healing processes to operate within the plant.

SECTION 5

Examination of the Epicuticular Wax of Watercress

5.1 Introduction

Cuticular wax is the surface layer of hydrophobic lipids covering leaves, fruits and other parts of higher plants. Being impervious it reduces water loss and protects against invasion by fungi and other organisms and physical damage.

Certain properties of the wax layer are due to its physical structure. The surface of glaucous plants is covered by random wax deposits which project outwards, whereas that of non-glaucous plants has a smooth layer of well defined orientation. Glaucous leaves appear shiny due to greater light scattering by the waxy projections. Using scanning electron microscopy the wax can be shown to exist as plates, rods, tubes or other forms projecting from the leaf surfaces. The microscopic appearance of the wax is often characteristic of the plant species, but environmental factors, such as humidity and temperature, greatly influence the morphology of the wax projections (Baker, 1974). Water repellancy is greatest when the wax surface has a rough structure. Surface structures such as ridges, trichomes and papillae also contribute to water repellancy (Holloway, 1970). This property of wax deposits and waxy hairs to prevent the wetting of leaves may be responsible for frost hardiness in certain species, for example Eucalyptus, (Grose, 1960).

Cuticular wax is generally a mixture of long chain hydrocarbons, esters, triterpenoids, fatty alcohols, ketones and acids. These are formed enzymically in the epidermal layer of cells and extruded by an unknown mechanism to the tissue surface. Chemical work on the waxy components of the plant cuticle dates from the end of the last century when waxes from material of commercial importance were

examined. In his examination of carnauba wax, Stürke (1884) found a preponderance of higher alcohols and a low content of paraffins. Until 1955 evidence from the analysis of plant waxes largely supported the view of Chibnall and his co-workers (1954) that waxes contained n-alkanes only of odd carbon numbers and n-primary alcohols only of even numbers. However, since then several plant waxes have been shown to contain both odd and even carbon-numbered alkanes and alcohols, and the previously held views on the biosynthesis of these compounds have now altered. Methods of isolation of the waxes have varied greatly and have caused confusion as to whether the material is solely from the cuticle, or also from cellular tissue. Recent work using modern analytical procedures has clarified past results and revealed even greater complexity in the constituents of plant waxes.

Being a complex mixture of compounds, the wax may contribute to the aroma of the fruit or leaf. Sometimes volatile compounds are distributed and disseminated from the epidermal cells of the perianth parts (Weichsel, 1956) and in many plants fragrant oils are secreted only from specialized glands called osmophors (Vogel, 1962). The relationship between the surface wax layer of fruits and their external aroma has been shown for cranberries (Croteau and Fagerson, 1971), apples (Paillard, 1975) and plums (Ismail et al, 1977).

The cuticular wax of watercress was analysed to determine whether it retains aroma components liberated from within the plant, or whether it contributes to the aroma itself by degradation of lipids (Forss, 1972; Ismail et al, 1977). Many plants possess specific structures for the secretion of lipophilic material, such as the glandular trichomes of Labiatae and secretory cavities in Leguminosae (Fahn,

(1979). The wax layer of watercress was examined using scanning electron microscopy for the presence of any structures capable of retaining flavour compounds.

5.2 An Investigation into the Volatile Constituents Present in Epicuticular Wax of Watercress

5.2.1 Materials

Watercress - grown in natural environment watercress bed simulation tanks (1.2.2). Green watercress was cut 12 - 14 weeks (young) and 16 - 18 weeks (old) from sowing, and flowering green watercress was also used. Florida type cress was cut after 14 - 16 weeks growth from stubble.

Diethyl ether - purified and redistilled (4.2) A.R., anhydrous, Koch Light Laboratories Ltd.

Sodium sulphate - purified by pyrolysis at 550^{o}C for 5h. A.R. Hopkin and Williams.

Silica gel G - type 60, Merck.

Benzene - redistilled, A.R. BDH.

Sulphuric acid - A.R. BDH.

Chloroform - redistilled, BDH.

Ethanol - redistilled, BDH.

2,4-dinitrophenyl hydrazine (DNPH) - A.R. BDH.

Ferric chloride - A.R. BDH.

Potassium ferricyanide - A.R. BDH.

Potassium permanganate - A.R. BDH.

Pyridine - Pierce.

Acetic anhydride	- 98% BDH.
Petroleum ether	- redistilled, boiling point 64 - 66°C, BDH.
N,O-bis-(trimethylsilyl)-acetamide (BSA)	- Supelco Inc.
Cholesterol caprylate	- 97% Aldrich Chemical Co.
Tetracosane	- 97% Aldrich Chemical Co.
β-amyrin acetate	- (Dr. J.A. Lamberton, CSIRO, Melbourne).
Brassica oleracea wax	- reference sample, (Dr. P.J. Holloway, L.A.R.S.).

5.2.2 Methods

5.2.2.1 Isolation of the Epicuticular Wax Layer

The epicuticular wax was removed from 100 g of watercress, previously washed in glass distilled water. Each sprig (c. 18 cm long) was individually immersed in 250 ml diethyl ether for 30 s with the cut stem ends held above the solvent. Sodium sulphate (25 g) was added to the ether washings which were stored overnight at -20°C. After vacuum sublimation (Section 4.3), the non-volatile wax remaining in the flask was dissolved in ether, transferred to a weighed ampoule and the ether evaporated off in an atmosphere of nitrogen (4.3.2).

5.2.2.2 Isolation of Wax from Watercress Flowers

The wax was removed from flowers by immersing 30 g of flower sprigs in 100 ml ether for 30 min and then using the same method as for the epicuticular wax (5.2.2.1).

5.2.2.3 Analyses of Wax Constituents

1) Thin Layer Chromatography (TLC)

A sample of wax (10 mg) was dissolved in chloroform (1 ml) and chromatographed on a 20 x 20 cm TLC plate coated with a 0.25 mm layer of Silica gel G, with benzene as the eluting solvent. The wax of Brassica oleracea and a pure sample of β-amyrin acetate (10 mg ml^{-1} chloroform) were used as standard reference compounds. After allowing the solvent to evaporate, the position of spots was determined by spraying with a 50% (v/v) aqueous solution of sulphuric acid; then by heating at 110°C for 3 min any triterpenoid acids present were visible as purple spots, triterpenols and triterpenol acetates as red to red-brown, and sterols and sterol esters as pink to red spots. Other unspecified organic compounds were visible as brown-black spots after charring at 180°C for 5 - 10 min.

A second TLC plate was spotted with the three wax samples (old, young and Florida), and each spot treated with DNPH solution (0.5% (v/v) ethanol). After development in benzene, drying and spraying with a 50% aqueous solution of sulphuric acid, the plate was charred at 100°C for 1 min. Any carbonyl compounds present appeared as red or orange spots.

2) Infra-red Analysis

Infra-red analysis of the total wax extract was carried out to obtain information on the chemical structures present.

The wax samples were dissolved in ether, evaporated on a sodium chloride plate, placed in an infra-red spectrophotometer (Perkin Elmer model 237) and scanned from 625 - 4000 cm^{-1} (wavenumber).

3) Preparative Layer Chromatography (PLC)

A mixture containing 2 mg of each wax sample was placed on a prewashed 20 x 20 cm glass plate coated with 0.75 mm Silica gel G. After developing in benzene and allowing the solvent to evaporate, the plate was stained with iodine vapour for 30 s to locate bands of aliphatic and lipid material. Bands containing compounds which absorb ultra violet (UV) radiation e.g. phenolics, were visible under a UV lamp.

Part of the bands was removed and compounds extracted from the Silica gel G by refluxing for 2 h in chloroform containing 2% ethanol, followed by filtration and evaporation of the bulk of the solvent at 50°C. The remaining portions of bands were sprayed with a mixture of equal volumes of 2% aqueous ferric chloride and 2% aqueous potassium ferricyanide with one drop of 1% aqueous potassium permanganate solution. Aromatic amines, phenols and phenolic steroids, if present, were detected as a blue coloured spot against a yellowish-blue background. Spraying with 50% aqueous sulphuric acid and charring at 180°C for 5 - 10 min intensified the blue colour and showed the position of the unspecified organic compounds as brown-black bands.

3a) TLC of Samples from PLC

The extracted compounds from the different bands on the PLC plate and the B. oleracea reference sample were spotted on to a 20 x 20 cm TLC plate coated with a 0.25 mm layer of Silica gel G. The plate was developed in benzene, dried, sprayed with 50% aqueous sulphuric acid and charred for 5 - 10 min at 180°C to check the classes of compounds present.

3b) Acetylation of PLC Samples

The PLC samples thought to contain alcohols were acetylated to esters, thus aiding their subsequent identification.

Samples were evaporated to dryness, 50 µl pyridine and 200 µl acetic anhydride added, and heated at 55°C overnight. After evaporation to half volume, 125 µl petroleum ether were added, forming an azeotropic mixture which evaporated completely under nitrogen. Finally the samples were each dissolved in 25 µl chloroform.

3c) Silylation of PLC samples

BSA reacts with hydroxyl compounds to form TMSi (trimethylsilyl) ethers, organic acids to form TMSi esters, and some enols to form TMSi ethers.

The PLC samples thought to contain hydroxyl compounds were silylated by adding 50 µl BSA to the sample and heating at 55°C for 1 h.

4) Gas Chromatographic and Mass Spectrometric Analyses

Columns : (length x internal diameter)

i) 90 x 0.2 cm glass column packed with 1% Dexsil 300 on 100-120 mesh Supelcoport (Supelco Inc).

ii) 90 x 0.32 cm stainless steel column packed with 1% Dexsil 300 on 100-120 mesh Supelcoport.

iii) 90 x 0.2 cm glass column packed with 3% SP2100 on 100-120 mesh Supelcoport.

iv) 180 x 0.2 cm glass column packed with 3% SP2100 on 100-120 mesh Supelcoport.

v) 56 m x 0.5 mm glass carbowax coated SCOT column.

vi) 70 m x 0.7 mm glass open tubular capillary column dynamically coated with SF-96-200 (non-polar silicone oil phase).

Instruments :

A. Hewlett-Packard 5730A gas chromatograph (g.c.) fitted with a flame ionisation detector (F.I.D.).

Temperature programme	: 130^{o}- 350^{o}C at 6^{o} min^{-1}
Carrier gas	: nitrogen (oxygen-free)
Flow	: 30 ml min^{-1}
Detector temperature	: 300^{o}C
Injection port temperature	: 250^{o}C

A mixture of the old, young and Florida wax samples was run on instrument A using columns i and iii, and the PLC samples using column iii.

B. Hewlett-Packard 5830 g.c. fitted with a F.I.D.

Temperature programme	: 65^{o}- 190^{o}C at 2^{o} min^{-1}
Carrier gas	: nitrogen (oxygen-free)
Flow	: 50 ml min^{-1}
Detector temperature	: 250^{o}C
Injection port temperature	: 250^{o}C

Samples of leaf wax and flower wax were run on instrument B using column vi.

C. LKB 9000 coupled gas chromatography - mass spectrometry (g.c.-m.s.) operated at 70 eV; scanning at 72 amu s^{-1} with an accelerating voltage of 3.5 kV.

Temperature programme	: 120^{o}- 270^{o}C at 6^{o} min^{-1}
Carrier gas	: helium (4.67 kg cm^{-2} at cylinder)
Flow	: 30 ml min^{-1}

Source temperature : a) 270°C b) 250°C

Injector temperature : a) 225°C b) 250°C

Separator temperature : a) 225°C b) 280°C

A mixture of the old, young and Florida wax samples was run on instrument C using column ii with source, injector and separator temperatures set at a). The PLC samples were also run on instrument C using column ii with source, injector and separator temperatures set at b).

D. Finnigan 4000 coupled g.c.-m.s. with a 2100 data system operated at 50 eV; scanning masses 33 - 400 in 2 s cycles.

Temperature programme : 1) 120°- 270°C at 6° min^{-1}

: 2) 130°- 275°C at 6° min^{-1}

: 3) 65°- 190°C at 2° min^{-1}

: 4) 65°- 180°C at 3° min^{-1}

Electron Impact (E.I.)

Carrier gas : helium (4.67 kg cm^{-2} at cylinder)

Flow (packed columns) : 20 ml min^{-1}

Flow (capill. columns): 2 ml min^{-1}

Chemical Ionisation (C.I.)

Carrier gas : methane (2.67 kg cm^{-2} at cylinder)

Flow : 20 ml min^{-1}

Source temperature : a) 270°C b) 280°C c) 250°C

Injector temperature : a) 250°C b) 250°C c) 225°C

Separator temperature : a) 280°C b) 280°C c) 225°C

A mixture of the old, young and Florida wax samples was run on instrument D using column ii with source, injector and separator temperatures set at a) and temperature programme 1). PLC samples were run using column iv, source, injector and separator temperatures set at b) and temperature programme 2).

Leaf and flower wax samples were run using columns v and vi, with source, injector and separator temperatures set at c). Temperature programme 3) was used for the leaf wax samples, and 4) for the flower wax samples.

5.2.3 Results and Discussion

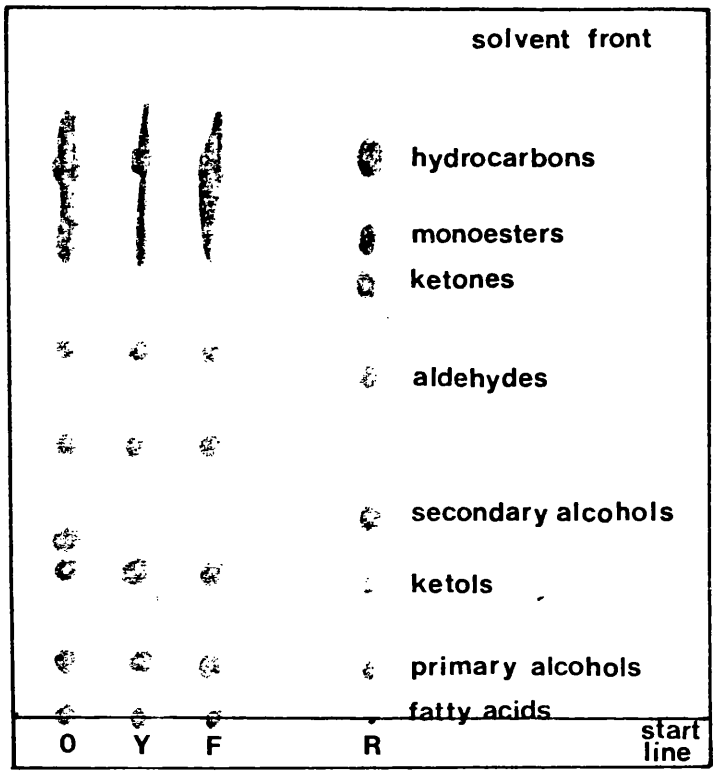
1) TLC

Figure 5.1 is a diagram of the results on the TLC plate. Together with the results of spraying and heating the plate, watercress wax was shown to be composed of fatty acids, primary alcohols, ketols, sterols, possibly amyrin, monoesters and long chain hydrocarbons after comparison with the reference sample. Other spots were visible from the wax samples which did not correspond to any of the reference sample spots. The old watercress wax sample had an extra spot, not visible in the other samples, which might correspond to the secondary alcohol spot in the reference. These results only provide tentative allocations of compound classes present in the samples which have to be confirmed by PLC and g.c.-m.s. analyses. The extra spot visible in the old watercress sample was not evident as an extra peak in chromatograms of this sample compared with the others, and this requires further investigation.

2) Infra-red Analysis

The results gave no indication of unsaturated compounds since there were no peaks above 3,000 cm^{-1} apart from a weak alcoholic OH peak at 3,300 cm^{-1}. There were strong CH_2, CH_3 absorptions at 2850 -

Fig. 5.1 Diagram of results on TLC plate for old (O), young (Y) and Florida (F) watercress wax samples, with B.oleracea wax reference sample (R), showing the classes of compounds present in the reference.



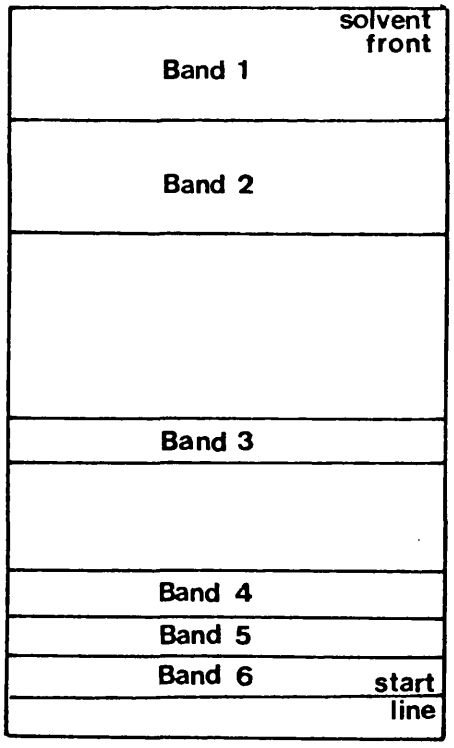
2950 cm^{-1}, stronger in the CH_2 region as expected for long, straight chained compounds.

3) PLC

Figure 5.2 shows the six bands on the PLC plate which were made visible by exposure to iodine vapour. Bands 1 and 2 absorbed UV radiation, possibly due to phenolic or flavanoid material, and also band 6, which probably contained pigmented material.

The ferric chloride / potassium ferricyanide spray for phenolics produced an intense response in band 6, and a slight response in bands 1, 4 and 5, suggesting phenolic or ring structures. From their R_f values, band 1 probably contained hydrocarbons, band 2 esters and trace alcohols, band 3 acetates, band 4 ketols and trace alcohols, band 5 primary alcohols, and band 6 sterols, primary alcohols and fatty acids.

Fig. 5.2 Diagram of the six bands visible on the PLC plate of the bulked wax sample.



3a) TLC of PLC Samples

Single spots were produced from the samples of bands 1,2 and 3, corresponding to the hydrocarbon, ester and acetate regions on the plate. The band 4 sample produced spots in the ketol and alcohol regions, the band 5 sample produced one spot in the alcohol region, and the band 6 sample spots in the alcohol, fatty acid and sterol regions.

4) Gas Chromatographic and Mass Spectrometric Analyses

Confirmation of the classes of compounds separated by PLC and their identification were obtained using g.c.-m.s.

Figure 5.3 shows the chromatograms of samples taken from the PLC plate. Band 1 sample was thought to contain hydrocarbons from its R_f value; identification of the compounds using g.c.-m.s. showed hydrocarbons with both odd and even numbers of carbon atoms. The most commonly found hydrocarbons in higher plant waxes are the C_{29} and C_{31} straight chain alkanes (Thompson, 1980), with the odd-carbon-numbers predominating (Eglinton and Hamilton, 1967). This was found in the watercress wax where C_{27}, C_{29} and C_{31} hydrocarbons were the largest peaks on the chromatogram (Fig. 5.3 i peaks 7,9 and 10). The phthalates detected (peaks 6 and 8) may be naturally occurring, although they are a common contaminant.

The band 2 sample was a low concentration sample, and consequently identification of compounds proved difficult (Fig. 5.3 ii). Phenethyl isothiocyanate was the major constituent (peak 1), trapped in the wax after release within cells in the plant, and, although esters were expected at this R_f value, only a hexadecanoate was identified (peak 10).

Fig. 5.3 Chromatograms of samples from PLC plate bands of 6 mg epicuticular wax of watercress. Sample size: 2 µl + 1 µl (1 mg ml^{-1}) tetracosane reference (C_{24}). Amplifier attenuation: 10^2 x 16. Column: 90 x 0.2 cm glass packed with 3% SP2100 on 100-120 mesh Supelcoport.

Peak identities : -

i) Band 1 sample

1 C_{19} hydrocarbon
2 C_{21} hydrocarbon
3 C_{22-23} hydrocarbon
4 C_{24} hydrocarbon + reference
5 C_{25} hydrocarbon
6 Phthalate + trace hydrocarbon impurity
7 C_{27} hydrocarbon
8 Phthalate + trace hydrocarbon impurity
9 C_{29} hydrocarbon
10 C_{31} hydrocarbon
11 C_{32} hydrocarbon + terpene
12 C_{33} hydrocarbon
13 C_{34} hydrocarbon + terpene
14 C_{35} hydrocarbon

ii) Band 2 sample

1 Phenethyl isothiocyanate
2 Phthalate
3 Unknown - possible M^+ 411 (36%) base peak m/e 57 (100%)
4 C_{23-25} alcohol terpene
5 Unknown + hydrocarbon (mol.wt. > 400)
6 Unknown - possible M^+ 439 (50%) base peak m/e 57 (100%)
7 Unknown - possible M^+ 453 (2.4%) base peak m/e 43 (100%)
8 Unknown - possible M^+ 466 (20%) base peak m/e 57 (100%)
9 Unknown - possible M^+ 430 (20%) base peak m/e 43 (100%)
10 A hexadecanoate + unknown

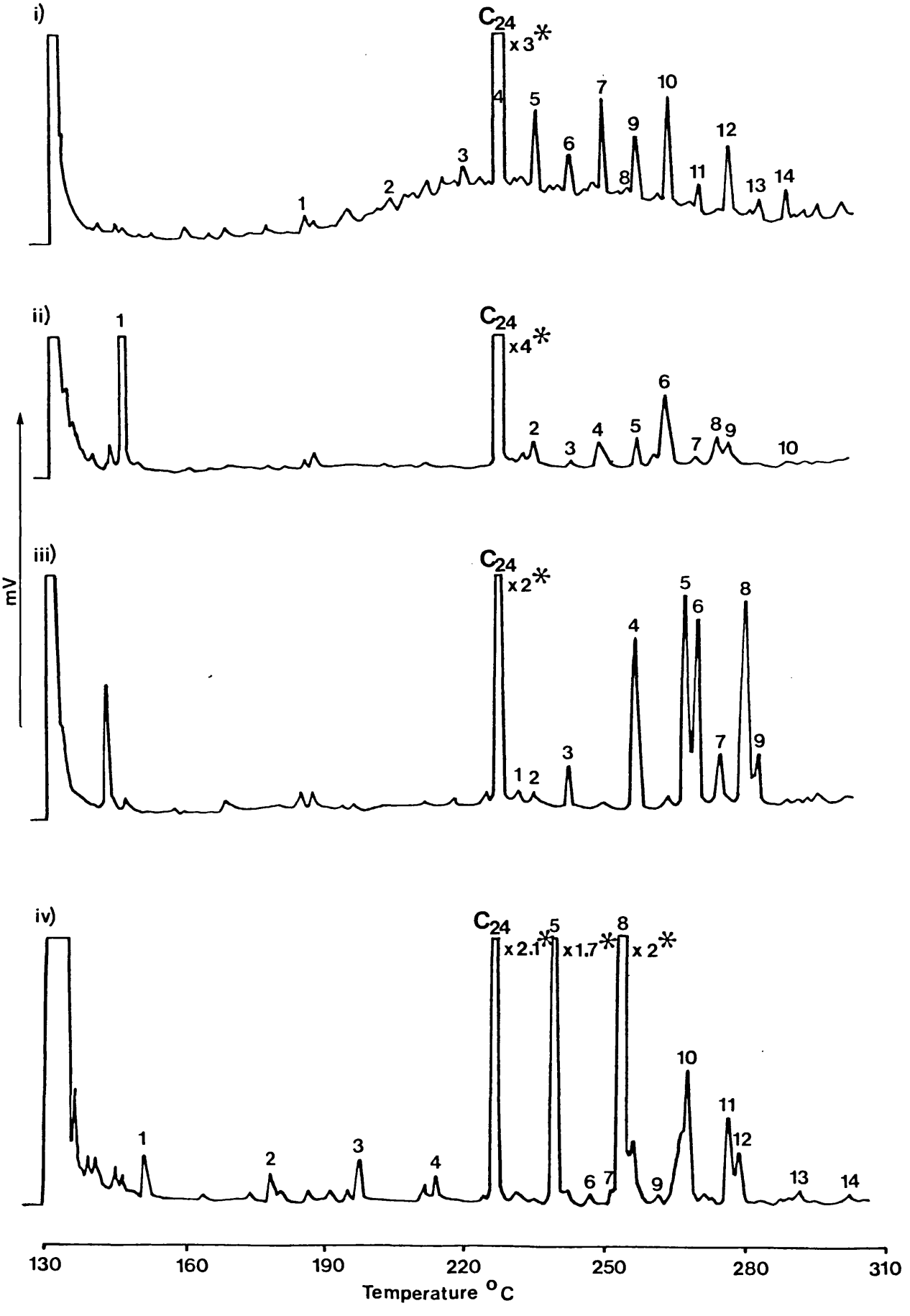
iii) Band 3 sample

1 Phthalate
2 Phthalate + C_{22} alcohol acetate
3 Terpene
4 C_{24} alcohol acetate
5 C_{26} alcohol acetate
6 C_{26} alcohol acetate + unknown
7 C_{27} alcohol acetate
8 C_{28} alcohol acetate + unknown
9 C_{29} alcohol acetate + unknown

iv) Band 4 sample (silylated)

1 Butyl phthalate + unknown
2 Unknown mixture
3 Phthalate + C_{16} primary alcohol TMSi
4 Phthalate + C_{16} primary alcohol TMSi
5 Unknown (base peak m/e 101) + hydrocarbon
6 C_{24} primary alcohol TMSi + trace unknown
7 Unknown + terpene (M^+ 486)
8 Unknown (base peak m/e 101) + hydrocarbon C_2H_4 up series from hydrocarbon in 5
9 C_{26} primary alcohol TMSi + trace acetate
10 Unknown (base peak m/e 101) + hydrocarbon C_2H_4 up series from hydrocarbon in 8
11 C_{27} primary alcohol TMSi
12 C_{27} primary alcohol TMSi + unknown
13 C_{29} primary alcohol TMSi + unknown C_2H_4 up series from unknown in 12
14 C_{31} primary alcohol TMSi

Fig. 5.3



* multiplication factor to give true peak height

Fig. 5.3 continued

v) Band 5 sample (acetylated)

1 C_{22} alcohol acetate
2 Terpene
3 C_{24} alcohol acetate
4 C_{25} alcohol acetate
5 C_{26} alcohol acetate + unknown
6 C_{26} alcohol acetate
7 C_{27} alcohol acetate + unknown
8 C_{28} alcohol acetate + unknown
9 C_{28} alcohol acetate
10 C_{30} alcohol acetate + unknown
11 β-amyrin acetate + unknown

vi) Band 5 sample (silylated)

1 C_{22} alcohol TMSi
2 C_{24} alcohol TMSi
3 C_{25} alcohol TMSi
4 C_{26} alcohol TMSi
5 C_{26} alcohol TMSi
6 C_{27} alcohol TMSi
7 C_{28} alcohol TMSi
8 C_{28} alcohol TMSi
9 C_{30} alcohol TMSi + β-amyrin

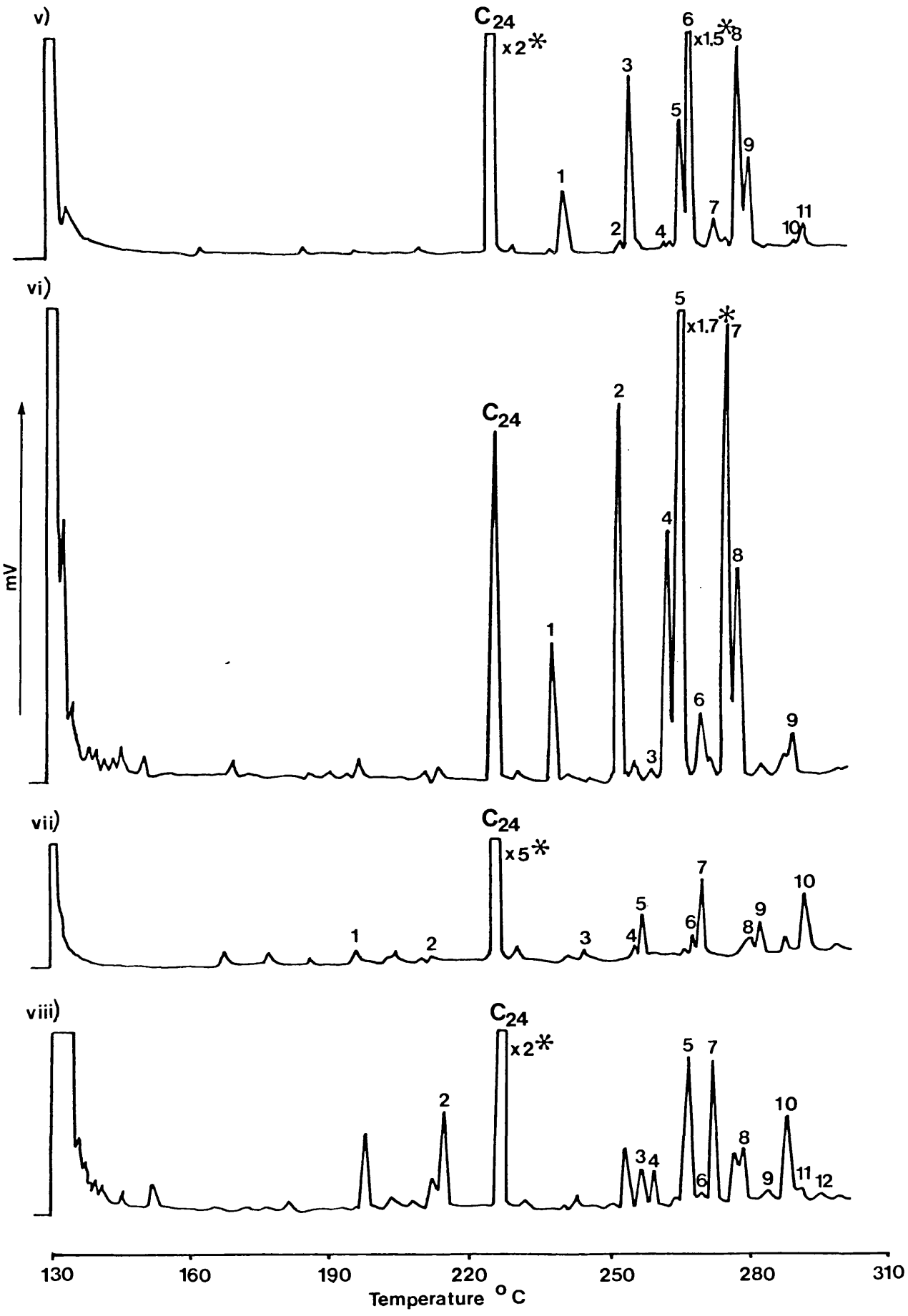
vii) Band 6 sample (acetylated)

1 Phthalate
2 Ethyl octadecanoate
3 C_{22} alcohol acetate + unknown
4 C_{24} alcohol acetate + unknown
5 C_{26} alcohol acetate + C_{22} aldehyde
6 C_{26} alcohol acetate + unknown
7 C_{28} hydrocarbon + C_{24} aldehyde
8 C_{28} alcohol acetate + unknown
9 C_{30} hydrocarbon + C_{26} aldehyde
10 C_{32} hydrocarbon + C_{28} aldehyde

viii) Band 6 sample (silylated)

1 C_{16} monobasic acid TMSi ester
2 C_{18} monobasic acid TMSi ester
3 Mixture of acid + unknown
4 Possibly C_{18} dihydroxymonobasic acid methyl ester TMSi ether (M^+-15 459)
5 Unknown base peak m/e 219, mol.wt. 472
6 Possibly C_{20} dihydroxymonobasic acid methyl ester TMSi ether (M^+-15 487)
7 Unknown base peak m/e 219, mol.wt. 514
8 Unknown base peak m/e 219, mol.wt. 528
9 Possibly C_{27} monobasic acid TMSi ester + unknown
10 Unknown mixture
11 Possibly C_{29} monobasic acid TMSi ester + unknown
12 Unknown mixture

Fig. 5.3 continued



Wax esters are usually saturated C_{24}, C_{26} and C_{28} primary alcohols esterified with saturated fatty acids (Thompson, 1980). However, in some plants, for example B.napus, ester chain lengths between C_{38} and C_{50} have been found (Holloway, Brown, Baker and Macey, 1977). The molecular ions (M^+) of the unknown compounds (peaks 3, 6, 7, 8 and 9) are given as a percentage of the base peak. A more concentrated sample of this band would be needed to confirm the identity of these compounds. Terpenoid compounds have been found in plant waxes, for example ursolic acid is a major component of apple wax (Silva Fernandes, Baker and Martin, 1964), so that the identification of terpenes in watercress wax is not unexpected (band 2 peak 4, band 3 peak 3, band 4 peak 7, band 5 (acetylated) peak 2).

The compounds in the sample from band 3 (Fig. 5.3 iii) were identified as alcohol acetates as expected from their R_f values. The major acetates were esters of even-carbon-numbered alcohols - C_{24}, C_{26} and C_{28}. The doublet peaks (5 and 6, 8 and 9), identified as C_{26} and C_{28} alcohol acetates, are possibly isomeric, and further g.c.-m.s. analyses and g.c. of authentic compounds are needed to confirm this possibility. Hydrolysis of the acetates to the free alcohols, and analysis as the TMSi ethers, would confirm their identities.

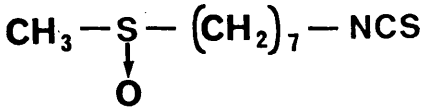
Compounds in band 4 (Fig. 5.3 iv) were silylated to form the TMSi ethers of alcohols present at this R_f value. Most of the peaks were found to consist of mixtures. Primary alcohols in plant waxes are mainly, but not exclusively, even-carbon-numbered chains (Eglinton and Hamilton, 1967). However, in the watercress wax sample both odd and even-carbon-numbered primary alcohols were identified, and the doublet peaks (11 and 12), both identified as C_{27} primary alcohol, may be isomers. The three major peaks (5, 8 and 10) contained mixtures

of an unknown with base peak m/e 101 and a homologous series of hydrocarbons. The unknowns were not silylated, which eliminates the presence of carboxyl, hydroxyl and enol groups in these compounds. They may be thiocyanates or isothiocyanates since their base peak is m/e 101 (Hamming and Foster, 1972), and confirmation could be obtained, for example, by chromatographic methods specific for these compounds such as those suggested by Kjaer (1960).

Figures 5.3 v and vi show chromatograms of the acetylated and silylated band 5 samples. Compounds were identified as odd and even-carbon-numbered alcohols; the even-carbon-numbered C_{24}, C_{26} and C_{28} were the major ones (Fig. 5.3 v peaks 3, 5 and 8, and Fig. 5.3 vi peaks 2, 4 and 7). The doublet peaks (v 5 and 6, 8 and 9, vi 4 and 5, 7 and 8) may be branched isomers. In this band β-amyrin was identified (v 11, vi 9) and it has also been found in the triterpenol fraction of several brassica species (Baker and Holloway, 1975; Holloway et al, 1977).

Band 6 (Fig. 5.3 vii) contained alcohols, hydrocarbons and aldehydes which should have had higher R_f values, but these compounds may have been inhibited by pigmented material on the base line. Fatty acids would remain on the base line, and C_{16} and C_{18} monobasic acids were identified by g.c.-m.s. in the silylated sample (Fig. 5.3 viii peaks 1 and 2). Tentative identifications were made of C_{27} and C_{29} monobasic acids (9 and 11) and C_{18} and C_{20} dihydroxy-monobasic acid methyl esters (4 and 6). Fatty acids found in plant waxes are usually C_{16}, C_{18} and the even-carbon-numbered members between C_{24} and C_{36} (Holloway 1980 - personal communication), so that further confirmation is required for those found in the watercress wax. The unknown peaks with a base peak m/e 219 (Fig. 5.3 viii peaks 5,

7 and 8) are thought to be long chain compounds which form a methylsulphinylisothiocyanate fragment

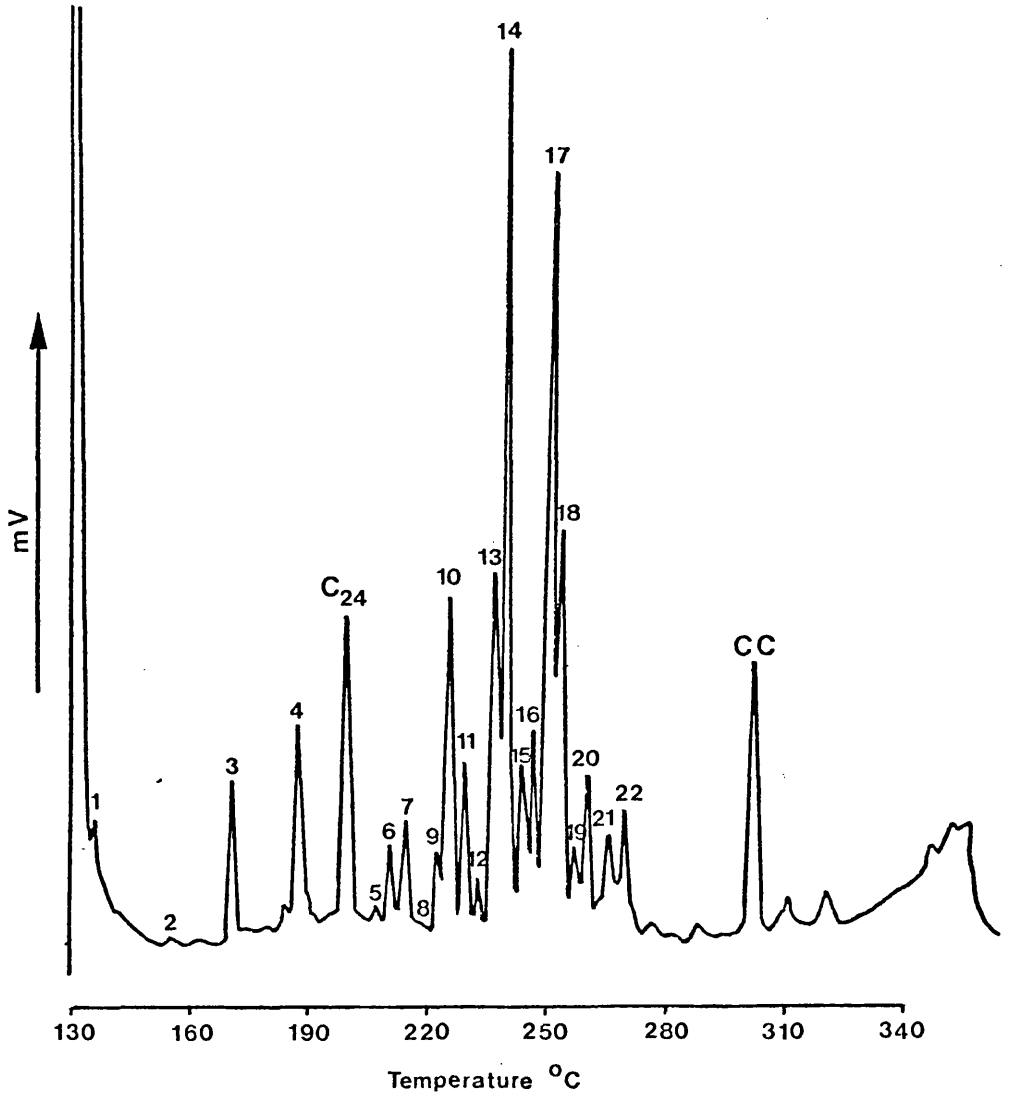


with a molecular weight of 219 (Spencer and Daxenbichler, 1980). The standard methods used to study plant waxes (Martin and Juniper, 1970) are not designed to detect thiocyanates or isothiocyanates. Specific separation techniques are required for these compounds.

Figure 5.4 is the chromatogram of a whole wax sample from watercress (bulked old, young and Florida cress sample). The peaks were identified using retention time data from the PLC samples and g.c.-m.s. results. Most of the peaks were mixtures with several unknown compounds. Further work using different columns may produce better resolution of peaks and identification of the unknowns.

The methods used for extracting and analysing plant waxes described in 5.2.2.3 detect high molecular weight compounds, but not those of low molecular weights. Work by Ismail et al (1977) suggests that relatively low molecular weight volatile compounds which contribute to the flavour and aroma of the material may be trapped in the cuticular wax of fruits and leaves. Therefore, samples of wax from watercress flowers and leaves, dissolved in diethyl ether, were analysed using capillary columns, and the results are shown in Fig. 5.5. Many compounds were identified in the leaf wax sample (Fig. 5.5 i) which were lower members of the classes of compounds found using TLC and PLC. The aldehydes identified (peaks 13, 22, 26, 44 and 50) have been reported in the volatiles of several other vegetables (Van Straten, 1977). Aliphatic aldehydes which contribute to both desirable and undesirable odours and flavours in natural

Fig. 5.4 Chromatogram of epicuticular wax of watercress. Sample size: 5 µl (0.1 mg) + 1 µl tetracosane reference (C_{24}) (1 mg ml^{-1}) + 1 µl cholesterol caprylate reference (CC) (1 mg ml^{-1}). Amplifier attenuation 10^2x 16. Column: 90 x 0.2 cm glass packed with 1% Dexsil 300 on 100-120 mesh Supelcoport.



Peak identities : -

1 Phenethyl isothiocyanate
2 Butyl phthalate + C_{19} hydrocarbon
3 C_{16} alcohol + C_{21} hydrocarbon
4 C_{22-23} hydrocarbon
5 C_{25} hydrocarbon
6 C_{22} alcohol + C_{22} acetate + phthalate
7 Unknown, base peak m/e 101 + hydrocarbon + phthalate
8 C_{23} alcohol
9 Unknown, base peak m/e 219 + C_{27} hydrocarbon
10 C_{24} alcohol + C_{24} acetate
11 Unknown, base peak m/e 101 + C_{29} hydrocarbon
12 C_{25} alcohol
13 Unknown, base peak m/e 219 + C_{31} hydrocarbon
14 C_{26} alcohol + C_{26} acetate
15 Unknown, base peak m/e 101 + C_{32} hydrocarbon
16 C_{27} alcohol + C_{27} acetate
17 Unknown, base peak m/e 219 + C_{33} hydrocarbon
18 C_{28} alcohol + C_{28} acetate
19 C_{34} hydrocarbon
20 Unknown
21 C_{30} alcohol + C_{35} hydrocarbon
22 ß-amyrin

Peak identifications : -

i) Leaf Wax

2 1,5-heptadiene-3,4-diol
9 Tetrahydro-2-methyl-furan
11 Methyl benzene
12 Octane
4 Ethyl benzene
13 Heptanal
14 Vinyl benzene
18 Dimethyl trisulphide
26 Benzaldehyde
30 Unknown, base peak m/e 43
31 1H-indene
32 Nonanol
22 Nonanal
44 Phenyl acetaldehyde
47 Acetophenone
48 Possibly hexyl isothiocyanate M^+ 143
50 Decanal
51 Unknown, base peak m/e 41 M^+ 141
56 Naphthalene
68 Benzyl alcohol
70 Possible isothiocyanate M^+ 157 (3%) base peak m/e 43 (100%) *2
71 Phenethyl alcohol
60 Thiocyanate or sulphide M^+ 155 (5%) base peak m/e 41 (100%) *1
78 Phenol
74 Benzothiazole
82 Possible isothiocyanate M^+ 171 (0.8%) base peak m/e 43 (100%) *2
83 Phenethyl nitrile
66 Thiocyanate or sulphide M^+ 169 (1.0%) base peak m/e 41 (100%) *1
84 1',1-biphenyl
85 1,2,3-propanetriol triacetate
86 Benzyl isothiocyanate
88 Undecane
89 Substituted phenol, base peak m/e 150
90 Unknown, base peak m/e 59
92 Phenethyl isothiocyanate
99 1H-indole
95 Diethyl-1,2-benzene dicarboxylate
97 Unknown hydrocarbon
98 Phenyl butyl isothiocyanate
91 Eicosane
100 7-methyl-thio-heptyl isothiocyanate
101 Pentacosane
102 Hexadecanol or hexadecene
103 8-methyl-thio-octyl isothiocyanate
104 Unknown hydrocarbon
93 Hexadecanoic acid

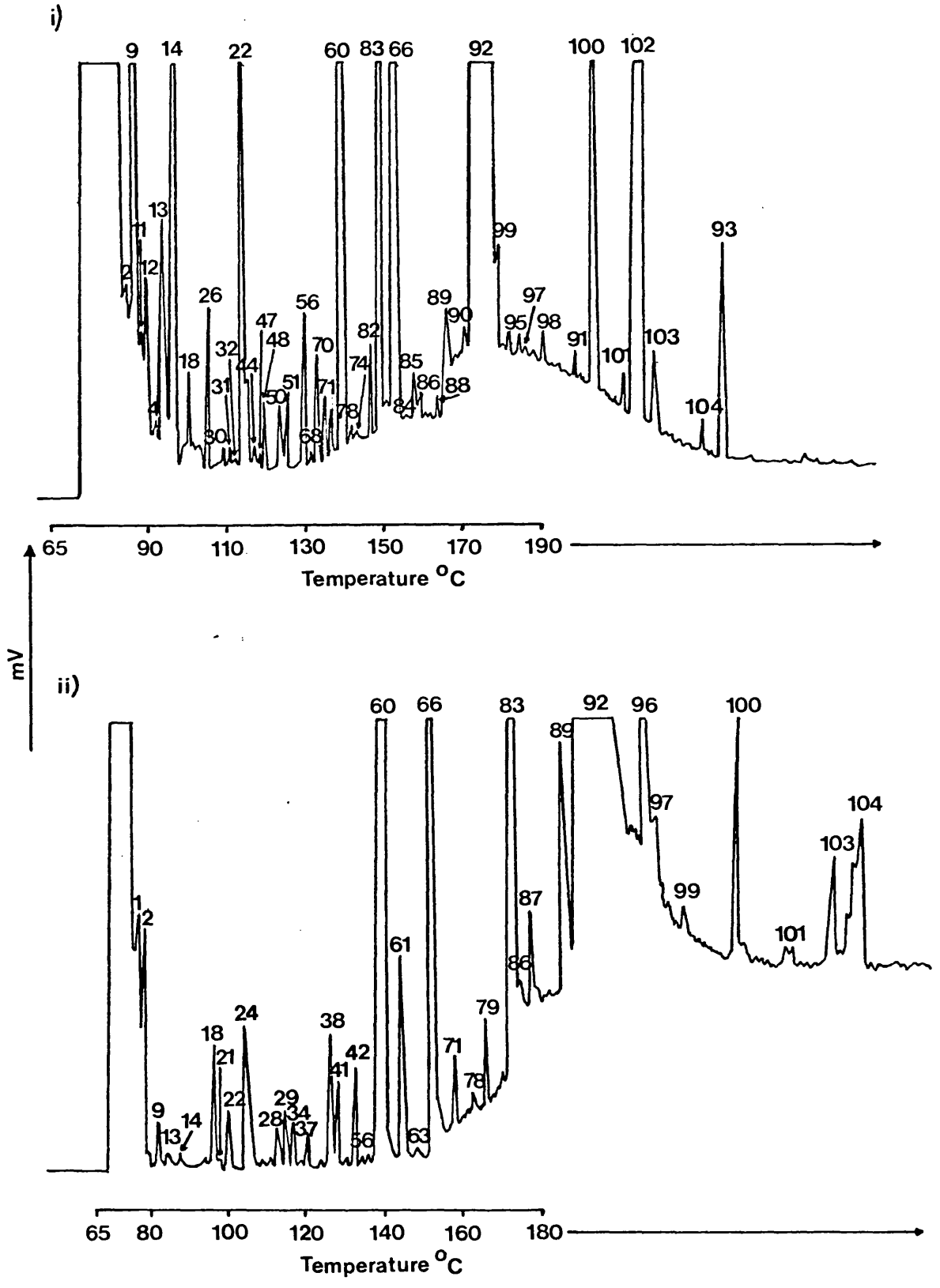
ii) Flower Wax

1 3-penten-2-one
2 1,5-heptadiene-3,4-diol
9 Tetrahydro-2-methyl-furan
13 Heptanal
14 Vinyl benzene
18 Dimethyl trisulphide
21 4-hydroxy-4-methyl-2-pentanone
22 Nonanal
24 Acetic acid
28 Unknown, base peak m/e 41
29 Unknown hydrocarbon
34 2-methyl propanoic acid
37 Thiocyanate or sulphide M^+ 94 (32%) base peak m/e 41 (100%) *1
38 Thiocyanate or sulphide M^+ 108 (5%) base peak m/e 41 (100%) *1
41 2-methyl butanoic acid
42 Unknown, base peak m/e 45
56 Naphthalene
60 Thiocyanate or sulphide M^+ 155 (3%) base peak m/e 41 (100%) *1
61 Possible aromatic aldehyde M^+ 148 (0.8%) base peak m/e 91 (100%)
63 Hexanoic acid
66 Thiocyanate or sulphide M^+ 169 (0.5%) base peak m/e 41 (100%) *1
71 Phenethyl alcohol
78 Phenol
79 Unknown, base peak m/e 43
83 Phenethyl nitrile
86 Benzyl isothiocyanate
87 Ethyl tetradecanoate
89 Substituted phenol, base peak m/e 150
92 Phenethyl isothiocyanate
96 Vinyl phenyl ether
97 Unknown hydrocarbon
99 1H-indole
100 7-methyl-thio-heptyl isothiocyanate
101 Pentacosane
103 8-methyl-thio-octyl isothiocyanate
104 Unknown hydrocarbon

*1 homologous series increasing by CH_2

*2 homologous series increasing by CH_2

Fig. 5.5 Chromatograms of wax samples from watercress leaves i) and flowers ii). Sample size 0.5 µl. Columns: SF-96-200 capillary column for leaf wax sample, carbowax SCOT capillary column for flower wax sample. Attenuation x 10.



products are derived from lipids by oxidation of unsaturated fatty acids and their esters (Forss, 1972). Galliard and Phillips (1976) showed that cucumber contains an enzyme system capable of causing cleavage of linoleic acid to trans-2-nonenal, which is responsible for the characteristic odour of cucumber. Hexanal is also formed by the enzymic cleavage of linoleic acid and Galliard, Matthew, Fishwick and Wright (1976) propose a sequence of enzymic reactions to explain the formation of carbonyl products in disrupted cucumber tissue. Fatty acids are formed by acyl hydrolase action on the lipid, whilst α-oxidation results in the production of $C_{12 - 17}$ aldehydes. Lipoxygenase action and cleavage result in the formation of C_6 and C_9 aldehydes. Similar mechanisms may be responsible for these classes of compounds found in watercress.

Nonanal has been identified as a major component in the cuticular wax of plums and is important in the overall aroma of this fruit (Ismail et al, 1977). It is also reported to be significant in the aroma of cauliflower and broccoli (Buttery et al, 1976) and nonanal may contribute to the aroma of watercress since it appears as a large peak in the leaf wax sample (Fig. 5.5 i peak 22), and it has a low threshold value (1 mg l^{-1} in paraffin oil, Stahl, 1973). Phenyl acetaldehyde (peak 44) has been identified in tomato volatiles (Kazeniac and Hall, 1970) and also benzaldehyde (peak 26) (Buttery et al, 1971). These compounds are thought to be formed by oxidative degradation of lipids; phenyl acetaldehyde being derived from phenyl alanine. This degradation may also occur in watercress as it is reported to contain 62 mg phenylalanine per 100 g edible tissue (Salunkhe, 1974).

There may be an alcohol - aldehyde relationship in watercress as observed in many fruits and vegetables, including tomato (Schornmüller and Grosch, 1965), cabbage (MacLeod and MacLeod, 1968) and pea (Ralls et al, 1965). In Fig. 5.5 i the following related alcohols and aldehydes were identified : nonanol (32) and nonanal (22), benzyl alcohol (68) and benzaldehyde (26), phenethyl alcohol (71) and phenyl acetaldehyde (44). However, recent work by Gil and MacLeod (in press) with garden cress (L. sativum) suggests that alcohol and aldehyde formation is not linked in that plant. Methyl-branched alcohols are probably derived from amino acids, while other alcohols are derived from C_{18} and C_{20} unsaturated fatty acids, either directly or via the aldehydes (Forss, 1972).

The lack of fatty acids in samples of watercress leaf wax examined by TLC and PLC was also evident in the sample analysed by capillary column g.c.-m.s.; only hexadecanoic acid (93) was identified. Fatty acids may be formed in foods by enzymic hydrolysis of triglycerides and other lipid components (Forss, 1972). A hexadecanoate and other possible acid esters were identified in the wax analysed by PLC. Hexadecanoates may be formed from hexadecanoic acid since direct esterification between a free acid and a fatty alcohol is one mechanism for wax ester synthesis (Kolattukudy, 1975). A similar explanation may account for the 1,2,3-propanetriol triacetate (85) identified in watercress wax because large quantities of acetic acid were present in the volatiles (Section 4).

Acetophenone was the only ketone identified in watercress leaf wax (Fig. 5.5 i peak 47). Its occurrence has been reported in tomato by Buttery et al (1971). In watercress, this compound, together with other aromatic compounds identified in the leaf wax - phenol (78),

methyl benzene (11), ethyl benzene (4), vinyl benzene (14) and tetrahydro-2-methyl-furan (9), may arise from the lipid material present (Forss, 1972).

Since long chain hydrocarbons are prevalent in most higher plant waxes, including watercress (Fig. 5.3 i), it was not surprising to find lower members (octane, 12 and undecane, 88) as well as C_{20} and C_{25} hydrocarbons (91 and 101) in the leaf wax sample. Naphthalene has been reported in the volatiles of many foods (Van Straten, 1977) and was found in the leaf wax of watercress (56).

Watercress, in common with other cruciferous vegetables, contains large amounts of organic sulphur (c. 130 mg per 100 g tissue, Paul and Southgate, 1978). The characteristic flavour and odour, particularly of cooked vegetables of the genus Brassica , have been attributed to sulphur-containing compounds which have been detected in their volatiles. However, the importance of these sulphur compounds with respect to flavour and aroma has not been fully established. Dimethyl disulphide occurs in a number of vegetables (Van Straten, 1977) and is considered a major contributor to the aroma of heated tomato products (Buttery et al, 1971) and cooked cabbage (Dateo et al, 1957). This compound was identified in the 7 day headspace collection of watercress volatiles (Section 4), but not in the wax. However, dimethyl trisulphide was identified in the watercress leaf wax sample (18). This compound has been detected in fresh cabbage by Bailey, Bazinet, Driscoll and McCarthy (1961) and in cooked cabbage by Maruyama (1970) who considers it to be important in the aroma of cooked brassicas.

Several isothiocyanates were positively identified in the leaf wax sample (86, 92, 100 and 103) and others need further investigation

for more than a tentative identification (70 and 82). Previous work on watercress volatiles by MacLeod and Islam (1975) identified phenethyl nitrile as the major compound. This is formed from the same precursor as phenethyl isothiocyanate, which was identified as the second major compound. Both these compounds were found in the watercress leaf wax (83 and 92), and also benzyl isothiocyanate (86) but not its nitrile, which were previously reported together in garden cress (MacLeod and Islam, 1976). Several glucosinolate products were found by Gil and MacLeod (in press) in garden cress seedlings which were identified in the watercress leaf wax i.e. benzaldehyde (26), benzyl alcohol (68), benzyl isothiocyanate (86) and phenethyl nitrile (83). In both garden cress and watercress a common metabolic pathway may exist for the production of these compounds which, in watercress, are subsequently trapped in the wax.

The two methylthioalkylisothiocyanates identified as peaks 100 and 103 in the leaf wax from published spectra (Kjaer et al, 1963) have been found in watercress seeds by Kaoulla, MacLeod and Gil (in press). The corresponding nitriles, detected in the seeds, were not identified in the wax although they have been found (without the isothiocyanates) in watercress leaves by MacLeod and Islam (1975). Phenyl butyl isothiocyanate, detected in horseradish roots (Grob and Matile, in press) was identified as peak 98. Peak 82 may be 8-methylthiooctanonitrile, but further work is required for identification of this peak and others thought to be isothiocyanates, sulphides or thiocyanates.

The degradation of glucosinolates to isothiocyanates or nitriles may depend on specificity of myrosinase enzymes, or specific cofactors, or, as suggested by Gil and MacLeod (1978), pH. At neutral pH isothiocyanate production is favoured, but as the pH decreases with

glucosinolate degradation and the release of sulphate ions, nitrile production increases and eventually exceeds isothiocyanate production. This process would occur during natural autolysis in the plant where, despite any buffering capacity, the pH decreases as glucosinolates decompose. The identification of nitriles and isothiocyanates in plants depends, therefore, on whether the extraction methods used alter pH, and also on the age of the material. Differences in the nitrile - isothiocyanate ratios in L. sativum as the plant ages have been reported by Gil and MacLeod (in press), although conflicting results were obtained with two types of garden cress ("plain" and "curled"). Results from the "plain cress" type indicated that the young seedling produces larger amounts of isothiocyanates than nitriles on autolysis, but as it ages, nitrile production increases and exceeds isothiocyanate production. Similar studies with "curled cress" seedlings produced contradictory results. Further detailed studies are required to understand all the contributing factors affecting the degradation of glucosinolates and the relative proportions of their products.

The petals of many flowers are known to have a fine waxy bloom due to a smooth, non-crystalline wax layer (Martin and Juniper, 1970). The sample of the wax from watercress flowers (Fig. 5.5 ii) contained many compounds found in the leaf wax (Fig. 5.5 i) in addition to several short chain fatty acids (Fig. 5.5 ii peaks 24, 34, 41 and 63), unidentified hydrocarbons (79, 97, 104) and two ketones (1, 21). Phenethyl isothiocyanate (92) was the major compound in both the flower and leaf waxes. Dimethyl trisulphide (18), phenethyl nitrile (83) and other isothiocyanates (86, 100, 103) identified in the leaf wax were also identified in the flower wax. Two other unidentified thiocyanates or sulphides belonging to the same series as the compounds in the major peaks 60 and 66 were found (37, 38).

Many plant flowers secrete lipophilic substances produced in epidermal or parenchyma cells, or by specialized secretory tissue (Fahn, 1979). Several flowers owe their fragrance to volatile terpenes which diffuse from the cytoplasm of the epidermal cells through the cell wall and cuticle to the surface. The flowers of watercress have little odour and no terpenes were found. Generally, the leaf and flower wax of watercress contained similar compounds; most being components of the epicuticular wax except for the glucosinolate degradation products.

The findings of this study warrant further investigation into those compounds trapped in the wax layer which are not part of its structure but contribute to the aroma and flavour of the watercress. Aroma was found to be a reliable indicator of watercress freshness (Section 3). The increasing level of watercress and seaweed odours as the plant deteriorates may be due to the release of sulphur compounds trapped in the wax. Since the flower wax was found to contain no fragrant terpenes, the floral odour observed as watercress deteriorates is not due to those compounds associated with flower fragrance, but most probably to the oxidation products of carotenes, the ionones, which are discussed in Section 4 and do not appear to be trapped in the plant wax.

5.2.4 Summary

The epicuticular wax of watercress contains many classes of compounds commonly found in plant waxes. However, the presence of glucosinolate degradation products, trapped in the wax after release within the plant, interferred with the standard methods of analysis and

identification. Future work using specific methods to separate these compounds would overcome this problem. Many components were identified which are possible lipid degradation products and others were plant volatiles trapped in the wax layer. The wax from watercress flowers contained very similar compounds to the leaf wax and no terpenes responsible for flower fragrance were identified. Several of the sulphur compounds trapped in the wax are reported to be significant in the flavour and aroma of different vegetables, and they probably contribute to watercress odour, particularly as they accumulate during plant senescence. Investigation into the changes in the composition of the wax as the plant deteriorates would increase the understanding of those mechanisms associated with autolysis, and point to possible means of delaying deterioration.

5.3 Ultrastructure of the Watercress Leaf Cuticular Wax

5.3.1 Materials

Watercress - as described in 5.2.1.

Sodium cacodylate - 97% BDH.

Glutaraldehyde - 25% purified for electron microscopy EMscope.

Acetone - A.R. BDH.

Instruments used - Cambridge Mark II A stereoscan electron microscope.

- Cambridge S4 stereoscan electron microscope.
- Polaron E 5000 critical point drier.
- Polaron sputter coater.

5.3.2 Methods

The ultrastructure of the wax layer was studied by scanning electron microscopy using both fresh and fixed material. The fresh material was prepared using methods described by Baker and Holloway (1971). Some material was also fixed before examination. Small squares (c. 3 x 3 mm) were cut from watercress leaves, fixed for 1 h in a mixture of 1 ml 0.2M sodium cacodylate, 1 ml glutaraldehyde and 8 ml deionised water; then dehydrated in 70%, 90% and three changes of 100% acetone (v/v aqueous solutions), each for 15 min before drying in a critical point drier. The specimens were mounted on aluminium stubs using double-sided tape and coated with gold in a sputter coater.

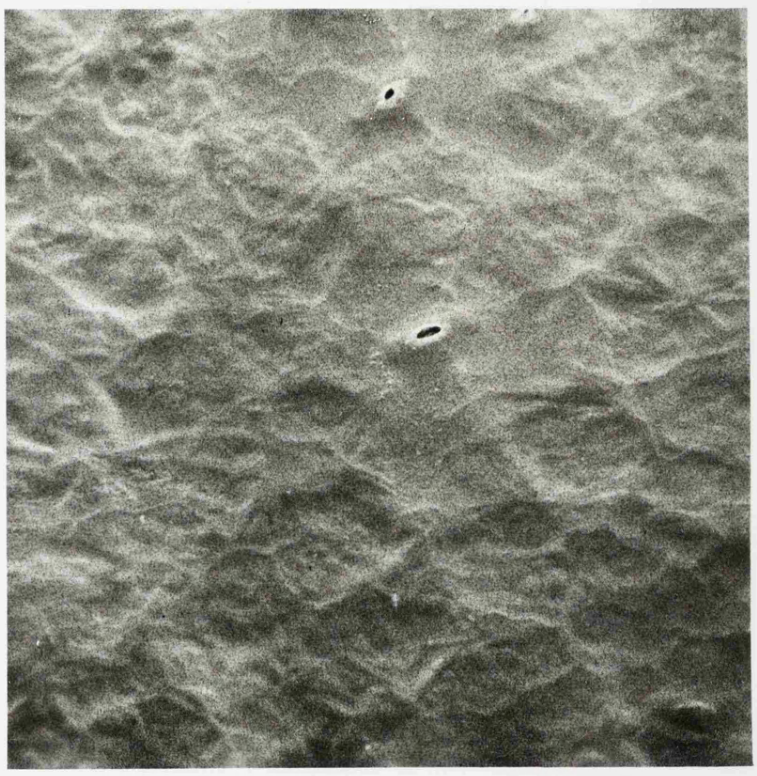
5.3.3 Results and Discussion

The surfaces of watercress leaves were examined for structures which may be responsible for the storage and secretion of volatile compounds. Both adaxial and abaxial surfaces of young, old and Florida watercress leaves were studied and found to be covered with a flat, even layer of wax without protruding structures. The wax was not composed of random deposits which form tubes, coral-like aggregates or rods in many other plants (Baker and Holloway, 1971). The weight of wax on watercress leaves (c. 0.5 $\mu g\ cm^{-2}$ leaf surface) is much less than that on brassica leaves (c. 50 - 60 $\mu g\ cm^{-2}$).

The adaxial and abaxial leaf surfaces of fresh and fixed material are shown in Plates 5.1 and 5.2 respectively. The process of fixing appears to cause the adhesion of foreign particles to the leaf surface; these particles were not present on the fresh material. However, both plates show a fairly flat surface with stomata on adaxial and abaxial

Plate 5.1 Scanning electron micrographs of the epicuticular wax on watercress leaves (fresh material).

a) Adaxial surface x 510
b) Abaxial surface x 455



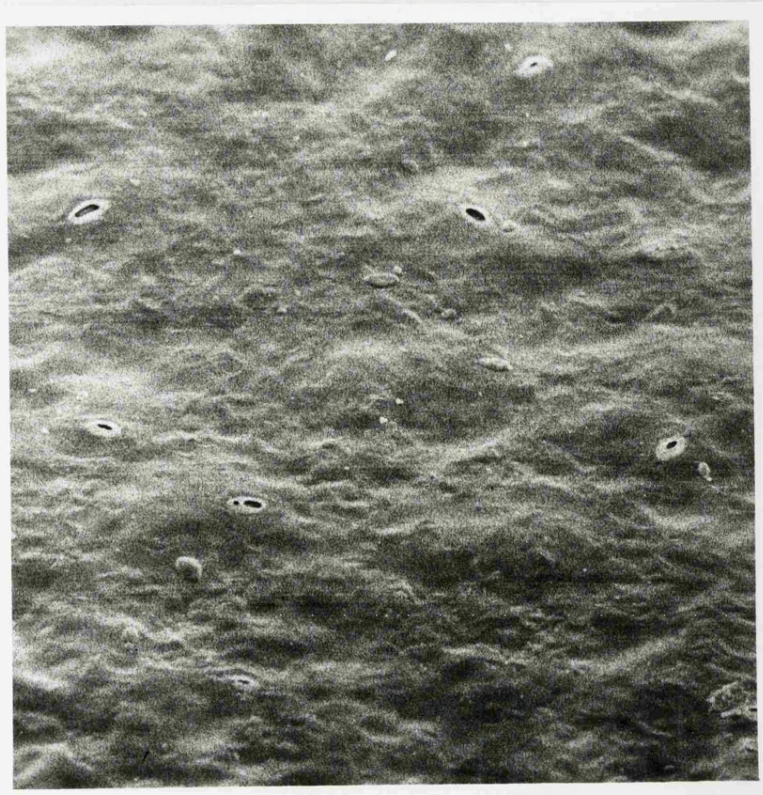


Plate 5.2 Scanning electron micrographs of the epicuticular wax on watercress leaves (fixed material).

a) Adaxial surface x 900

b) Abaxial surface x 900

surfaces. The wax surface has a more "cushioned" appearance in the photomicrographs of the fixed material (Plate 5.2) which may be due to the fixation process.

The formation of crystalline wax deposits depends on both temperature and rate of crystallisation. Work by Baker (1974) supports the view that wax is carried through the cuticle in a solvent. Alternative suggestions are that wax is extruded in a softened form through a smooth cuticle (Müller, Carr and Loomis, 1954), or through special pores (Hall and Donaldson, 1962). Baker suggests that the solvent transporting the wax may be composed of volatile compounds such as short chain aldehydes, ketones, alcohols and sulphur compounds. This suggestion may account for the presence of many volatile compounds in the watercress wax (Section 5.2) which were also detected by head-space collection and ether washing (Section 4).

The surface properties of plants are important in relation to cuticular transpiration, colonisation by microorganisms and the deposition, distribution and absorption of applied chemicals. Watercress leaves do not need a thick wax layer to reduce cuticular transpiration because of their aquatic environment. Few microorganisms were found on the leaf surfaces of watercress (Section 4.6), and this may be due to the absence of protective crevices on the flat wax surface.

The results obtained from the analyses of watercress wax and the examination of its ultrastructure suggest that both its chemical composition and structure are very different from those of brassica species studied. This supports the hypothesis that the structure of the wax surface is strongly dependent on its chemical composition.

5.3.4 Summary

The surfaces of watercress leaves are covered by a thin, flat layer of wax which has no protruding structures for the storage or secretion of volatile compounds. The ultrastructural examination of the watercress wax combined with chemical analyses suggest a correlation between structure and composition, when compared with the structure and composition of other plant waxes.

GENERAL DISCUSSION

Although several different areas of work have been studied, they each developed from work in the previous section. The introductory experiment showed that different seasonal daylengths during crop growth may affect its post-harvest life, but further work combining daylength effects with seasonal temperatures is needed to relate this to the natural environment in commercial beds.

During a study of several commercial distribution chains it was found that watercress was exposed to great variations in conditions from harvesting to sale at wholesale and retail markets, which overshadowed the effects of refrigerated and unrefrigerated transport, and differences in the length of time from harvesting to retailing. There appears to be a greater need for better handling by the wholesaler and retailer to increase the shelf-life of watercress.

Physiological methods used along the distribution chains were unable to detect small changes in quality but sensory assessments, using the profile developed during this work, may be of more value. Further large-scale testing of the sensory profile on bunches of watercress from different retail outlets and after storage in different conditions will decide whether alterations are needed to cover the range of quality encountered.

The value of aroma assessment in determining watercress freshness was unexpected, but found to be a reliable indicator, particularly when used with odour reference samples. In order to identify these aroma compounds, volatiles released by the plant were collected and also the cuticular wax analysed for its contribution to aroma. Results from both these areas of work suggest inadequate techniques were used

by other workers. Previous methods for the collection of watercress volatiles were destructive and resulted in the identification of glucosinolate degradation products as the major components. In this study many compounds, not previously reported in watercress, were identified, including α and β-ionones which may be responsible for the primrose odour observed in the sensory work, and sulphur compounds resulting in seaweed and rotting aromas.

Isothiocyanates and thiocyanates, found to be trapped in the wax and contributing to the aroma of the plant material, were not separated from the wax components by standard methods of analysis. This became apparent by their interference when using standard cuticular wax analyses.

The large quantities of acetic acid found in the samples of volatiles were not produced by microorganisms on the watercress, but by the plant. Unlike the surface of many Cruciferae, watercress wax was found to be an amorphous layer without structures capable of retaining flavour compounds. However, compounds are trapped in the wax layer which contribute to the flavour and aroma of the plant material.

The dual headspace apparatus was effective in determining the effects of controlled post-harvest conditions on watercress. Storage in the dark at 10^{o}C in a current of air kept the watercress fresh and crisp apart from yellowing of leaves, which was prevented by cytokinin treatment. Many compounds contribute to the different aromas observed as watercress deteriorates and more positive identification of volatiles and wax constituents will lead to an understanding of the production mechanisms of different compounds and to the detection of quality indicators.

Areas of future work are suggested below : -

1) Plant production using nutrient film technique for physico-chemical examination and for commercial purposes.

2) The combined effects of daylength and temperature on growth and shelf-life of watercress, developing improved methods for measuring shelf-life.

3) A study of the effects of different storage conditions on watercress in prepacks, particularly dark storage at 10^{o}C in an air current. This should include an examination of the effect of storage conditions on the environment in the prepacks and the plant microflora.

4) Further testing of the sensory profile and odour reference samples on watercress covering a wide quality range.

5) Further examination of cuticular wax using separation methods for glucosinolate degradation products.

6) Analysis of wax and collection of volatiles from yellow watercress leaves.

7) Determination of the position of myrosin cells in watercress by localising the enzyme using lead sulphate (Hall, 1978) or electron autoradiographic analysis of labelled glucosinolates (Iversen, 1973).

8) Determination of the effect of plant constituents, particularly isothiocyanates, on the insect population on watercress, especially virus-transmitting aphids. Aphid attack may be related to plant age because the aphids appeared to be attracted to older plants in the simulation tanks.

9) Monitoring ascorbic acid and carotene levels during storage, and the effect of cytokinin treatments on the levels of these compounds.

10) Collection of volatiles for further identification of compounds, particularly those containing sulphur and nitrogen, and examination of the effect of sulphate nutrition on watercress volatiles.

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APPENDIX

Raw data from the study of commercial distribution chains of watercress (Section 2).

Coding of data :

1st digit	2nd digit	3rd digit	4th digit
1 = unrefrigerated long chain	1 = March	1 = Cutting/Pulling	(reading)
	2 = April	2 = Arrival at Packhouse	
2 = refrigerated long chain	3 = May	3 = After bunching	
3 = unrefrigerated short chain	4 = June	4 = After hydrocooling	
	5 = July	5 = After cold storage	
4 = prepack B chain		6 = Lorry change	
5 = prepack A chain		7 = Arrival at market	
		8 = Selling at market	
		9 = Arrival at Bath	

a) Cress temperature
Relative humidity
Chlorophyll content
Carbon dioxide level
Water vapour level
} for unrefrigerated v refrigerated long chains

b) Cress temperature
Relative humidity
Chlorophyll content
Carbon dioxide level
Water vapour level
} for prepack chains

c) Cress temperature
Relative humidity
Chlorophyll content
Carbon dioxide level
Water vapour level
} for unrefrigerated short chain

d) Greenness values for data from all distribution chains for calculation of hue angle : 4th digit = a value
5th digit = b value

a)

cresstemp

1 1 1 10	1 3 3 16	2 3 8 11.5	
1 1 1 12	1 3 3 15.5	2 3 8 11.5	
1 1 1 11	1 3 3 16	2 3 8 11.5	
1 1 2 11	1 3 4 4	2 3 9 15.5	
1 1 2 11	1 3 4 4.5	2 3 9 15.0	
1 1 2 11	1 3 4 3.5	2 3 9 14.5	
1 1 3 13	1 3 5 7	2 4 1 11.5	
1 1 3 13	1 3 5 6.7	2 4 1 11.5	
1 1 3 12.5	1 3 5 6.7	2 4 1 11.3	
1 1 4 6	1 3 6 13.4	2 4 3 12.0	
1 1 4 6	1 3 6 13.4	2 4 3 11.7	
1 1 4 3	1 3 6 13.8	2 4 3 12.1	
1 1 5 6	1 3 8 15.6	2 4 4 7.0	
1 1 5 6	1 3 8 14.6	2 4 4 7.5	
1 1 5 6	1 3 8 14.7	2 4 4 5.8	
1 1 6 7.5	1 3 9 17.4	2 4 5 7.2	
1 1 6 8	1 3 9 16.8	2 4 5 6.7	
1 1 6 6	1 3 9 16.2	2 4 5 6.5	
1 1 8 6	1 4 2 14	2 4 8 10.5	
1 1 8 6	1 4 2 14	2 4 8 9.5	
1 1 8 6	1 4 2 13	2 4 8 10.0	
1 1 9 9.5	1 4 3 15	2 4 9 14.5	
1 1 9 8	1 4 3 15	2 4 9 13.5	
1 1 9 9.5	1 4 3 14.5	2 4 9 15.0	
1 2 1 8.7	1 4 4 3.2		
1 2 1 8.7	1 4 4 4		
1 2 1 9	1 4 4 3.6		
1 2 2 7.5	1 4 5 5.6		
1 2 2 8.5	1 4 5 6		
1 2 2 7.5	1 4 5 5.4		
1 2 3 12.5	1 4 6 11		
1 2 3 12.5	1 4 6 10.5		
1 2 3 11.5	1 4 6 10.6		
1 2 4 2.5	1 4 8 14.9		
1 2 4 2.3	1 4 8 15		
1 2 4 3.2	1 4 8 14.4		
1 2 5 4.9	1 4 9 17.6		
1 2 5 5.2	1 4 9 16		
1 2 5 5.3	1 4 9 16		
1 2 6 9.2	2 3 1 14.5		
1 2 6 8.2	2 3 1 15.6		
1 2 6 8.0	2 3 1 15.5		
1 2 8 12.6	2 3 3 14.3		
1 2 8 13	2 3 3 14.5		
1 2 8 12.8	2 3 3 14.8		
1 2 9 18.5	2 3 4 9.0		
1 2 9 18.9	2 3 4 9.8		
1 2 9 17.8	2 3 4 9.7		
1 3 1 16.5	2 3 5 9.0		
1 3 1 18	2 3 5 8.5		
1 3 1 18	2 3 5 8.9		

relativeturgidity

1 1 2 96.394	1 2 4 98.979	1 3 8 89.242	2 3 3 94.510
1 1 2 99.012	1 2 4 96.457	1 3 8 91.127	2 3 3 91.283
1 1 2 100	1 2 4 96.883	1 3 8 92.718	2 3 4 87.422
1 1 2 100	1 2 4 97.662	1 3 8 89.072	2 3 4 95.585
1 1 2 100	1 2 4 96.143	1 3 9 91.787	2 3 4 91.946
1 1 3 100	1 2 5 96.073	1 3 9 90.432	2 3 4 90.432
1 1 3 98.595	1 2 5 97.112	1 3 9 90.259	2 3 4 93.710
1 1 3 100	1 2 5 97.429	1 3 9 89.461	2 3 5 94.017
1 1 3 100	1 2 5 99.13	1 3 9 88.206	2 3 5 91.247
1 1 3 100	1 2 5 99.438	1 4 1 94.598	2 3 5 94.17
1 1 4 100	1 2 6 96.24	1 4 1 86.458	2 3 5 91.479
1 1 4 100	1 2 6 95.104	1 4 1 85.185	2 3 5 92.97
1 1 4 100	1 2 6 95.897	1 4 1 87.5	2 3 8 91.928
1 1 4 100	1 2 6 91.866	1 4 1 86.304	2 3 8 93.555
1 1 4 98.554	1 2 6 96.977	1 4 3 91.105	2 3 8 91.063
1 1 5 100	1 2 8 96.709	1 4 3 91.542	2 3 8 95.011
1 1 5 90.698	1 2 8 96.573	1 4 3 91.228	2 3 8 91.694
1 1 5 98.163	1 2 8 97.457	1 4 3 92.447	2 3 9 95.594
1 1 5 93.705	1 2 8 94.656	1 4 3 91.729	2 3 9 95.673
1 1 5 100	1 2 8 97.839	1 4 4 98.56	2 3 9 94.42
1 1 6 99.277	1 2 9 95.710	1 4 4 94.226	2 3 9 89.361
1 1 6 94.588	1 2 9 94.764	1 4 4 98.369	2 3 9 92.411
1 1 6 92.938	1 2 9 94.262	1 4 4 98.284	2 4 1 91.210
1 1 6 98.571	1 2 9 95.549	1 4 4 96.765	2 4 1 91.208
1 1 6 90.524	1 2 9 96.892	1 4 5 91.489	2 4 1 91.587
1 1 8 95.082	1 3 1 87.553	1 4 5 96	2 4 1 89.473
1 1 8 90.068	1 3 1 86.035	1 4 5 96.252	2 4 1 93.762
1 1 8 97.007	1 3 1 83.516	1 4 5 91.063	2 4 3 93.446
1 1 8 100	1 3 1 86.634	1 4 5 94.145	2 4 3 91.263
1 1 8 96.912	1 3 1 86.067	1 4 6 95.217	2 4 3 90.101
1 1 9 100	1 3 3 84.165	1 4 6 94.63	2 4 3 88.884
1 1 9 93.231	1 3 3 82.389	1 4 6 92.715	2 4 3 91.111
1 1 9 93.850	1 3 3 81.29	1 4 6 93.36	2 4 4 90.791
1 1 9 94.382	1 3 3 83.677	1 4 8 93.75	2 4 4 92.395
1 1 9 96.107	1 3 3 84.804	1 4 8 98.8	2 4 4 92.627
1 2 1 100	1 3 4 85.365	1 4 8 94.672	2 4 4 92.975
1 2 1 94.976	1 3 4 84.89	1 4 8 91.598	2 4 4 97.052
1 2 1 94.976	1 3 4 83.903	1 4 8 95.185	2 4 5 93.429
1 2 1 96.977	1 3 4 88.89	1 4 9 90.769	2 4 5 94.409
1 2 1 96.543	1 3 4 82.834	1 4 9 90.566	2 4 5 93.295
1 2 1 97.542	1 3 5 91.355	1 4 9 93.612	2 4 5 95.041
1 2 2 94.699	1 3 5 92.857	1 4 9 94.343	2 4 5 93.491
1 2 2 92.874	1 3 5 93.095	1 4 9 92.207	2 4 8 91.875
1 2 2 96.642	1 3 5 91.729	2 3 1 88.04	2 4 8 95.881
1 2 2 100	1 3 5 89.578	2 3 1 87.481	2 4 8 94.360
1 2 2 97.56	1 3 6 92.241	2 3 1 98.503	2 4 8 94.020
1 2 3 96.842	1 3 6 87.922	2 3 1 95.09	2 4 8 94.020
1 2 3 100	1 3 6 83.903	2 3 1 89.643	2 4 9 92.121
1 2 3 96.009	1 3 6 85.542	2 3 3 93.561	2 4 9 95.345
1 2 3 100	1 3 6 88.281	2 3 3 91.671	2 4 9 94.303
1 2 3 100	1 3 8 89.4	2 3 3 92.059	2 4 9 95.304
			2 4 9 95.564

a) continued

chlorophyll

1 1 2	.0334	1 2 4	.03294	1 3 8	.03876	2 3 3	.04521				
1 1 2	.03363	1 2 4	.04017	1 3 8	.03866	2 3 3	.03576				
1 1 2	.03668	1 2 4	.03458	1 3 8	.0373	2 3 4	.03443				
1 1 2	.03478	1 2 4	.03495	1 3 9	.04028	2 3 4	.03555				
1 1 2	.04323	1 2 5	.03238	1 3 9	.03453	2 3 4	.03576				
1 1 3	.03499	1 2 5	.03315	1 3 9	.03591	2 3 4	.03512				
1 1 3	.03563	1 2 5	.03627	1 3 9	.0406	2 3 4	.03647				
1 1 3	.0343	1 2 5	.03468	1 3 9	.04539	2 3 5	.03683				
1 1 3	.03363	1 2 5	.03343	1 4 1	.03273	2 3 5	.03614				
1 1 3	.03648	1 2 6	.03904	1 4 1	.03457	2 3 5	.03694				
1 1 4	.03614	1 2 6	.03532	1 4 1	.0365	2 3 5	.0396				
1 1 4	.0376	1 2 6	.04101	1 4 1	.03606	2 3 5	.03884				
1 1 4	.0395	1 2 6	.0346	1 4 1	.0359	2 3 8	.04058				
1 1 4	.03878	1 2 6	.0372	1 4 3	.03284	2 3 8	.03794				
1 1 4	.03658	1 2 8	.03371	1 4 3	.03595	2 3 8	.04252				
1 1 5	.03263	1 2 8	.03415	1 4 3	.03514	2 3 8	.04117				
1 1 5	.03499	1 2 8	.04027	1 4 3	.03361	2 3 8	.03561				
1 1 5	.03366	1 2 8	.04166	1 4 3	.03125	2 3 9	.03576				
1 1 5	.03524	1 2 8	.02828	1 4 4	.03506	2 3 9	.03763				
1 1 5	.03099	1 2 9	.03192	1 4 4	.03187	2 3 9	.03599				
1 1 6	.03543	1 2 9	.03409	1 4 4	.03243	2 3 9	.03978				
1 1 6	.03612	1 2 9	.0416	1 4 4	.03238	2 3 9	.03496				
1 1 6	.04517	1 2 9	.03432	1 4 4	.02466	2 4 1	.03755				
1 1 6	.03576	1 2 9	.0378	1 4 5	.03363	2 4 1	.03211				
1 1 6	.03519	1 3 1	.03945	1 4 5	.03373	2 4 1	.03219				
1 1 8	.02869	1 3 1	.04247	1 4 5	.03391	2 4 1	.03058				
1 1 8	.0333	1 3 1	.04847	1 4 5	.03514	2 4 1	.03053				
1 1 8	.03361	1 3 1	.0415	1 4 5	.03422	2 4 3	.03470				
1 1 8	.03355	1 3 1	.05208	1 4 6	.03519	2 4 3	.03540				
1 1 8	.03337	1 3 3	.04549	1 4 6	.03049	2 4 3	.03412				
1 1 9	.0396	1 3 3	.04616	1 4 6	.03614	2 4 3	.03422				
1 1 9	.03536	1 3 3	.03514	1 4 6	.03576	2 4 3	.03238				
1 1 9	.0374	1 3 3	.0395	1 4 6	.03791	2 4 4	.03611				
1 1 9	.0356	1 3 3	.03945	1 4 8	.0353	2 4 4	.03488				
1 1 9	.03166	1 3 4	.04268	1 4 8	.03443	2 4 4	.03550				
1 2 1	.03817	1 3 4	.04047	1 4 8	.03509	2 4 4	.03587				
1 2 1	.02995	1 3 4	.04109	1 4 8	.03525	2 4 4	.03514				
1 2 1	.02747	1 3 4	.04713	1 4 8	.03243	2 4 5	.03376				
1 2 1	.02952	1 3 4	.03509	1 4 9	.03714	2 4 5	.03565				
1 2 1	.03125	1 3 5	.03504	1 4 9	.0355	2 4 5	.03550				
1 2 2	.03466	1 3 5	.04134	1 4 9	.0325	2 4 5	.03550				
1 2 2	.03153	1 3 5	.03473	1 4 9	.03181	2 4 5	.03624				
1 2 2	.03325	1 3 5	.03503	1 4 9	.03068	2 4 8	.03624				
1 2 2	.04222	1 3 5	.04222	2 3 1	.03432	2 4 8	.03740				
1 2 2	.0351	1 3 6	.04434	2 3 1	.03248	2 4 8	.03661				
1 2 3	.03639	1 3 6	.04511	2 3 1	.03519	2 4 5	.03652				
1 2 3	.03303	1 3 6	.04657	2 3 1	.03776	2 4 8	.03540				
1 2 3	.03766	1 3 6	.04672	2 3 1	.03243	2 4 9	.03704				
1 2 3	.03102	1 3 6	.05049	2 3 3	.03591	2 4 9	.03529				
1 2 3	.03397	1 3 8	.0376	2 3 3	.03422	2 4 9	.03794				
1 2 4	.03732	1 3 8	.04534	2 3 3	.0334	2 4 9	.03606				
						2 4 9	.03647				

Carbon Dioxide

1 1 4	0.2	1 4 6	0.28				
1 1 4	0.25	1 4 6	0.5				
1 1 4	0.25	1 4 6	0.8				
1 1 5	1.0	1 4 8	0.5				
1 1 5	1.5	1 4 8	1.3				
1 1 5	1.2	1 4 8	0.8				
1 1 6	0.1	1 4 9	1.2				
1 1 6	0.08	1 4 9	2.0				
1 1 6	0.05	1 4 9	1.6				
1 1 8	0.6	2 3 4	0.4				
1 1 8	0.4	2 3 4	0.2				
1 1 8	0.6	2 3 4	0.5				
1 1 9	0.6	2 3 5	0.1				
1 1 9	0.7	2 3 5	0.08				
1 1 9	0.5	2 3 5	0.08				
1 2 4	0.13	2 3 8	0.3				
1 2 4	0.1	2 3 8	0.15				
1 2 4	0.2	2 3 8	0.15				
1 2 5	1.3	2 3 9	0.3				
1 2 5	1.0	2 3 9	0.5				
1 2 5	0.8	2 3 9	0.4				
1 2 6	0.5	2 4 4	0.32				
1 2 6	0.23	2 4 4	0.26				
1 2 6	0.4	2 4 4	0.29				
1 2 8	2.0	2 4 5	0.18				
1 2 8	2.0	2 4 5	0.10				
1 2 8	2.0	2 4 5	0.12				
1 2 9	4.0	2 4 8	0.13				
1 2 9	5.8	2 4 8	0.02				
1 2 9	4.8	2 4 8	0.05				
1 3 4	0.1	2 4 9	0.6				
1 3 4	0.17	2 4 9	0.3				
1 3 4	0.2	2 4 9	0.45				
1 3 5	1.5						
1 3 5	1.0						
1 3 5	2.0						
1 3 6	1.7						
1 3 6	0.9						
1 3 6	1.2						
1 3 8	1.5						
1 3 8	1.2						
1 3 8	1.6						
1 3 9	2.5						
1 3 9	2.4						
1 3 9	2.5						
1 4 4	0.18						
1 4 4	0.28						
1 4 4	0.2						
1 4 5	0.32						
1 4 5	0.6						
1 4 5	0.4						

Water Vapour

1 1 4	9.0	1 4 6	6.0				
1 1 4	8.0	1 4 6	7.0				
1 1 4	5.0	1 4 6	5.0				
1 1 5	5.0	1 4 8	9.0				
1 1 5	5.0	1 4 8	7.0				
1 1 5	6.0	1 4 8	8.0				
1 1 6	5.0	1 4 9	12.0				
1 1 6	6.0	1 4 9	12.0				
1 1 6	5.0	1 4 9	12.5				
1 1 8	5.0	2 3 4	7.0				
1 1 8	5.0	2 3 4	6.0				
1 1 8	5.0	2 3 4	9.0				
1 1 9	10.0	2 3 5	5.0				
1 1 9	10.0	2 3 5	5.0				
1 1 9	7.0	2 3 5	5.0				
1 2 4	5.0	2 3 8	7.0				
1 2 4	5.0	2 3 8	7.0				
1 2 4	6.0	2 3 8	7.0				
1 2 5	5.0	2 3 9	11.0				
1 2 5	5.0	2 3 9	13.0				
1 2 5	5.0	2 3 9	14.0				
1 2 6	5.0	2 4 4	7.0				
1 2 6	5.0	2 4 4	6.0				
1 2 6	6.0	2 4 4	5.0				
1 2 8	10.0	2 4 5	5.0				
1 2 8	8.0	2 4 5	4.0				
1 2 8	8.0	2 4 5	2.5				
1 2 9	15.0	2 4 8	6.0				
1 2 9	17.0	2 4 8	6.0				
1 2 9	17.0	2 4 8	7.0				
1 3 4	6.0	2 4 9	10.0				
1 3 4	5.0	2 4 9	10.0				
1 3 4	6.0	2 4 9	8.0				
1 3 5	5.0						
1 3 5	5.0						
1 3 5	5.0						
1 3 6	12.0						
1 3 6	12.0						
1 3 6	12.0						
1 3 8	7.0						
1 3 8	9.0						
1 3 8	6.0						
1 3 9	14.0						
1 3 9	12.0						
1 3 9	15.0						
1 4 4	5.0						
1 4 4	5.						
1 4 4	8.0						
1 4 5	5.0						
1 4 5	5.0						
1 4 5	5.0						

b)

Cress	Temp
4 4 2	15.5
4 4 5	15.6
4 4 5	14.4
4 4 5	16.6
4 4 8	12.8
4 4 8	10.4
4 4 8	11.8
4 4 9	13.5
4 4 9	13.5
4 4 9	13.6
4 5 2	13.0
4 5 2	12.0
4 5 2	12.5
4 5 4	4.0
4 5 4	5.0
4 5 4	4.5
4 5 5	10.0
4 5 5	11.0
4 5 5	10.5
4 5 8	13.2
4 5 8	13.8
4 5 9	20.0
4 5 9	20.0
4 5 9	20.0
5 4 2	15.0
5 4 2	15.0
5 4 5	16.5
5 4 5	14.2
5 4 5	15.0
5 4 6	9.5
5 4 6	9.8
5 4 6	9.8
5 4 8	16.7
5 4 8	18.2
5 4 8	18.0
5 4 9	24.2
5 4 9	23.8
5 4 9	23.8
5 5 2	11.0
5 5 2	11.0
5 5 2	11.5
5 5 5	12.5
5 5 5	12.0
5 5 5	14.0
5 5 8	13.3
5 5 8	13.2
5 5 8	12.7
5 5 9	17.4
5 5 9	18.0
5 5 9	18.0

Relative Turgidity			
4 4 2	94.503	5 4 2	99.277
4 4 2	91.213	5 4 2	97.222
4 4 2	93.006	5 4 2	97.326
4 4 2	98.876	5 4 4	92.840
4 4 2	88.069	5 4 4	96.073
4 4 4	97.022	5 4 4	95.907
4 4 4	94.843	5 4 4	93.794
4 4 4	98.275	5 4 4	94.471
4 4 4	98.871	5 4 5	98.740
4 4 4	99.071	5 4 5	95.410
4 4 5	97.931	5 4 5	96.969
4 4 5	98.591	5 4 5	97.129
4 4 5	95.544	5 4 5	94.103
4 4 5	100.00	5 4 6	96.954
4 4 5	100.00	5 4 6	96.984
4 4 8	100.00	5 4 6	98.160
4 4 8	99.783	5 4 6	96.997
4 4 8	100.00	5 4 6	96.261
4 4 8	99.777	5 4 8	95.890
4 4 8	99.378	5 4 8	92.974
4 4 9	99.518	5 4 8	96.287
4 4 9	98.305	5 4 8	94.000
4 4 9	96.99	5 4 8	89.485
4 4 9	96.923	5 4 9	94.414
4 4 9	96.190	5 4 9	92.982
4 5 2	100.00	5 4 9	90.400
4 5 2	95.980	5 4 9	91.606
4 5 2	100.00	5 4 9	90.151
4 5 2	98.396	5 5 2	100.00
4 5 2	100.00	5 5 2	100.00
4 5 4	100.00	5 5 2	95.701
4 5 4	100.00	5 5 2	97.403
4 5 4	100.00	5 5 2	99.782
4 5 4	100.00	5 5 4	96.101
4 5 4	100.00	5 5 4	97.468
4 5 5	97.756	5 5 4	100.00
4 5 5	98.611	5 5 4	98.646
4 5 5	100.00	5 5 4	98.018
4 5 5	94.840	5 5 5	98.810
4 5 5	100.00	5 5 5	98.853
4 5 8	98.969	5 5 5	92.952
4 5 8	99.490	5 5 5	97.489
4 5 8	93.333	5 5 5	94.588
4 5 8	100.00	5 5 8	100.00
4 5 8	100.00	5 5 8	98.429
4 5 9	93.443	5 5 8	97.382
4 5 9	94.684	5 5 8	98.237
4 5 9	92.031	5 5 8	98.922
4 5 9	94.183	5 5 9	94.293
4 5 9	92.992	5 5 9	96.970
5 4 2	96.675	5 5 9	95.641
5 4 2	92.558	5 5 9	94.656
		5 5 9	91.314

Chlorophyll			
4 4 2	.03817	5 4 2	.02864
4 4 2	.04104	5 4 2	.03084
4 4 2	.03728	5 4 2	.03376
4 4 2	.03681	5 4 4	.04053
4 4 2	.04063	5 4 4	.03637
4 4 4	.03758	5 4 4	.03976
4 4 4	.03340	5 4 4	.03702
4 4 4	.03340	5 4 4	.03883
4 4 4	.03484	5 4 5	.03565
4 4 4	.03824	5 4 5	.03486
4 4 5	.03689	5 4 5	.03376
4 4 5	.03807	5 4 5	.03098
4 4 5	.03766	5 4 5	.03443
4 4 5	.03787	5 4 6	.03668
4 4 5	.04354	5 4 6	.03726
4 4 8	.03804	5 4 6	.03632
4 4 8	.03863	5 4 6	.03945
4 4 8	.04017	5 4 6	.04060
4 4 8	.03837	5 4 8	.03711
4 4 8	.03788	5 4 8	.03586
4 4 9	.03776	5 4 8	.03381
4 4 9	.03919	5 4 8	.03791
4 4 9	.03491	5 4 8	.03750
4 4 9	.03863	5 4 9	.02897
4 4 9	.03755	5 4 9	.03069
4 5 2	.03470	5 4 9	.02736
4 5 2	.03450	5 4 9	.02705
4 5 2	.03340	5 4 9	.03096
4 5 2	.03380	5 5 2	.03505
4 5 2	.03126	5 5 2	.02921
4 5 4	.03459	5 5 2	.03228
4 5 4	.03318	5 5 2	.03242
4 5 4	.03207	5 5 2	.03133
4 5 4	.0298	5 5 4	.03387
4 5 4	.03372	5 5 4	.03164
4 5 5	.03461	5 5 4	.03874
4 5 5	.03271	5 5 4	.03631
4 5 5	.03287	5 5 4	.03381
4 5 5	.03213	5 5 5	.03592
4 5 5	.03448	5 5 5	.03459
4 5 8	.03126	5 5 5	.03105
4 5 8	.03542	5 5 5	.03156
4 5 8	.03284	5 5 5	.03315
4 5 8	.03346	5 5 8	.03059
4 5 8	.03377	5 5 8	.03045
4 5 9	.03484	5 5 8	.02855
4 5 9	.03638	5 5 8	.03269
4 5 9	.03686	5 5 8	.02905
4 5 9	.03760	5 5 9	.03604
4 5 9	.03310	5 5 9	.03633
5 4 2	.03189	5 5 9	.03725
5 4 2	.03058	5 5 9	.03284
		5 5 9	.03545

Carbon Dioxide	
4 4 5	1.7
4 4 5	1.1
4 4 5	1.8
4 4 8	0.4
4 4 8	0.2
4 4 8	0.1
4 4 9	2.0
4 4 9	1.7
4 4 9	0.8
4 5 5	0.1
4 5 5	0.2
4 5 5	0.16
4 5 8	0.4
4 5 8	0.4
4 5 8	0.4
4 5 9	0.34
4 5 9	0.20
4 5 9	0.27
5 4 5	0.10
5 4 5	0.13
5 4 5	0.18
5 4 6	0.24
5 4 6	0.30
5 4 6	0.38
5 4 8	0.60
5 4 8	0.25
5 4 8	0.20
5 4 9	0.60
5 4 9	1.10
5 4 9	0.90
5 5 5	0.20
5 5 5	0.20
5 5 5	0.30
5 5 8	0.36
5 5 8	0.40
5 5 8	0.80
5 5 9	1.50
5 5 9	1.80
5 5 9	1.00

Water	Vapour
4 4 5	12.0
4 4 5	12.0
4 4 5	15.0
4 4 8	15.0
4 4 8	10.0
4 4 8	10.0
4 4 9	20.0
4 4 9	20.0
4 4 9	20.0
4 5 5	10.0
4 5 5	10.0
4 5 5	10.0
4 5 8	10.0
4 5 8	10.0
4 5 8	10.0
4 5 9	20.0
4 5 9	15.0
4 5 9	17.5
5 4 5	9.0
5 4 5	15.0
5 4 5	15.0
5 4 6	6.0
5 4 6	6.0
5 4 6	6.0
5 4 8	15.0
5 4 8	12.0
5 4 8	15.0
5 4 9	20.0
5 4 9	20.0
5 4 9	22.0
5 5 5	10.0
5 5 5	10.0
5 5 5	11.0
5 5 8	8.0
5 5 8	6.0
5 5 8	8.0
5 5 9	12.0
5 5 9	14.0
5 5 9	15.0

c)

Cress Temp

3	1	1	9.0	3	3	3	13.4
3	1	1	9.0	3	3	3	13.4
3	1	1	9.0	3	3	3	13.5
3	1	2	10.0	3	3	4	5.5
3	1	2	10.0	3	3	4	4.5
3	1	2	11.0	3	3	4	4.0
3	1	3	13.0	3	3	5	6.2
3	1	3	13.0	3	3	5	6.7
3	1	3	14.0	3	3	5	4.0
3	1	4	11.0	3	3	7	10.0
3	1	4	11.0	3	3	7	8.7
3	1	4	11.0	3	3	7	8.0
3	1	6	8.0	3	3	8	12.5
3	1	6	6.0	3	3	8	12.4
3	1	6	6.0	3	3	8	12.6
3	1	6	11.0	3	3	9	18.0
3	1	8	12.0	3	3	9	18.5
3	1	8	11.0	3	3	9	18.5
3	1	9	7.0	3	5	2	17.5
3	1	9	8.0	3	5	2	16.5
3	1	9	7.0	3	5	2	18.0
3	2	1	8.0	3	5	3	18.0
3	2	1	8.5	3	5	3	17.7
3	2	1	7.5	3	5	3	16.2
3	2	2	7.5	3	5	4	5.5
3	2	2	6.5	3	5	4	7.0
3	2	2	6.5	3	5	4	5.0
3	2	3	9.5	3	5	5	6.5
3	2	3	8.5	3	5	5	8.2
3	2	3	9.5	3	5	5	5.5
3	2	4	4.0	3	5	7	13.8
3	2	4	3.5	3	5	7	12.5
3	2	4	3.5	3	5	7	12.0
3	2	5	4.5	3	5	8	21.5
3	2	5	4.5	3	5	8	21.6
3	2	5	4.5	3	5	8	21.4
3	2	6	4.0	3	5	9	22.0
3	2	6	4.5	3	5	9	22.0
3	2	6	5.5	3	5	9	22.0
3	2	8	7.0				
3	2	8	7.0				
3	2	8	6.5				
3	2	9	6.5				
3	2	9	6.5				
3	2	9	7.0				
3	3	1	12.5				
3	3	1	12.0				
3	3	1	12.5				
3	3	2	12.0				
3	3	2	11.8				
3	3	2	11.8				

Relative Turgidity

3	1	1	86.829	3	2	4	96.313	3	3	9	94.554
3	1	1	89.743	3	2	4	96.698	3	3	9	97.237
3	1	1	90.476	3	2	4	100.00	3	3	9	88.427
3	1	1	97.849	3	2	4	99.738	3	5	1	89.164
3	1	1	97.495	3	2	6	95.454	3	5	1	94.362
3	1	3	100.00	3	2	6	97.304	3	5	1	94.573
3	1	3	95.025	3	2	6	94.025	3	5	1	95.833
3	1	3	88.293	3	2	6	95.685	3	5	1	96.875
3	1	3	100.00	3	2	6	96.984	3	5	2	89.373
3	1	3	85.500	3	2	8	98.648	3	5	2	93.366
3	1	4	95.556	3	2	8	97.596	3	5	2	87.990
3	1	4	78.571	3	2	8	100.00	3	5	2	77.562
3	1	4	98.770	3	2	8	100.00	3	5	2	92.225
3	1	4	99.502	3	2	8	100.00	3	5	3	98.162
3	1	4	95.238	3	2	9	91.016	3	5	3	93.654
3	1	5	95.089	3	2	9	95.419	3	5	3	96.192
3	1	5	91.827	3	2	9	92.345	3	5	3	94.724
3	1	5	100.00	3	2	9	92.326	3	5	3	93.673
3	1	5	94.595	3	2	9	96.068	3	5	4	99.466
3	1	5	98.522	3	3	1	89.397	3	5	4	99.753
3	1	6	92.344	3	3	1	91.421	3	5	4	100.00
3	1	6	100.00	3	3	1	86.842	3	5	4	100.00
3	1	6	93.534	3	3	1	90.862	3	5	4	100.00
3	1	6	82.014	3	3	1	90.450	3	5	5	96.202
3	1	6	99.000	3	3	2	97.984	3	5	5	95.554
3	1	8	93.333	3	3	2	94.930	3	5	5	97.044
3	1	8	94.652	3	3	2	97.037	3	5	5	97.721
3	1	8	93.158	3	3	2	100.00	3	5	5	97.323
3	1	8	97.340	3	3	2	92.250	3	5	7	92.665
3	1	8	91.477	3	3	4	100.00	3	5	7	94.520
3	1	9	92.784	3	3	4	100.00	3	5	7	93.924
3	1	9	93.103	3	3	4	100.00	3	5	7	96.816
3	1	9	100.00	3	3	4	99.491	3	5	7	90.886
3	1	9	92.271	3	3	4	100.00	3	5	8	99.484
3	1	9	93.103	3	3	5	96.162	3	5	8	98.533
3	2	1	99.163	3	3	5	92.624	3	5	8	96.049
3	2	1	86.593	3	3	5	98.019	3	5	8	90.155
3	2	1	86.666	3	3	5	98.639	3	5	8	95.739
3	2	1	87.473	3	3	5	95.822	3	5	9	94.324
3	2	1	90.745	3	3	7	100.00	3	5	9	93.465
3	2	2	83.959	3	3	7	100.00	3	5	9	95.161
3	2	2	83.979	3	3	7	97.932	3	5	9	93.779
3	2	2	86.989	3	3	7	97.593	3	5	9	93.298
3	2	2	83.000	3	3	7	95.702				
3	2	2	85.185	3	3	8	94.793				
3	2	3	99.343	3	3	8	97.578				
3	2	3	93.361	3	3	8	96.978				
3	2	3	100.00	3	3	8	98.364				
3	2	3	100.00	3	3	8	98.117				
3	2	3	96.912	3	3	9	96.341				
3	2	4	96.402	3	3	9	90.404				

c) continued

Chlorophyll

3 1 1 .02549	3 2 4 .03305	3 3 9 .04885
3 1 1 .02795	3 2 4 .03709	3 3 9 .04119
3 1 1 .02877	3 2 4 .03292	3 3 9 .04485
3 1 1 .02554	3 2 4 .03951	3 5 1 .03100
3 1 1 .02638	3 2 6 .02969	3 5 1 .03379
3 1 3 .02797	3 2 6 .03620	3 5 1 .03203
3 1 3 .02662	3 2 6 .03712	3 5 1 .03709
3 1 3 .03043	3 2 6 .03780	3 5 1 .03284
3 1 3 .03069	3 2 6 .03643	3 5 2 .03669
3 1 3 .02943	3 2 8 .03612	3 5 2 .03591
3 1 4 .02951	3 2 8 .04086	3 5 2 .03678
3 1 4 .02833	3 2 9 .03694	3 5 2 .03458
3 1 4 .02881	3 2 8 .03796	3 5 2 .03566
3 1 4 .02792	3 2 8 .03919	3 5 3 .03525
3 1 4 .02843	3 2 9 .03924	3 5 3 .03463
3 1 5 .02941	3 2 9 .04481	3 5 3 .03642
3 1 5 .02418	3 2 9 .04258	3 5 3 .03443
3 1 5 .03038	3 2 9 .03881	3 5 3 .03571
3 1 5 .02956	3 2 9 .03755	3 5 4 .03874
3 1 5 .02316	3 3 1 .04278	3 5 4 .03787
3 1 6 .03192	3 3 1 .04232	3 5 4 .03485
3 1 6 .03402	3 3 1 .03727	3 5 4 .03587
3 1 6 .03230	3 3 1 .04012	3 5 4 .03320
3 1 6 .03223	3 3 1 .03712	3 5 5 .03697
3 1 6 .03484	3 3 2 .04089	3 5 5 .03462
3 1 8 .02966	3 3 2 .04150	3 5 5 .03815
3 1 8 .03329	3 3 2 .04309	3 5 5 .04221
3 1 8 .03099	3 3 2 .04703	3 5 7 .03771
3 1 8 .03422	3 3 2 .04119	3 5 7 .03637
3 1 8 .03307	3 3 4 .04211	3 5 7 .03581
3 1 9 .03507	3 3 4 .04201	3 5 7 .03765
3 1 9 .03335	3 3 4 .03965	3 5 7 .03673
3 1 9 .03027	3 3 4 .03814	3 5 8 .04202
3 1 9 .03476	3 3 4 .04580	3 5 8 .04307
3 1 9 .03299	3 3 5 .04488	3 5 8 .03633
3 2 1 .03679	3 3 5 .03981	3 5 8 .03966
3 2 1 .04022	3 3 5 .03650	3 5 8 .03705
3 2 1 .04104	3 3 5 .04534	3 5 9 .03258
3 2 1 .03925	3 3 5 .03981	3 5 9 .03513
3 2 1 .04012	3 3 7 .04263	3 5 9 .03294
3 2 2 .03996	3 3 7 .03828	3 5 9 .03479
3 2 2 .03770	3 3 7 .03862	3 5 9 .03053
3 2 2 .03530	3 3 7 .03848	
3 2 2 .03832	3 3 7 .04698	
3 2 2 .03983	3 3 8 .04109	
3 2 3 .03961	3 3 8 .04281	
3 2 3 .03515	3 3 8 .04340	
3 2 3 .03827	3 3 8 .04626	
3 2 3 .03814	3 3 8 .03791	
3 2 3 .03404	3 3 9 .04637	
3 2 4 .03869	3 3 9 .03965	

Carbon Dioxide

3 1 4 0.10
3 1 4 0.20
3 1 4 0.14
3 1 6 1.05
3 1 6 1.0
3 1 6 0.9
3 1 8 0.55
3 1 8 0.45
3 1 8 0.30
3 1 9 0.35
3 1 9 0.37
3 1 9 0.38
3 2 4 0.15
3 2 4 0.2
3 2 4 0.2
3 2 6 0.3
3 2 6 0.8
3 2 6 0.6
3 2 8 1.3
3 2 8 1.4
3 2 8 1.4
3 2 9 0.5
3 2 9 1.4
3 2 9 0.8
3 3 4 0.12
3 3 4 0.18
3 3 4 0.18
3 3 5 0.4
3 3 5 0.48
3 3 5 0.25
3 3 7 0.4
3 3 7 0.55
3 3 7 0.65
3 3 8 2.0
3 3 8 2.0
3 3 8 2.3
3 3 9 2.0
3 3 9 2.5
3 3 9 2.5
3 5 4 0.25
3 5 4 0.16
3 5 5 0.55
3 5 5 0.35
3 5 7 0.2
3 5 7 0.12
3 5 8 2.3
3 5 8 3.0
3 5 9 2.1
3 5 9 2.3

Water Vapour

3 1 4 6.0
3 1 4 9.0
3 1 4 5.0
3 1 6 4.0
3 1 6 5.0
3 1 6 4.0
3 1 8 5.0
3 1 8 5.0
3 1 8 6.0
3 1 9 5.0
3 1 9 4.0
3 1 9 5.0
3 2 4 5.0
3 2 4 5.0
3 2 4 4.0
3 2 6 5.0
3 2 6 5.0
3 2 6 5.0
3 2 8 5.0
3 2 8 5.0
3 2 8 5.0
3 2 9 5.0
3 2 9 5.0
3 2 9 5.0
3 3 4 5.0
3 3 4 6.0
3 3 4 5.0
3 3 5 5.0
3 3 5 6.0
3 3 5 6.0
3 3 7 6.0
3 3 7 6.0
3 3 7 5.0
3 3 8 9.0
3 3 8 8.0
3 3 8 8.0
3 3 9 18.0
3 3 9 17.0
3 3 9 17.0
3 5 4 5.0
3 5 4 6.0
3 5 5 6.0
3 5 5 5.0
3 5 7 10.0
3 5 7 12.0
3 5 8 15.0
3 5 8 15.0
3 5 8 15.0
3 5 9 16.0
3 5 9 15.0
3 5 9 16.0

d)

1 1 2 -15.3 8.1	1 2 4 -14.3 6.8	1 3 8 -16.7 9.4	2 3 3 -15.8 8.4	2 4 9 -16.7 7.6
1 1 2 -15.9 9.1	1 2 4 -15.6 7.0	1 3 8 -16.2 8.5	2 3 3 -15.9 8.8	3 1 1 -15.0 8.3
1 1 2 -15.4 8.8	1 2 4 -15.6 8.4	1 3 8 -16.6 8.9	2 3 4 -15.6 5.8	3 1 1 -14.6 7.8
1 1 2 -15.4 8.8	1 2 4 -12.4 7.2	1 3 9 -15.8 8.5	2 3 4 -15.6 8.4	3 1 1 -15.3 7.2
1 1 2 -16.5 9.5	1 2 5 -15.7 8.1	1 3 9 -14.5 6.8	2 3 4 -14.4 7.1	3 1 1 -15.8 8.8
1 1 3 -15.9 8.9	1 2 5 -15.1 7.7	1 3 9 -15.7 7.4	2 3 4 -15.8 8.7	3 1 1 -15.8 8.8
1 1 3 -16.0 8.5	1 2 5 -15.5 8.1	1 3 9 -17.6 9.2	2 3 4 -16.5 9.5	3 1 3 -17.0 10.7
1 1 3 -15.1 8.9	1 2 5 -16.0 8.7	1 3 9 -15.7 8.3	2 3 5 -15.2 6.8	3 1 3 -15.1 8.7
1 1 3 -15.8 8.4	1 2 5 -14.9 7.3	1 4 1 -16.0 6.6	2 3 5 -17.4 9.8	3 1 3 -15.4 8.4
1 1 3 -15.3 8.1	1 2 6 -14.7 6.8	1 4 1 -15.1 6.0	2 3 5 -15.9 7.3	3 1 3 -15.2 9.2
1 1 4 -15.8 8.9	1 2 6 -14.1 6.5	1 4 1 -15.9 6.3	2 3 5 -14.5 6.9	3 1 3 -15.8 8.9
1 1 4 -14.9 8.8	1 2 6 -15.2 7.3	1 4 1 -15.4 6.7	2 3 5 -15.6 6.6	3 1 4 -15.5 9.4
1 1 4 -14.9 8.7	1 2 6 -14.8 7.8	1 4 1 -16.0 7.3	2 3 8 -16.5 8.9	3 1 4 -15.3 8.7
1 1 4 -15.6 9.7	1 2 6 -15.3 7.8	1 4 3 -15.8 7.2	2 3 8 -15.4 7.1	3 1 4 -15.5 7.9
1 1 4 -16.5 10.2	1 2 8 -15.1 7.2	1 4 3 -15.7 7.5	2 3 8 -16.4 9.2	3 1 4 -16.5 9.6
1 1 5 -16.3 9.0	1 2 8 -15.3 7.5	1 4 3 -15.5 7.8	2 3 8 -15.3 6.9	3 1 4 -15.4 9.1
1 1 5 -16.0 9.1	1 2 8 -16.4 8.5	1 4 3 -15.6 6.9	2 3 8 -16.2 8.9	3 1 5 -15.5 9.5
1 1 5 -16.3 9.2	1 2 8 -14.8 7.2	1 4 3 -15.2 6.2	2 3 9 -17.4 9.5	3 1 5 -15.5 9.4
1 1 5 -16.5 8.8	1 2 8 -15.3 8.6	1 4 4 -15.5 7.0	2 3 9 -16.3 7.7	3 1 5 -16.7 9.3
1 1 5 -16.0 8.6	1 2 9 -14.3 6.9	1 4 4 -16.6 0.8	2 3 9 -15.8 7.4	3 1 5 -14.8 7.7
1 1 6 -15.8 8.9	1 2 9 -15.4 7.1	1 4 4 -17.0 8.4	2 3 9 -16.4 8.8	3 1 5 -15.8 9.9
1 1 6 -16.2 8.8	1 2 9 -16.2 7.9	1 4 4 -16.1 8.2	2 3 9 -16.4 8.0	3 1 6 -15.5 9.7
1 1 6 -16.1 9.2	1 2 9 -16.0 9.2	1 4 4 -16.0 7.9	2 4 1 -16.5 6.1	3 1 6 -16.0 10.1
1 1 6 -16.4 9.1	1 2 9 -17.7 11.3	1 4 5 -16.5 7.4	2 4 1 -15.8 8.1	3 1 6 -15.6 9.3
1 1 6 -16.6 9.3	1 3 1 -15.4 6.7	1 4 5 -16.3 8.0	2 4 1 -16.8 6.2	3 1 6 -15.5 7.9
1 1 8 -16.0 8.4	1 3 1 -14.4 6.6	1 4 5 -16.7 8.4	2 4 1 -16.1 8.1	3 1 6 -16.0 8.7
1 1 8 -16.8 8.7	1 3 1 -15.5 8.2	1 4 5 -16.0 7.6	2 4 1 -15.3 7.5	3 1 8 -15.7 10.1
1 1 8 -16.0 8.7	1 3 1 -14.1 5.9	1 4 5 -16.4 8.1	2 4 3 -16.1 7.5	3 1 8 -15.6 8.9
1 1 8 -16.4 9.3	1 3 1 -16.7 9.3	1 4 6 -16.7 9.1	2 4 3 -15.5 8.2	3 1 8 -15.1 8.6
1 1 8 -16.8 7.4	1 3 3 -15.9 8.3	1 4 6 -16.7 8.0	2 4 3 -15.3 7.9	3 1 8 -15.9 9.7
1 1 9 -16.0 8.4	1 3 3 -13.6 5.8	1 4 6 -15.9 7.0	2 4 3 -16.4 8.4	3 1 8 -15.8 9.9
1 1 9 -17.0 10.0	1 3 3 -14.7 7.7	1 4 6 -16.7 8.2	2 4 3 -15.3 7.7	3 1 9 -15.9 9.1
1 1 9 -17.7 10.3	1 3 3 -14.5 5.2	1 4 6 -16.5 7.3	2 4 4 -15.4 7.7	3 1 9 -15.4 8.2
1 1 9 -16.2 8.5	1 3 3 -13.9 5.5	1 4 8 -16.6 8.1	2 4 4 -16.6 7.2	3 1 9 -16.2 9.2
1 1 9 -16.8 9.1	1 3 4 -12.8 5.3	1 4 8 -17.2 8.0	2 4 4 -15.8 7.7	3 1 9 -16.0 9.2
1 2 1 -14.7 7.8	1 3 4 -16.7 8.8	1 4 8 -16.4 7.1	2 4 4 -15.0 8.0	3 1 9 -17.2 9.7
1 2 1 -14.7 7.7	1 3 4 -15.1 6.9	1 4 8 -16.8 7.9	2 4 4 -17.2 8.5	3 2 1 -15.1 7.4
1 2 1 -15.9 8.4	1 3 4 -14.8 6.6	1 4 8 -16.4 8.4	2 4 5 -15.9 8.0	3 2 1 -14.5 6.8
1 2 1 -15.3 7.9	1 3 4 -13.3 7.0	1 4 9 -16.8 8.3	2 4 5 -16.0 8.4	3 2 1 -15.1 7.0
1 2 1 -16.3 8.6	1 3 5 -14.4 7.5	1 4 9 -17.4 8.8	2 4 5 -16.1 7.4	3 2 1 -14.2 7.3
1 2 2 -15.9 6.5	1 3 5 -14.9 7.1	1 4 9 -16.5 7.7	2 4 5 -15.7 8.6	3 2 1 -15.4 6.9
1 2 2 -16.7 8.0	1 3 5 -15.4 8.1	1 4 9 -16.9 7.9	2 4 5 -16.1 7.4	3 2 2 -15.6 7.5
1 2 2 -13.1 6.6	1 3 5 -15.8 7.5	1 4 9 -16.5 7.7	2 4 8 -16.9 8.1	3 2 2 -14.6 6.7
1 2 2 -16.4 9.3	1 3 5 -15.1 6.7	2 3 1 -16.3 7.7	2 4 8 -16.8 8.6	3 2 2 -14.5 6.3
1 2 2 -12.8 7.0	1 3 6 -15.4 7.0	2 3 1 -15.5 8.9	2 4 8 -15.5 7.0	3 2 2 -14.0 5.7
1 2 3 -14.6 6.7	1 3 6 -14.4 6.3	2 3 1 -16.5 8.8	2 4 8 -16.0 8.2	3 2 2 -15.3 7.6
1 2 3 -14.9 8.6	1 3 6 -15.6 6.4	2 3 1 -15.1 6.6	2 4 8 -15.7 7.8	3 2 3 -13.9 6.1
1 2 3 -14.5 7.7	1 3 6 -16.7 9.7	2 3 1 -14.8 6.4	2 4 9 -16.0 7.0	3 2 3 -14.1 7.2
1 2 3 -15.0 8.4	1 3 6 -14.7 7.1	2 3 3 -14.7 7.5	2 4 9 -17.6 9.1	3 2 3 -14.3 8.4
1 2 3 -14.9 7.5	1 3 8 -16.3 8.4	2 3 3 -15.4 7.9	2 4 9 -15.1 6.6	3 2 3 -14.4 6.2
1 2 4 -14.9 8.4	1 3 8 -16.4 8.6	2 3 3 -14.7 8.0	2 4 9 -15.6 7.9	3 2 4 -14.7 7.0

d) continued

3	2	4	-14.7	6.8	3	3	9	-15.2	7.3	4	4	4	-17.4	9.3	5	4	5	-15.6	6.9
3	2	4	-15.1	7.7	3	3	9	-15.4	7.7	4	4	5	-16.1	7.7	5	4	5	-15.5	6.9
3	2	4	-15.1	8.5	3	5	1	-15.4	7.4	4	4	5	-17.5	10.1	5	4	5	-16.4	7.1
3	2	4	-14.8	7.3	3	5	1	-15.5	7.0	4	4	5	-17.1	8.6	5	4	5	-17.8	8.4
3	2	6	-16.2	8.9	3	5	1	-16.1	7.8	4	4	5	-17.2	9.2	5	4	5	-16.8	8.1
3	2	6	-15.1	8.1	3	5	1	-16.6	8.2	4	4	5	-17.2	8.8	5	4	6	-16.5	8.4
3	2	6	-16.0	8.6	3	5	1	-16.2	7.9	4	4	8	-16.5	8.6	5	4	6	-16.2	7.8
3	2	6	-15.6	8.5	3	5	2	-15.5	7.3	4	4	8	-17.2	9.4	5	4	6	-16.8	8.3
3	2	6	-16.3	9.2	3	5	2	-16.9	9.2	4	4	8	-16.1	8.3	5	4	6	-16.6	8.3
3	2	8	-15.7	8.0	3	5	2	-15.3	7.1	4	4	8	-17.8	10.1	5	4	6	-15.5	7.3
3	2	8	-15.5	7.7	3	5	2	-15.9	8.0	4	4	8	-16.5	8.3	5	4	8	-16.2	7.8
3	2	8	-15.4	8.7	3	5	2	-16.5	7.5	4	4	9	-15.9	8.3	5	4	8	-15.5	7.5
3	2	8	-15.9	7.9	3	5	3	-16.8	9.3	4	4	9	-18.0	10.0	5	4	8	-15.6	6.7
3	2	8	-15.0	7.8	3	5	3	-16.2	8.9	4	4	9	-15.6	7.5	5	4	8	-17.2	9.2
3	2	9	-15.5	7.4	3	5	3	-15.6	8.1	4	4	9	-16.4	8.1	5	4	8	-15.3	6.8
3	2	9	-14.3	6.7	3	5	3	-15.7	7.7	4	4	9	-16.0	7.1	5	4	9	-17.8	9.0
3	2	9	-14.8	6.6	3	5	3	-15.9	7.6	4	5	2	-15.6	7.8	5	4	9	-17.6	8.8
3	2	9	-14.4	6.9	3	5	4	-16.2	8.1	4	5	2	-15.4	7.8	5	4	9	-19.0	10.8
3	2	9	-15.5	8.2	3	5	4	-16.3	8.5	4	5	2	-14.5	6.0	5	4	9	-18.2	10.4
3	3	1	-15.3	7.5	3	5	4	-16.0	8.4	4	5	2	-14.7	7.1	5	4	9	-17.4	9.4
3	3	1	-16.6	8.2	3	5	4	-15.8	7.9	4	5	2	-15.1	6.2	5	5	2	-16.1	7.6
3	3	1	-16.5	8.2	3	5	4	-16.8	8.1	4	5	4	-14.4	7.4	5	5	2	-16.6	8.3
3	3	1	-16.2	8.4	3	5	5	-17.1	8.9	4	5	4	-15.5	8.2	5	5	2	-16.4	6.9
3	3	1	-16.1	7.6	3	5	5	-17.2	10.0	4	5	4	-16.1	7.7	5	5	2	-16.1	8.1
3	3	2	-14.8	7.1	3	5	5	-17.3	9.7	4	5	4	-15.8	7.5	5	5	2	-17.3	10.0
3	3	2	-15.3	7.6	3	5	5	-15.6	7.8	4	5	4	-16.6	8.2	5	5	4	-17.7	10.8
3	3	2	-15.1	7.3	3	5	5	-16.5	8.7	4	5	5	-16.6	8.6	5	5	4	-16.7	6.6
3	3	2	-15.2	7.8	3	5	7	-16.8	8.1	4	5	5	-15.4	7.3	5	5	4	-15.5	7.2
3	3	2	-14.8	7.0	3	5	7	-15.8	7.1	4	5	5	-15.8	7.7	5	5	4	-15.1	7.7
3	3	4	-15.8	8.0	3	5	7	-16.3	8.2	4	5	5	-16.2	7.9	5	5	4	-15.3	6.9
3	3	4	-15.5	8.0	3	5	7	-15.6	6.8	4	5	5	-15.7	6.9	5	5	5	-15.5	7.9
3	3	4	-15.3	8.4	3	5	7	-16.2	7.6	4	5	8	-15.7	6.9	5	5	5	-16.2	8.3
3	3	4	-15.4	7.2	3	5	8	-15.9	8.2	4	5	8	-16.1	7.9	5	5	5	-16.4	8.5
3	3	4	-15.8	7.7	3	5	8	-15.8	8.2	4	5	8	-15.4	6.4	5	5	5	-15.8	8.3
3	3	5	-15.0	7.2	3	5	8	-16.2	8.6	4	5	8	-16.1	7.6	5	5	5	-15.6	8.5
3	3	5	-15.7	7.5	3	5	8	-16.3	8.5	4	5	8	-15.0	7.5	5	5	8	-15.9	7.4
3	3	5	-15.8	8.2	3	5	8	-16.3	8.0	4	5	9	-14.8	7.3	5	5	8	-15.4	7.2
3	3	5	-15.5	7.8	3	5	9	-16.9	9.6	4	5	9	-15.0	7.3	5	5	8	-16.6	8.5
3	3	5	-15.6	7.8	3	5	9	-17.8	9.3	4	5	9	-15.3	7.3	5	5	8	-16.2	8.1
3	3	7	-15.1	6.6	3	5	9	-16.7	8.1	4	5	9	-15.8	8.3	5	5	8	-16.9	8.9
3	3	7	-15.6	8.0	3	5	9	-16.8	8.2	4	5	9	-16.3	8.7	5	5	9	-16.3	8.7
3	3	7	-15.7	8.2	3	5	9	-16.9	8.9	5	4	2	-17.8	10.0	5	5	9	-15.6	7.5
3	3	7	-15.3	8.0	4	4	2	-16.8	8.4	5	4	2	-16.8	8.5	5	5	9	-16.3	8.4
3	3	8	-16.4	8.0	4	4	2	-16.7	7.4	5	4	2	-17.2	8.3	5	5	9	-16.5	9.1
3	3	8	-16.0	7.9	4	4	2	-16.0	7.5	5	4	2	-16.2	8.1	5	5	9	-16.3	9.8
3	3	8	-15.6	8.0	4	4	2	-15.7	6.9	5	4	2	-17.2	8.4					
3	3	8	-15.5	7.3	4	4	2	-15.9	7.4	5	4	4	-16.3	6.6					
3	3	8	-17.1	8.6	4	4	4	-16.7	8.7	5	4	4	-15.9	7.1					
3	3	9	-16.8	9.4	4	4	4	-16.2	8.4	5	4	4	-16.4	7.7					
3	3	9	-16.0	8.1	4	4	4	-16.9	8.5	5	4	4	-15.6	6.2					
3	3	9	-15.9	7.5	4	4	4	-16.2	6.9	5	4	4	-16.1	6.6					