A STUDY OF FACTORS AFFECTING S-METHYL CYSTEINE SULPHOXIDE CONTENT OF KALE (*BRASSICA OLERACEA*).

by

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ABSTRACT

Factors affecting S-methyl cysteine sulphoxide (SMCO) content in Maris Kestrel kale were studied in field and glasshouse trials. SMCO is responsible for kale anaemia in ruminants.

Higher plant populations reduced whole plant and stem SMCO in six trials, especially at harvests after October. Increasing plant density from 10 to 80 plants/m^2 reduced whole plant SMCO in November or December by approximately 25%.

Nitrogen (N) fertiliser increased SMCO and dry matter (DM) yields in six out of seven trials, with SMCO increases ranging from 8 to 68% depending on soil fertility.

There was a high correlation between SMCO (Y) and N (x) levels in mature kale from different sites ($r = 0.92$, $n=26$). Using the relationship $Y = 7.53x - 3.20$, plant N could be measured as an estimate of the SMCO level.

On free draining soils low in available sulphate at sowing (<12 ppm), applied sulphur (S) increased SMCO in two out of six trials, although only at early autumn harvests. On a soil with impeded drainage (sulphate = 12.5 ppm), S tended to increase ($P < 0.10$) SMCO at a December harvest.

As the crop aged, SMCO increased especially in leaves. This was not caused by frosting, but may have been affected by floral initiation, as stems with small inflorescences had higher ($P < 0.05$) SMCO levels than non-flowering stems.

July sown kale had less SMCO than June sown kale in September, but tended to have more SMCO in November, especially at higher populations and particularly in stems.

SMCO was not affected by varying growing temperature (-6 to 15°C), moisture availability, chopping or wilting for 96 hours.

Chrysol (13.1 g/kg DM) tended to have a higher SMCO content than Canson, Maris Kestrel, Merlin, Vulcan or Bittern (mean = 9.2 g/kg DM). The lower yielding cultivars, Chrysol and Canson, contained most of their SMCO in leaf tissue. SMCO varied in different plant components, with SMCO in petioles having the highest correlation with whole plant SMCO ($r = 0.73$, $n=8$ and 18).

Near infrared reflectance (NIR) analysis accurately predicted N and moisture contents in different kale tissue types, with correlation coefficients (r) ≥ 0.95 between NIR predicted and Kjeldahl N or oven-dried moisture values. SMCO was only predicted satisfactorily by NIR for plant breeding purposes in entire leaves and young leaves, which had average standard errors of 2.3 and 1.7 respectively and correlations between SMCO values predicted by NIR and the autoanalysis method of $r = 0.87$ to 0.91. In stems and whole plants, which had average standard errors of 2.9 and 3.9 respectively and correlations (r) ranging from 0.69 to 0.84, NIR could only be used to separate low and high SMCO samples.

DECLARATION

This thesis has been composed by myself and all results presented are from my own studies.

R.C. McDonald

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CHAPTER I

Introduction

1.1 History of Kale Anaemia

1.1.1 Early reports

The first report of deleterious effects on cattle fed with kale (*Brassica oleracea*, var. *acephala*), was made in Germany by Rosenberger (1939) at a time when food supplies were limited and dairy cattle were often fed predominantly on kale. He reported severe haemolytic anaemia in cattle and low red blood cell counts of approximately 1.5 million/mm^3, compared with the normal level of about 7 million/mm^3 (Rosenberger, 1943).

The first reports of haemolytic anaemia in cattle in Britain, were also on kale (Evans, 1951; Gregor, 1952). In Germany and Yugoslavia, Schubert (1954) and Rebesko (1958) reported anaemia in dairy cows grazing white cabbage (*Brassica oleracea*, var. *capitata*), while in Canada, Schofield (1947) reported anaemia in cattle on rape (*B. napus*). Haemolytic anaemia has since been reported in ruminants on most brassica species (Greenhalgh, 1971; Smith, 1974).

Reviews on kale anaemia by Clegg and Evans (1962), Penny *et al.* (1964), Clegg (1966), Grant *et al.* (1968), Greenhalgh (1969) and Smith (1974) have summarised knowledge on susceptibility of different animals, the clinical picture, pathological effects and aetiology. The main findings are summarised below.

1.1.2 Susceptibility

The earliest reports of kale anaemia were in cattle (Rosenberger, 1939; Evans, 1951; Gregor, 1952), however Stamp and Stewart (1953) reported anaemia in sheep grazing kale. Anaemia has since been reported in most ruminants, although generally cattle and goats are more susceptible than sheep (Smith, 1974). Non-ruminants such as rats, rabbits, pigs and mice do not become anaemic on brassica diets.

The most severe kale anaemia has been on heavily pregnant cows, recently calved cows and other high yielding animals (Clegg, 1966). This has probably been due to a greater intake in these animals per unit liveweight (Greenhalgh *et al.*, 1970; Smith, 1974).

1.1.3 The clinical picture

Anaemia is most severe in animals grazing on kale or other brassicas during the first three or four weeks on these crops. During this time Heinz-Ehrlich (Heinz) bodies are formed. These were first reported by Penny *et al.* (1961) and are spherical refractile aggregates within red blood cells, precipitated from haemoglobin after irreversible oxidative changes. In severe cases, all red blood cells may contain one or more Heinz bodies which may account for a high proportion of cell's haemoglobin (Greenhalgh, 1969). With the formation of Heinz bodies, the blood haemoglobin level in cattle can fall from the normal 11 g/100 ml to 3 g or lower. In acutely poisoned animals, haemoglobinuria (redwater) may be seen and animals may die, although usually only sub-clinical signs occur. If kale feeding is ceased, haemoglobin returns to normal levels in 3-4 weeks, but if kale feeding is continued, haemoglobin levels recover in 3-4 weeks to a level slightly below normal (Greenhalgh, 1969; Greenhalgh *et al.*, 1970; Smith, 1976; Barry *et al.*, 1981a).

Greenhalgh (1969) reported that ruminants, and cattle in particular, have low levels of the enzyme glutathione-reductase which reduces glutathione, a substance that helps prevent the oxidation of haemoglobin. Ruminants therefore are more susceptible than non-ruminants to toxins which generate severe oxidative stress, as probably occurs with Heinz body formation. Tucker and Kilgour (1973) found that sheep with hereditary low levels of glutathione, were more prone to Heinz body

formation and appeared more susceptible to anaemia when grazing kale, than sheep with high levels of glutathione.

1.1.4 Clinical signs of anaemia

As mentioned previously, an obvious clinical sign of severe kale poisoning in animals is haemoglobinuria and a red-brown discoloured urine which later may become dark and black. Clegg (1966) stated that other signs may include loss of appetite and thus a rapid loss in condition, sunken eyes, animals appearing dull and having a lustreless coat. As animals become weaker, their walk may be slow and unsteady and scouring may occur. Pulse rate may be very high and respirations shallow and frequent. Heart sounds may be tumultuous as to be heard some distance away and extremities may be cold. Collapse and sudden death sometimes occurs.

Milk production usually only falls if cows become severely ill and pinkish discolouration of milk has been reported (Clegg, 1966). A main effect of kale anaemia is the lowering of liveweight gains of both sheep and cattle, achievable on the crop in relation to its high digestibility (75-90%) and nitrogen content (2.5 - 4.0% of dry matter). Nicol and Barry (1979) reported that liveweight gains on kale in all published New Zealand trials were only 100-150 g/day with sheep and 0.3 - 0.6 kg/day with cattle, as compared with gains of up to twice as much on pasture having a similar digestibility and nitrogen content.

There have been several reports of infertility in animals on kale (Boyd and Reed, 1961; Melrose and Brown, 1962; Williams *et al.*, 1965; Greenhalgh, 1971), however David (1965) found that most infertility factors associated with kale, such as poor conception rates and embryonic mortality, were not associated with anaemia, but with goitrogenic problems which could be overcome by iodine supplementation.

1.1.5 Pathological effects

Possible effects of severe kale anaemia on an animal's carcase, bladder, spleen, kidneys and liver have been detailed by Clegg (1966). Many of the organs may have black discolourations and necrosis, especially in the liver, may occur.

1.1.6 Aetiology

One of the earliest ideas as to the possible cause of kale anaemia was mould infection on the crop, similar to *Polydesmus exitiosus* infection of rape, which was said to cause illness in cattle and sheep (Volker, 1950).

Another widely held view was that frosting was related to kale anaemia, possibly through affecting blood circulation through the capillaries of the liver, or possibly the lower temperatures put further strains on already debilitated animals (Clegg, 1966). Evans (1951) reported anaemia in cattle after ingestion of frosted kale and Gregor (1952) reported anaemia after a period of inclement weather. Clegg and Evans (1962) and Dunbar *et al.* (1963) also believed frosted kale was particularly toxic, although Penny *et al.* (1964) found no relationship between the numbers of red blood cells having Heinz bodies in cattle fed with kale and periods of coldest temperatures. Smith (1974, 1976, 1977) considered that it was probably kale maturity and not frosting in late winter, that was responsible for higher levels of the toxin in kale and hence more cases of anaemia in animals. There has been no work reported, however, on the effects of temperature on kale toxins.

The intake of large volumes of cold water by pregnant cows on some feeding systems was known to cause haemoglobinuria (Wright, 1961), hence this was also considered as a possible cause of kale anaemia.

After drinking the water, the animals would produce dark urine within one or two hours which sometimes resulted in the animals collapsing and dying.

Low phosphorus (P) diets such as provided by turnips, fodder beets and alfalfa caused post-parturient haemoglobinuria in cows (Madsen and Nielsen, 1944; Parkinson and Sutherland, 1954; Mullins and Ramsey, 1959) and this was thought to be related to kale anaemia. Clegg and Evans (1962) considered that the high calcium (Ca) : P ratios in kale were a more likely precursor to kale anaemia, however haemoglobinuria had never been reported in any other diets with a high Ca : P ratio. The failure of mineral supplements to prevent anaemia suggested a course of disease more consistent with the presence of some toxic substance in the brassica diet (Greenhalgh, 1969).

Brassicas are known to contain high levels of both glucosinolates which are responsible for iodine deficiencies in animals (Allcroft and Salt, 1961; Williams *et al.*, 1965; Josefsson, 1967; Paxman and Hill, 1974) and nitrates (Dodd and Coup, 1957; Te Velde, 1967). Greenhalgh (1969), however, could not induce haemolytic anaemia after oral administration of the thiocyanate-type glucosinolate to a goat, nor could he obtain anaemic responses in kale fed cattle supplemented with nitrate, hence he concluded that these two substances were not responsible for kale anaemia.

The substance in kale responsible for anaemia was finally identified at the Rowett Research Institute as S-methyl cysteine sulphoxide (SMCO) (Smith, 1974). SMCO was only isolated after kale was divided into small fractions and each tested for haemolytic activity. This was a difficult process as there was no satisfactory laboratory technique to test such activity, hence each fraction had to be fed to

a ruminant and in this case a goat was used. When synthetic SMCO and synthetic S-methyl cysteine (SMC), the reduced form of SMCO, were fed to goats, both substances produced haemolytic anaemia similar to that produced by kale.

SMCO had previously been isolated from cabbage in 1956 by Synge and Wood, however, its effect on ruminants had not been tested due to the difficulty in producing large amounts of synthetic SMCO for a feeding trial.

The actual toxin which is responsible for the anaemia was identified by Smith (1974) as dimethyl disulphide, which is formed from SMCO by bacterial organisms in the rumen. SMCO itself in brassicas is probably not toxic to ruminants (Smith, 1978), although when synthetic SMCO has been fed in large quantities it has been found to be toxic to dogs (Pirie, 1932) and rats (Smith, 1974). Organic disulphides have caused anaemia in many animals including cattle (Koger, 1956), dogs (Gruhzit, 1931), horses (Thorp and Harshfield, 1939; Pierce *et al.*, 1972) and sheep (Van Kampen *et al.*, 1970).

The rate at which dimethyl disulphide is formed from SMCO is not yet known, neither has the rate at which it is absorbed into the blood been ascertained. These are probably affected by rumen flora, dietary composition, SMCO content and possibly other bacterial substrates such as sucrose (Smith, 1974; Smith and Greenhalgh, 1977).

1.1.7 The distribution of SMCO in plants

SMCO is found only in Liliaceae, where it has been identified in onions and garlic, and in Cruciferae mainly in the genera *Brassica* and *Raphanus* (Smith, 1974). In 1956 SMCO was first isolated in turnips, cabbage, cauliflower, radish, mustard, broccoli and kohlrabi (Morris and Thompson, 1956; Synge and Wood, 1956).

SMC is mainly found in Leguminosae and especially in beans (Thompson *et al.*, 1956) in which it does not cause haemolytic anaemia in ruminants.

1.1.8 The formation of SMCO

SMCO is a non-protein amino-acid (AA) which is synthesised at all stages of growth of brassica plants, including germinating seedlings (Mae *et al.*, 1971a). Details of its formation in brassicas is not clear, but its synthesis has been shown by several pathways.

In turnips and spinach, SMCO has been formed *in vitro* from methylmercaptan and O-acetyl serine (Thompson and Moore, 1968; Giovanelli and Mudd, 1967). In radish, Thompson and Gering (1966), using radioactive methyl groups, showed that SMCO could be synthesised *in vivo* by methylation of cysteine or methionine (via cysteine).

SMCO has also been formed from methylmercaptan and serine both *in vivo* in yeast (Wolf *et al.*, 1956) and *in vitro* with turnips and yeast (Thompson and Moore, 1968). The latter workers found the reaction only one-hundredth the speed of the similar reaction using O-acetyl serine as the substrate instead of serine. Sugii *et al.* (1963) considered that they had also formed SMCO *in vivo* in garlic from serine and methylmercaptan. They found radioactive sulphur(S) in SMCO after injecting radioactive methionine into garlic leaves and assumed that the methionine formed methylmercaptan which reacted with serine. They may, however, have had a transmethylation reaction similar to that of Thompson and Gering (1966).

Mae *et al.* (1971b) have shown using isotopically labelled SMCO, that both SMCO and SMC (via SMCO) can be metabolised to cysteine by demethylation. Demethylation of SMCO occurred in brassicas and in other plants, such as tomatoes, tobacco and cucumbers, in which SMCO

has not been found. SMCO can also be degraded to pyruvate, methyl methanethiosulphinate and ammonia by the enzyme cysteine sulphoxide lyase (Mazelis, 1963).

Mae *et al.* (1971a,b) stated that SMCO is an important soluble pool for organic sulphur and can be actively synthesised or metabolised depending on the soluble S content to which it is proportional.

A diagram summerising work on the syntheis and metabolism of SMCO, together with the main chemical formulae, is shown in Table 1.

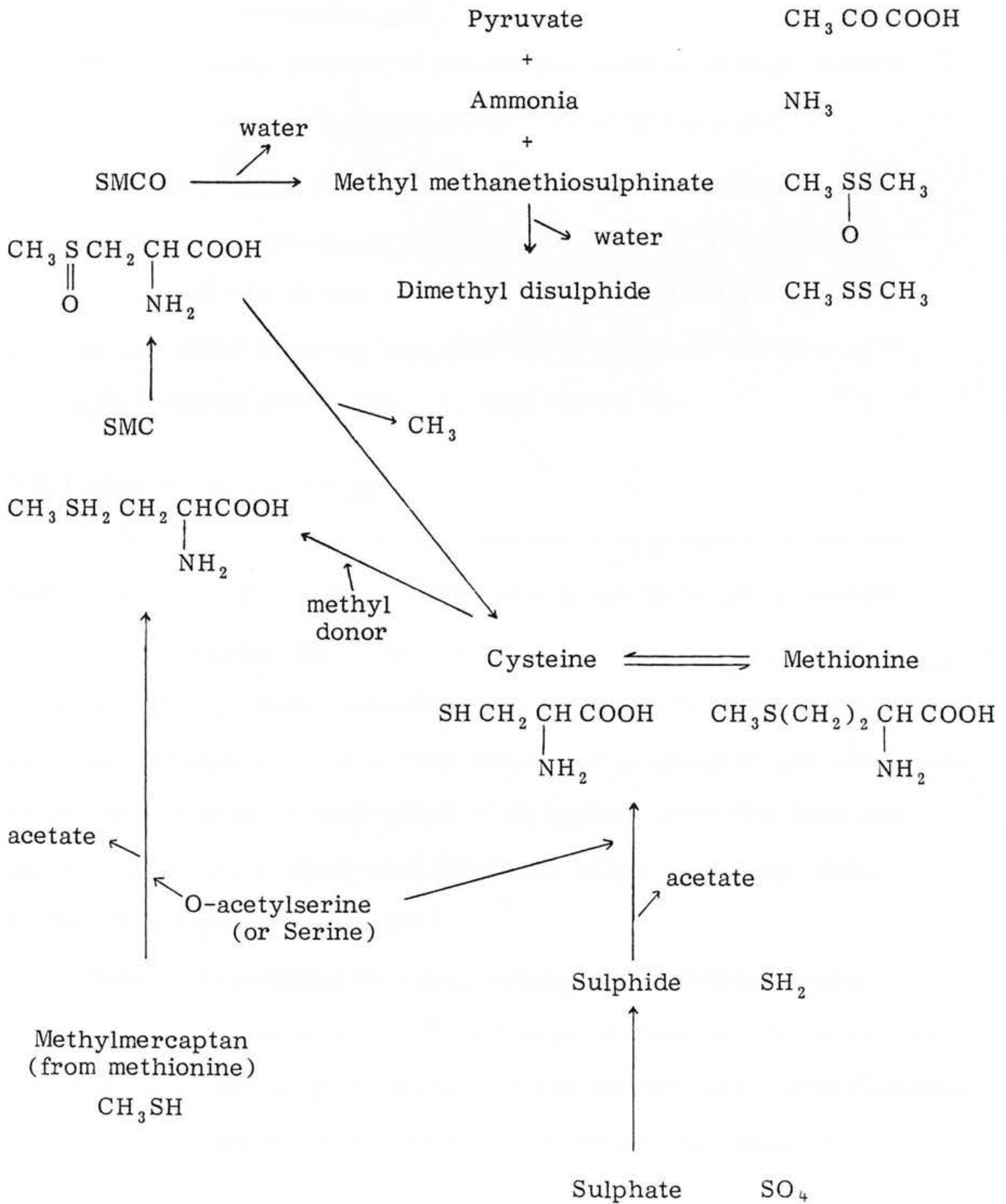
1.1.9 The importance of SMCO

As stated previously, animal performance on kale throughout the world has not been high in relation to the nutritive value of the crop in terms of digestibility and nitrogen content. Barry *et al.* (1981a) considered that the main factor responsible for this poor animal performance was the high S content of kale (0.6 - 1.0% of dry matter). Sulphur occurs in both protein and non-protein compounds including SMCO and glucosinolates. The major ways in which the high S levels in kale were considered to depress animal growth were, in order of priority, a high SMCO content, a depression of true availability of copper (Suttle and McLaughlan, 1976; Barry *et al.*, 1981a,b) and a high glucosinolate content (Barry *et al.*, 1981a).

The importance of SMCO was shown in recent work by Barry (personal communication), who found that sheep intake and liveweight gains were inversely proportional to the level of synthetic SMC added to a kale diet. He also found that SMC had no effect on animals fed a lucerne diet, suggesting that other factors are also involved in the formation of dimethyl disulphide from SMCO.

Other characteristics of kale which could affect animal performance are the high Ca : P ratios especially in kale leaves (Cornforth *et al.*,

TABLE 1: Pathways involved in synthesis and metabolism of SMCO (summerised from available literature).



1978), high nitrate levels or a palatability factor. The main factor, however, is probably the high SMCO levels.

1.2 Methods of Overcoming Kale Anaemia

The most likely methods of reducing anaemia in animals grazing kale are, apart from limiting the proportion of brassica fed, to:

(a) modify the rumen flora and prevent rumen organisms converting SMCO to dimethyl disulphide;

(b) breed cultivars of kale with very low levels of SMCO; or

(c) reduce SMCO levels by changing the growing environment of kale by agronomic means, i.e. crop husbandry.

1.2.1 Modifying rumen flora

Work at the Rowett Research Institute is in progress to try and modify the rumen flora within ruminant animals, in order to prevent organisms converting SMCO to dimethyl disulphide (Anon., 1978a). Three rumen organisms - *Lactobacillus, Megasphaera elsdenii* and *Veillonella alcalescens* - have been isolated as involved in this conversion. These three organisms were added to an isolated germ-free lamb which had been previously dosed with SMCO and within a few days Heinz bodies and anaemia were detected.

There is no practical way yet, however, of modifying rumen organisms to prevent dimethyl disulphide production and hence preventing anaemia in animals grazing kale. There appears to be little likelihood that this work will result in a practical solution to kale anaemia.

1.2.2 Plant breeding

- a) Genetic variation:

There are significant differences in SMCO levels between forage crucifers, between cultivars of a particular crop type (e.g. kale), and between plants of a particular cultivar.

Kales generally have higher levels of SMCO than the other forage crucifers; forage rapes, radicole (*Raphanobrassica*), swedes (*B. napus*), stubble-turnips, turnips (*B. compestris*, ssp. *rapifera*) or fodder radish (*Raphanus sativus*) (Smith, 1976; Whittle *et al.*, 1976). The North of Scotland College of Agriculture (NSCA) reported that rape and especially radicole, usually contain the next highest concentration of SMCO after kale (Anon., 1978b).

Variation in SMCO between kale and rape cultivars was shown clearly by Whittle *et al.* (1976). Generally the thousand-head types of kale had higher levels of SMCO than the marrow-stem cultivars. This was confirmed by Bradshaw and Borzuki (1980). SMCO levels of between 0.4 and 2.0% of the DM have been reported in kales (Smith and Greenhalgh, 1977). Gosden and Johnston (1977) found that SMCO concentration varied more than four-fold ranging between 0.25 and 1.1% in 130 plants of the kale cultivar Proteor. There is probably therefore a large range of material available for breeding, depending on the heritability of SMCO content, although it is not known how much of the variation is genetic and how much environmental.

Glucosinolate levels also vary between brassicas (Van Etten *et al.*, 1969, 1976; Mullin and Sahasrabudhe, 1977; Chong and Bible, 1978), between cultivars (Josefsson and Appelqvist, 1968; Paxman and Hill, 1974; Van Etten *et al.*, 1976; Chong and Bible, 1978; Daxenbichler *et al.*, 1979) and between plants within cultivars (Josefsson, 1967; Gosden and Johnston, 1977).

Genetic investigations with kale by Gosden and Johnston (1977) found SMCO to be a moderately heritable character, however, more recently they have suggested that its heritability at the best is very low (Anon., 1978c; Gosden, pers. comm.). In comparison, the thiocyanate level in kale is definitely a heritable character (Johnston and Gosden, 1975).

In kale, Gosden and Johnston (1977) found no significant correlation between any two of the following characters: thiocyanates, isothiocyanates, SMCO, soluble carbohydrates and crude proteins. They concluded therefore that each of the undesirable constituents of brassicas would have to be selected for independently and that selection would not result in any marked change in the content of protein or carbohydrate. Whittle *et al*. (1976) also reported no difference in SMCO content in two kale cultivars selected specifically for high and low thiocyanate content. With rapes, however, they found that cultivars bred for low thiocyanate had significantly lower SMCO levels throughout their growing season than cultivars with high thiocyanate levels.

Further work on the relationship between SMCO and glucosinolates in kale and other brassicas is required. Both components are non-protein S containing compounds, hence if both are related to soil S status especially when S is limiting, differences in abilities of brassica varieties to take up S could cause significant correlations between the two components.

Breeding kales and other brassicas with low levels of SMCO is a sure way of reducing incidences of anaemia in animals grazing these crops. However, due to the probable low heritability of SMCO, it may never be successful or it may take many years to achieve.

- *b) Plant component variation:*

Several workers have found that SMCO levels are higher in the young leaves than in the older leaves of brassicas (Morris and Thompson, 1956; Mae *et al.*, 1971a; Whittle *et al.*, 1976; Gosden and Johnston, 1977). In Germany in 1954, Schubert noticed that anaemia was more likely to occur in cattle eating the inner smaller leaves of white cabbage (*B. oleracea,* var. *capitata*), than in cattle eating the outer leaves. SMCO levels in flowering parts of plants are especially high and can be several times the levels found in other parts of the plant (Morris and Thompson, 1956; Whittle *et al.*, 1976).

The relationship between SMCO levels in leaves and stems of kale plants and leaves and 'bulbs' of swedes, turnips and fodder radish is not clear, although it appears to vary with maturity, i.e. age. In young plants, stems or bulbs generally have similar or higher SMCO levels than leaves, whereas in more mature plants, SMCO levels are usually higher in leaves than stems or bulbs (Whittle *et al.*, 1976; Smith and Greenhalgh, 1977; Anon., 1979a; Barry *et al.*, 1981a). The relationship between SMCO levels in different parts of plants differs with cultivars, however, especially in kales where there is a big variation in leaf : stem ratios.

In swedes it has been found that there is no correlation between SMCO levels in 'bulbs' and leaves of individual plants (Gosden, 1977).

Glucosinolates also vary in different parts of brassica plants and in kale the level of thiocyanates, which is the major glycogen formed from glucosinolates in this crop, is mugh higher in leaves than in stems (Josefsson, 1967; Paxman and Hill, 1974). Chong and Bible (1978) stated that levels of glucosinolates are generally higher in the young flowering parts of brassicas than in the older parts of the plants.

Crude protein levels also vary in different parts of brassica plants. Frame and Robinson (1966a) reported that crude protein levels were higher in laminae than petioles and, within stems, higher in pith than cortex tissue. They also reported that crude protein levels decreased with maturity, mainly through the loss of protein rich leaves.

- *c) Sampling procedure:*

In order to make comparisons of SMCO levels in different kale or rape cultivars, brassica breeders at the Welsh Plant Breeding Station (Gosden, 1977) and the Scottish Crop Research Institute (McNaughton, pers. comm.), have sampled from plants only the young leaves approximately one-third expanded. It was proposed to study the relationship between SMCO levels in these young leaves and levels in other parts of the plants and especially in whole plants, using several kale cultivars. It is important that the part of the plant sampled gives the same relationship between breeding lines as sampling the whole plant would give.

Young leaves have been sampled for freeze drying and toxic factor analyses prior to the main plant harvest. The main purpose has been to obtain samples early, hence allowing maximum time for freeze drying and chemical analyses which are very time consuming. It has been important to obtain results of chemical analyses prior to sowing the following year's crop in the spring. Harvesting of only young leaves as opposed to whole plants, would also allow plants to be grown on to produce seed for the next generation.

1.2.3 Agronomic factors

In this section, possible ways of reducing SMCO levels in kale by cultural procedures are outlined. Whereas modifying rumen flora and

breeding kale with low SMCO levels are probably long term methods of overcoming kale anaemia, changing the growth environment of kale could be a much quicker method, hence finding effective ways of doing this is the main objective of this study.

Most of the following work has been carried out on factors affecting SMCO levels within the plant. However, as SMCO and glucosinolates are both non-protein sulphur (NPS) compounds, previous work on glucosinolates which could be applicable to SMCO is also described.

Work on some of the agronomic factors which could affect SMCO levels in kale is summarised below. Table 2 gives a summary of some reported SMCO levels (g/kg DM) in kale and factors affecting them.

- *a) Effects of fertilisers:*

I. Sulphur: Plants take up sulphur from the soil only in the form of sulphate, which when in the plant, is synthesised in the leaf chloroplasts mainly into the organic S proteins, cysteine and methionine (Table 1). In plants other than brassicas, excess S in the plant is stored as inorganic sulphate which in most plants is at a level proportional to available sulphate in the soil (Esminger and Freney, 1966; Bouma, 1975). In brassicas excess S is stored as SMCO and glucosinolates as well as sulphate.

The level of glucosinolates in brassicas has been found to be directly related to the availability of soil sulphate (Josefsson and Appelqvist, 1968; Finlayson *et al.*, 1970; Freeman and Mossadeghi, 1972). Josefsson (1970) found this relationship to be greater on sandy or lighter soils than on heavier soils. Josefsson and Appelqvist (1968) showed that when the glucosinolate content was reduced by a low application of sulphate fertiliser, the protein still approached normal

TABLE 2: Summary of SMCO levels and factors affecting SMCO in kale.

Factor	Rate (kg/ha)	Whole plant SMCO (g/kg DM)	Source
1. Sulphur (kg S/ha)	0	5.4	McDonald *et al*. (1981) (low soil S site)
	50	7.4	
	150	8.6	
2. Nitrogen (kg N/ha)	0	4.8	Anon. (1978b) (harvested in October)
	75	6.9	
	150	10.2	
	225	11.0	
3. Environment	-	4.1 - 12.7	Whittle *et al.* (1976)
	Date of harvest		
4. Harvest date	Aug	5.9	Whittle *et al*. (1976) (mean of 8 varieties)
	Oct	6.9	
	Dec	9.6	
	Jan	12.1	
	Sep	6.6	Bradshaw and Borzuki (1980) (mean of 7 marrow-stem varieties)
	Nov	8.3	
	Jan	11.2	
	Mar	10.6	
	Sowing date		
5. Sowing date	22 Jun	4.6	Anon. (1978b) (harvested in October)
	5 Jul	6.7	

values, thus indicating that plants deficient in S may use this primarily for protein biosynthesis and secondly for glucosinolate production.

Mae *et al.* (1971a) found in trials of plants in pots, that the level of SMCO in Chinese cabbage was also proportional to the availability of S. They were able to reduce the level of SMCO to zero by putting cabbage plants into a S deficient culture, although growth was also restricted and levels of all S compounds in the plants decreased.

Recently, McDonald *et al.* (1981) showed in a field trial in New Zealand, that SMCO in Medium Stemmed marrow-stem kale was directly related to available S on a light S deficient soil (soil sulphate = 4 ppm). However, on a soil with a high availability of S (soil sulphate = 22 ppm) there was no relationship. The SMCO level in the kale at the S deficient site was increased significantly (SE = 0.45) when S fertiliser as gypsum was applied. Dry matter (DM) yield (mean = 5.3 t/ha, SE = 0.21) was not increased by the addition of S fertiliser, showing that even when no S was applied, there was adequate S in the soil for maximum dry matter production. Associated with the increase in SMCO, they reported a similar increase in total plant S and the maintenance of the total protein content.

An objective of this study is to confirm and extend these findings and to look at the following:

1. The level to which SMCO can be reduced in kale by limiting available S, before plant quality and growth are restricted. It has been suggested (Saalbach, 1970) that growth is restricted when the sulphate content in plants is less than 0.35 to 0.40%.

2. The level of soil S at which SMCO becomes dependent on S available.

3. The practicability of reducing kale SMCO on a soil with a reasonably high S status, by restricting S fertilisers and by growing several kale or high S uptake crops.

II. Nitrogen and other minerals: The application of nitrogen (N) fertiliser has recently been shown to increase SMCO levels in kale (Table 2), rape and stubble-turnips (Anon., 1978b).

Trial results on the effects of N fertiliser on glucosinolates in brassicas, however, have been conflicting. Some workers have reported increases in glucosinolate levels in *Brassica oleracea* with higher rates of N fertiliser (Allcroft and Salt, 1961; Sedlack *et al.*, 1964), while others have shown, using both kale and other brassicas, that N decreases glucosinolate levels (Eaton, 1942 a,b; Trzebny, 1964; Josefsson, 1970; Wojtych *et al.*, 1973). Eaton (1942 a,b) suggested N decreased glucosinolates due to 'dilution', as at a high rate of N, the production in plants of other substances rose more than the glucosinolate production. Similarly, Josefsson (1970) suggested that N fertiliser possibly maximised protein production and thus glucose was limiting for glucosinolate synthesis. There have been no reasons given to explain these conflicting results on the effects of N fertiliser application, except that Josefsson (1970) suggested the effect of N may be dependent on the brassica crop type.

McDonald *et al.* (1981) showed that N fertiliser increased SMCO in kale in a soil high in available S (Table 2), however, in a S deficient soil they found that the effect of N was related to whether S fertiliser was applied or not. It is possible therefore that the level of S in the soil will govern whether N will increase or decrease glucosinolates and SMCO. Josefsson (1970) did not obtain a N x S interaction on glucosinolate levels as this theory would suggest, although the trial

was of pot grown plants and S may have been limiting at all levels. Possibly N fertiliser will only increase the non-protein sulphur compounds SMCO and glucosinolates when available S is surplus to that required for maximum protein production. One of the objectives of this work will be to study the effects of N fertilisers on SMCO in kale at different S levels.

Results at NSCA on the effects of phosphoric and potassic fertilisers on SMCO levels have been variable. Trials in one year showed that neither phosphate nor potassium affected SMCO in kale, rape or stubble-turnips (Anon., 1978b). In trials in the following year (Anon., 1979b), phosphate increased both SMCO and thiocyanates in leaves and stems of forage rape, but nitrogen had no effect on either factor.

Nitrogen fertiliser often has other effects on brassica plants such as increasing total dry matter yield and crude protein content and decreasing the proportion of leaf yield and dry matter content (Willey, 1964).

- *b) Factors affecting soil sulphate availability:*

Due to the possible importance of soil S on SMCO levels in brassicas (Mae *et al.*, 1971a; McDonald *et al.*, 1981), any fertiliser or factor which affects the availability of soil sulphate could affect SMCO levels in the plant, especially when available sulphate is at low levels. In most soils, approximately 60-70% of S is permanently unavailable to plants and usually less than 10% is available as sulphate in the top 25 cm layer (Anderson, 1978), although in lower soil horizons the amount of sulphate is usually greater. The rest of the S is mainly organic S which is not available to plants until it is oxidised to sulphate by various biological and physical processes. Its rate of oxidation is dependent on

factors such as water, pH, temperature, C : S ratios and the presence of certain cations, all of which will be discussed briefly.

I. Water: This probably has most influence on available sulphate in free draining soils as sulphate is very easily leached (Hague and Walmsley, 1974). Water also affects the oxidation of S, which is maximised near field moisture capacity and reduced by drier or wetter conditions (Attoe and Olsen, 1966).

II. pH: The availability of soil sulphate from organic S is usually increased by the addition of calcium carbonate (Williams and Steinberg, 1962; Kamprath *et al.*, 1956; Freney and Stevenson, 1966). The latter authors suggested possible reasons being,

1. a higher pH favours S oxidising micro-organisms;
2. a higher pH favours chemical reactions which oxidise S;
3. a higher pH releases sulphate from soil exchange sites; or
4. sulphate is added with calcium carbonate.

Williams and Steinberg (1962) stated that the maximum adsorption of sulphate occurred at pH 2-4 and decreased as pH increased beyond these levels.

The effect of pH on sulphate availability is related to particle size of added fertilisers, as Attoe and Olsen (1966) showed that an increase in pH only increased available sulphate when applied lime was in small particles. The importance of particle size on S availability has been confirmed by Bertramson *et al.* (1950) and Kittams and Attoe (1965).

The type of plant is also important in relation to the effect of pH on S availability, as pH had little effect on S uptake in beans (Biddulf *et al.*, 1956)

III. Temperature: Sulphate is not released from organic matter below 10°C but its rate of release increases up to 35°C (Attoe and Olsen, 1966; Freney and Stevenson, 1966; Williams, 1967).

IV. C : S ratio: Barrow (1960) and Freney and Stevenson (1966) reported that a C : S ratio of greater than 400 caused S to remain in the organic matter, whereas a ratio of less than 200 favoured the accumulation of sulphate in the soil. The mineralisation of S, however, depended on the S content of the soil as well as the C : S ratio (Nelson, 1964).

V. Presence of cations: Sulphate is retained in significant amounts by aluminium and iron hydrous oxides in soils provided that pH is below about 7.0 (Harward and Reisenauer, 1966). Also barium and calcium ions can obscure the release of sulphate by the formation of insoluble sulphates (Cairns and Richer, 1960; Harward and Reisonauer, 1966).

VI. Other factors: The number of S oxidising bacteria is thought to be a major factor responsible for different rates of S oxidation in different soils (Kittams and Attoe, 1965; Attoe and Olsen, 1966). Depressing S oxidising bacteria in order to reduce available sulphate, however, would also affect the availability of nitrate, as similar bacteria are involved in the formation of nitrate and sulphate from organic N and S (Starkey, 1966).

The addition of sugars to soils also depresses S oxidation (Attoe and Olsen, 1966; Freney and Stevenson, 1966).

- *c) Effects of plant population:*

Many workers have shown that the total dry matter yield of kale is increased by increasing plant population (Hunter-Smith and Rhys Williams, 1927; Tamás, 1976). Holliday (1960), reporting on results of Roebuck (1958), suggested that yield showed a curvilinear response to population, although Bleasdale and Nelder (1960) considered that such an asymptotic relationship may not be valid at either very low or very high plant populations. Some workers have failed to obtain a yield response from changing population, especially when kale plants were thinned within rows (Robinson and Frame, 1966; Johnston, 1971).

In kale, plant composition is also generally affected by plant population. Increasing population tends to increase dry matter content and fibre content, but decrease crude protein level and to a lesser extent digestibility and levels of minerals (Roebuck, 1958; Willey, 1964; Frame and Robinson, 1966 a,b). The latter authors considered that there was only a slight advantage in thinning kale in terms of composition and that any advantages were outweighed by the increase in plant utilisation that could be achieved by animals, when grazing a non-thinned, dense, thin stemmed kale crop.

No studies have been conducted on the effect of different plant populations on SMCO levels in brassicas. Glucosinolates, however, have been found to vary significantly with different populations in both kale and rape (Josefsson, 1967). In a trial reported by Josefsson *et al*. (1972), thin stemmed plants appeared to contain more glucosinolates than thick stemmed plants.

An objective of this thesis is to study the effect of population on SMCO levels in kale. The most likely ways that population could affect SMCO are if the dry matter yield is increased S may be diluted and

SMCO reduced, especially if available S is limited, or the leaf : stem ratio may be changed and thus possibly affect SMCO levels and distribution.

- *d) Environmental effects:*

Whittle *et al*. (1976) reported a wide range of SMCO levels in Maris Kestrel kale grown on different farms (Table 2). Reasons given for possible variations in SMCO included different stages of maturity arising from different sowing dates, different manurial treatments or possibly different microclimate effects. An objective of this work is to study the importance of S levels in different soils on plant SMCO levels, as this could have been a factor causing the variation in kale SMCO levels found by Whittle *et al*. (1976).

The effects of rainfall, soil type and temperature on SMCO levels have not been studied, although, as mentioned previously, many believed frosting of kale increased the incidence of anaemia in animals grazing the crop (Evans, 1951; Gregor, 1952; Clegg and Evans, 1962; Dunbar *et al*., 1963). There has been no scientific evidence to support this, however, hence another objective of this work is to look at the effects of varying periods of frosting and low temperatures on levels of SMCO in kale and its distribution within plants. It has been suggested that frosts can cause protein degradation, due to dehydration and an increase in the concentration of electrolytes (Street and Opik, 1970). Possibly these denatured proteins form SMCO. It has also been shown with mint, that protein breakdown can be increased by low night temperatures and by long nights and short days (Steward *et al*., 1959).

The uptake of total S in peas, barley, clover and roses is directly related to temperature in low S soils, although this does not occur with

rape (Herath and Ormrod, 1971). This presumably is due to temperature increasing the availability of sulphate, as mentioned previously.

Josefsson and Appelqvist (1968) found that glucosinolate levels in seeds of oil-seed types of rape and turnip rape, varied by only about 15% in different environments, with the exception of one soil which was very low in S and had a very low glucosinolate level. Neil and Bible (1972) found that radishes grown on organic soils had higher levels of thiocyanate than radishes grown on loam soils. They thought that this was possibly due to the availability of S. Chong and Bible (1978) stated that natural goitrogens in cruciferous vegetables have been found to be related to soil type, temperature, rainfall, irrigation frequency and S nutrition.

e) Harvest date effects:

SMCO as a percentage of total dry matter, increases in brassicas as they mature (Smith, 1974, 1976; Whittle *et al.*, 1976; Bradshaw and Borzuki, 1980). Whittle *et al.* (1976) found that SMCO levels in kales more than doubled between August and January from 5-6 g/kg DM to 11-14 g/kg DM (Table 2). Recent results from the Rowett Research Institute showed that the SMCO ratio for leaf : stem increased five-fold between 63 and 147 days after sowing (Anon., 1979a). They reported levels at the end of the grazing season, of 52.5 g/kg DM in Lair rape and 45.0 g/kg DM in radicole. These are the highest reported SMCO levels in forage crucifers.

Whittle *et al.* (1976) described the increase in SMCO content with kale maturity as probably being associated with secondary growth and the development of flowers. However, the reason why SMCO increases as brassicas mature is not known. There have been several reports of the transfer of S in rape from older to younger shoots as

the crop has matured (Panak and Szafranek, 1967; Herath and Ormrod, 1971). As plants age, proteins in older leaves are hydrolysed to soluble amino acids which are transported to younger parts of the plants where they are used to resynthesise proteins (Bonner, 1950). Possibly SMCO is formed from these amino acids either in the old leaves or in the new leaves where protein is resynthesised. An objective of this work is to study why SMCO increases with maturity, especially in the younger parts of the plants.

Glucosinolate levels in brassicas do not change as much with maturity as SMCO levels. In kales and rapes, glucosinolate levels, unlike SMCO, are generally highest in young plants (Josefsson, 1967; Paxman and Hill, 1974).

- *f) Sowing date effects:*

Trials conducted by NSCA (Anon., 1978b) with kale (Table 2), rape, radicole, fodder radish and stubble-turnips, showed that plants from a later sowing (July) had higher SMCO levels in October than those sown earlier (June), despite the later sown crops being lower yielding and less mature. These results appear to conflict with the fact that SMCO increases with later harvests on more mature brassicas (Whittle *et al*., 1976). No conclusions for the surprising effect of sowing date on SMCO levels have been given.

Possibly the sowing date effect is related to the fact that later sowings of kale have higher nitrate levels at any particular harvest (Boelcke, 1972), hence if ample sulphur were available more SMCO may be produced.

- *g) Effects of wilting, chopping and ensiling:*

It has been suggested by some farmers and researchers that cutting and wilting kale, prior to feeding it to animals, will reduce its toxicity (NZDA, 1955). Gregor (1952) observed that cows ceased to be anaemic when kale was cut and carried to another field and allowed to wilt before being fed. There has been no detailed work done on the effect of wilting on SMCO, however, except for that reported by Allison and Borzuki (1978) who measured SMCO levels in inflorescences of Maris Kestrel kale stored at room temperature. They found that kale flowers lost no SMCO when left up to seven hours before freezing at -10°C or freeze drying, although when left 24 hours, 10% of the SMCO was lost.

The only report on the effect of chopping on the loss of SMCO in brassicas is by Schubert (1954), who reported that when white cabbage plants were chopped they produced less anaemia in dairy cows. SMCO levels in ensiled kale have not been measured, although NZDA (1955), Steger *et al*. (1965, 1968) and Greenhalgh (1969) have reported that ensiled kale is non-toxic to animals compared with fresh kale. Josefsson *et al*. (1972) reported significantly lower glucosinolate levels in kale which had been ensiled for 3 to 5 months, although Steger *et al*. (1968) found that both fresh and ensiled kales increased the thiocyanate level of cows' blood by more than 10-fold. A possible disadvantage with chopping and wilting kale is a loss of palatability, which Willey (1964) suggested could happen in 24 hours.

As well as knowing if a cut and carry system involving wilted or chopped kale would be an effective way of reducing SMCO levels, it is also important for researchers sampling brassicas to know the time that chopped kale can be stored prior to freezing before a significant amount of SMCO is lost.

Little work has been done relating the time frozen or freeze dried kale samples can be stored with the loss of SMCO from such samples. Gosden (1979) reported that freeze dried samples stored in sealed bottles showed no change in SMCO content during the first three months of storage and has since suggested that there is probably little change in SMCO content during six months of storage (Gosden, pers. comm.). Greenhalgh *et al.* (1969) reported that fresh kale, after being stored at -20°C for four months, still caused anaemia in cattle and goats, hence freezing kale below a certain temperature must prevent or at least significantly decrease any degradation of SMCO.

The means by which SMCO can be maintained or destroyed by drying at different temperatures is not clear. Pelletier and Martin (1973) obtained anaemia in sheep fed with kale dried at a low temperature (25-35°C) for 48 hours while both Piatkowski and Steger (1965) and Steger *et al.* (1965) appeared to destroy the toxin by drying at a high temperature. Greenhalgh (1969), however, found that autoclaved kale (120°C for 30 mins) was still toxic to animals.

1.3 Measurement of SMCO

1.3.1 Chemical techniques

SMCO was first isolated and measured quantitatively by Synge and Wood (1956) and by Morris and Thompson (1956). They extracted SMCO from brassicas by macerating the crop material in ethanol and then passing the effluent through an ion exchange column to separate the different amino acids. They found that SMCO was one of the first amino acids eluted from the column and measured its concentration by paper chromatography. Smith (1974) when isolating the toxic factor in kale also extracted SMCO using an ion exchange column, after

dividing the kale into small fractions and testing each for haemolytic activity. After identifying the toxic factor, Smith (1974) and his co-workers measured SMCO quantitatively in different crops using an amino acid analyser, which was first developed and tested by Spackman *et al.* (1958).

Because of the slowness and expense involved in measuring SMCO concentrations on amino acid analysers, faster methods were developed to enable large numbers of measurements to be made, especially for plant breeding purposes. Whittle *et al.* (1976) developed a reasonably rapid electrophoresis method for quantitatively measuring SMCO. They extracted SMCO by repeated maceration of kale in water and then separated it from other components of the deproteinised extract by electrophoresis on a silica gel impregnated glass-fibre sheet. After visualising with a ninhydrin reagent, the SMCO spots were quantified by reflectance mode densitometry. SMCO values measured by this method were found to be reasonably well correlated with those measured by ion-exchange chromatography on an amino acid analyser.

Another method for measuring SMCO was developed by Matheson and Moir (1976). This was faster than that developed by Whittle *et al.* (1976) but less accurate. It was based on thin-layer chromatography and visual comparison of isolated SMCO spots with standards run simultaneously on the same plate. The SMCO was extracted from the kale by the same method as used by Whittle *et al.* (1976). Extracts of kale containing SMCO were spotted onto glass plates covered with cellulose powder, the plates were developed in an acid solution, sprayed with ninhydrin and the colour was allowed to develop overnight. SMCO migrated more slowly than the other common amino acids and thus separated itself from them. This technique was further improved

by Matheson (1980) who developed a simple quick extraction technique prior to thin layer chromatography, involving an ethanolic extraction of SMCO from fresh material.

A more accurate method of measuring SMCO, based on automation of the ion exchange column, has recently become the most popular among researchers desiring to do large numbers of measurements (Gosden and Johnston, 1977; Gosden, 1979). This method takes advantage of the polar side chain of SMCO to develop a simplified amino acid analyser using a short column with a cycle time of less than ten minutes, in order to achieve a separation of SMCO from other amino acids. The column elutant is developed with ninhydrin reagent and the absorbance of SMCO measured. It is a much less labour intensive method than the electrophoresis or the thin layer plate chromatography methods. Gosden and Johnston (1977) found the accuracy of 150 analyses performed by this technique to be ± 7%, although Gosden (1979) later reported the technique to have an accuracy of about ± 5%. They extracted the SMCO by treating freeze dried kale with a buffered hydrogen peroxide solution, whereas previously SMCO was extracted from frozen kale by repeated maceration with water (Whittle *et al.*, 1976; Matheson and Moir, 1976).

1.3.2 Near infrared reflectance analysis

Near infrared reflectance (NIR) analysis involves the use of instruments to measure a physical property of matter, and the relating of the data to chemical composition. The physical property measured is the ability of matter, usually a particular chemical group, to absorb, transmit or reflect infrared radiation. For a molecule to interact with infrared radiation it must have a permanent dipole movement which involves either vibrations about a bond or rotations around a bond.

Some matter, such as metals, do not produce an infrared spectrum, but most chemical bonds, such as hydroxyl, methyl or α-amino, absorb infrared radiation at one or more specific wavelength areas within the near infrared range of 1100 to 2500 nm. Most compounds absorb near infrared radiation at several wavelengths, at varying intensities.

The basic premise in NIR analysis is to construct a mathematical model relating energy absorption at several points in the near infrared spectrum to the concentration of one of a number of chemical components. The individual correlation between any absorption bond and the concentration of the molecular group which causes that absorption is low and a multiple linear regression is used to distinguish the effect of the constituent of interest from other chemical groups which may interfere.

The NIR instrument is calibrated for each parameter against a population of dried ground samples of known concentrations. This calibration population should include samples with concentrations of the parameter evenly distributed over as wide a range as possible. The multiple linear regression calibration equation is checked against another population of samples with known concentrations of the parameter of interest. This check population should also contain evenly distributed samples with a range similar to those in the calibration population.

The degree of accuracy of NIR analysis for any parameter has usually been based on correlations and standard errors of differences between predicted and known values in check populations. Correlations, however, are dependent on the range of sample values within a population. A valuable statistic for estimating the usefulness of NIR is the range divided by the standard error, called the range error ratio or RER (Starr *et al.*, 1981).

The major advantages of NIR analysis are: a) speed (once a machine is calibrated, a sample can be processed by an operator in 1 to 2 minutes depending on the machine); b) its non-destructive nature; c) the lack of requirement for either any chemicals or any weighing; and d) the ability to determine several quality parameters simultaneously.

A major use of NIR analysis has been in the determination of nitrogen content in forages (Norris *et al.*, 1976; Barton and Burdick, 1979; Winch and Major, 1981), cereals (Gill *et al.*, 1979; Starr *et al.*, 1981), oil-seeds (Hunt *et al.*, 1978) and to a lesser extent forage brassicas (Starr *et al.*, 1981). Other factors such as moisture (Williams and Thompson, 1978), lysine (Gill *et al.*, 1979) and malting quality (Allison *et al.*, 1978) in cereal grain, plus oil in oil-seeds (Starr *et al.*, 1981) and several forage quality factors (Norris *et al.*, 1976) have all been successfully measured by this technique. There are no reports of NIR analysis having been used to measure SMCO or moisture in brassicas.

The correlation coefficients between predicted and reference nitrogen values in reported check populations of all crops have been greater than $r = 0.95$, suggesting that nitrogen can be accurately determined by NIR analysis. Reported standard errors between predicted and reference nitrogen values have varied between 0.03 - 0.07 for cereals (Gill *et al.*, 1979; Starr *et al.*, 1981), 0.07 - 0.10 for kale leaves and stems (Starr *et al.*, 1981) and 0.16 - 0.20 for forages (Norris *et al.*, 1976; Shenk and Barnes, 1977; Winch and Major, 1981). The calculated RER values on N predictions for the three groups varied between 15-24 for cereals, 14-16 for kale and 15-25 for forages.

Winch and Major (1981) reported that nitrogen was predicted more accurately in both grasses and legumes, when the material was ground to a fine uniform particle size through a Udy cyclone mill, rather than coarsely ground through a 'Christy and Norris' mill fitted with a 1 mm screen. Similar results were obtained by Hunt *et al.* (1978) when predicting nitrogen in wheat. Winch and Major (1981) showed that moisture levels of check samples should all be within the range of moistures in the calibration samples, in order to obtain best predictions.

CHAPTER II

Chemical Analyses

2.1 Analysis of SMCO Using Autoanalysis Method

Introduction

The main method used for measuring SMCO in kale samples in this thesis was an autoanalysis method. This technique is a slightly modified version of that used by Gosden (1979) and is used by the Scottish Crop Research Institute (SCRI), Pentlandfield, for screening brassica samples for SMCO (Allison *et al.*, 1980).

The technique involved first extracting SMCO from a known weight of freeze dried material for approximately three hours using a pH 1.8 citric acid buffer. This extract was passed through a short resin filled ion exchange column using a pH 2.5 citric acid elution buffer. At this pH SMCO will pass through the column with the solvent front, the other amino acids being held by the column. An alkali wash released the amino acids in one group and these and SMCO were separately estimated by ninhydrin in a colorimeter and the results graphed on a chart recorder. Commercially available SMC (Sigma Chemical Company) was oxidised with hydrogen peroxide and used as a standard. The approximate cycle time of the assay was 10 minutes per sample.

Prior to using this method to analyse trial samples, the following factors relating to the extraction of SMCO from the kale were examined:

a) Concentration of material to extract.

b) Effect of hydrogen peroxide oxidation. The purpose of adding hydrogen peroxide to the extraction buffer was to oxidise any SMC in the material to SMCO in order that it might be included in the assay (Gosden, 1979). SMC is also toxic and can not be easily separated from the other amino acids unless oxidised to SMCO.

The addition and omission of hydrogen peroxide enabled the amount of SMC in the samples to be determined.

c) Effect of time between extraction and assay. The effect of storage on extracted solutions, either with or without hydrogen peroxide, was not known.

These factors were examined in two experiments, using Maris Kestrel kale samples. Studies were made on whole plant, leaf and stem samples to see if different tissue types affected the results.

Experimental

In the first experiment, the effect of the concentration of material extracted on SMCO was studied. From previously screened samples, five samples of each tissue type were chosen containing approximately 4, 8, 12, 16 and 20 g SMCO/kg DM. Each of the 15 samples was extracted using 100 mg and 200 mg of material in 20 ml of extraction buffer. All 30 extracts were assayed on the same day as extracted. Gosden (1979) had previously used 100 mg of plant material with 10 ml of extraction buffer.

In the second experiment, the effects of hydrogen peroxide and time between extraction and assay were studied. Three samples of each tissue type were extracted, both with and without hydrogen peroxide. Each sample was extracted using 100 mg of material in 20 ml of extraction buffer. The 18 samples were extracted and assayed on the same day and assayed again after 1, 2, 3 and 4 days storage at -20°C.

To determine the experimental precision of the autoanalysis technique, duplicate samples of leaves, stems and whole plants were extracted and assayed on the same day, with duplicates being assayed

successively. The errors involved with weighing and extracting were expressed in these results. To compare the precision of the technique with samples analysed on different days, leaf samples from the same material were both extracted and assayed on successive days.

Results and discussion

- *a) Concentration of material to extract:*

Detailed results of the first experiment are not available. However, there was a tendency at higher levels of SMCO (16 and 20 g/kg DM) for more SMCO to be measured when 100 mg, rather than 200 mg, of material was extracted with 20 ml of extraction buffer. This trend was evident in each of the three types of kale material, hence in all subsequent SMCO analyses, 100 mg of material was extracted in 20 ml of buffer. This was a lower concentration of material than that used by Gosden (1979).

The results of the experiment in which the effects of hydrogen peroxide oxidation and time between extraction and assay were examined, are presented in Table 3.

- *b) Effect of hydrogen peroxide oxidation:*

The addition of hydrogen peroxide caused an immediate increase of between 5 and 9% in the amount of measured SMCO. This indicates that SMC was present in these concentrations in the samples.

- *c) Effect of time between extraction and assay:*

When no hydrogen peroxide was added to the extraction buffer, maximum SMCO levels were reached in all tissue types after one day's storage. Levels remained at a maximum for another day with leaves and whole plants and for another three days with stems.

TABLE 3: Effect of hydrogen peroxide and storage time after extraction on SMCO in kale (g/kg freeze dried material).

Tissue type		Storage time (days) 0	1	2	3	4	Mean
Leaves	+HP†	19.3 abc	19.7 abc	20.0 ab	18.3 bcd	18.0 bcd	19.1
	-HP	17.7 cd	20.7 a	19.0 abcd	17.7 cd	17.0 d	18.4
	Mean	18.5	20.2	19.5	18.0	17.5	18.7
	Significant effects:		*Time**			*SE*	*1.33*
Stems	+HP	14.7 a	14.7 a	15.3 a	14.7 a	12.7 c	14.4
	-HP	13.3 bc	14.3 ab	15.3 a	15.3 a	14.3 ab	14.5
	Mean	14.0	14.5	15.3	15.0	13.5	14.5
	Significant effects:		*Time**,	*Time x HP**		*SE*	*0.77*
Whole plants	+HP	14.0 c	14.3 bc	14.3 bc	13.3 c	12.3 c	13.6
	-HP	13.3 c	16.0 a	15.7 ab	14.0 c	13.7 c	14.5
	Mean	13.6	15.2	15.0	13.6	13.0	14.1
	Significant effects:		*Time**,	*HP***		*SE*	*0.94*

† HP = Hydrogen Peroxide. Lower case letters show significant differences at the 5% level (LSD test) for the Time x HP interaction.

* and ** = Effects significant at 5% and 1% levels of significance respectively.

Hydrogen peroxide addition caused maximum apparent amounts of SMCO to be produced immediately in all extracts. SMCO levels in extracts remained at a maximum for two days with leaves and whole plants and for three days with stems, after which levels tended to be reduced.

As a result of this experiment, it was decided that with all subsequent analyses, hydrogen peroxide would be included in the extraction buffer and extracted solutions would be assayed either immediately or after one or two days storage.

- *d) Precision of autoanalysis method:*

The results of analysing kale leaf, stem and whole plants in duplicate are shown in Table 4.

TABLE 4: Errors involved in analysing SMCO in kale using the autoanalysis method.

Tissue type	No. of samples	SMCO (g/kg freeze dried material)				
		Mean	Range	SE	RER	CV%
A. Duplicates on same day:						
Leaves	60	10.9	4.5-20.0	0.63	24.6	5.8
Stems	59	9.1	1.5-20.5	0.86	22.1	9.4
Whole plants	60	8.9	3.5-15.5	0.55	21.8	6.2
B. Duplicates on successive days:						
Leaves	30	9.0	3.0-20.0	0.97	17.5	10.8

When duplicates were analysed on the same day, the variation was similar to the 5% and 7% reported for the same technique by Gosden and Johnston (1977) and Gosden (1979) respectively. Stem samples had

a higher standard error and coefficient of variation than leaf or whole plant samples. Because stems had a wider range of values, similar RER values to the other tissue types were obtained. When leaf duplicates were analysed on different days, the standard error (SE) and coefficient of variation (CV) were higher. With samples analysed on different days, further errors occurred which could have been due to changes with time in either the chemicals or the ion exchange column.

Conclusions

Experiments on varying the extraction technique showed that SMCO levels obtained could be significantly altered if a standard technique was not maintained. More SMCO was extracted in high SMCO samples when 100 mg, rather than 200 mg of material, in 20 ml of extraction buffer was used. The time between extraction and assay also affected SMCO levels, as did the inclusion of hydrogen peroxide in the extraction buffer. For all subsequent samples, the extraction technique was standardised by always using 100 mg of freeze dried material in 20 ml of extraction buffer which included hydrogen peroxide and ensuring that the time delay between extraction and assay was not more than two days.

An acceptable variation of between approximately 6 and 9% was found for this technique when duplicate samples were analysed on the same day. A slightly higher variation occurred when duplicates were analysed on different days. It was concluded therefore, that in order to obtain the most accurate results, samples to be compared should be analysed over as short a time scale as possible. When comparing samples analysed over several days, a larger analytical error must be taken into account.

2.2 Use of Near Infrared Reflectance for Estimating Nitrogen, SMCO and Moisture

2.2.1 *Nitrogen*

Introduction

It has been reported that SMCO levels in brassicas are related to levels of nitrogen (N) fertiliser applied (Anon., 1978b; McDonald *et al.*, 1981). Levels of N in brassicas are also related to levels of N in the soil. In some of the trials reported in this thesis, there was a significant correlation between SMCO and N within plants. It is also important to know how crude protein levels are affected by various treatments aimed at reducing SMCO levels in kale. The amount of nitrogen in various tissue types was examined, as brassica breeders are interested in knowing levels of toxins and nutrients in different parts of kale plants.

The nitrogen content in samples is normally measured by the Kjeldahl method, however it is quite time consuming, hence the use of NIR analysis for predicting N was studied.

Experimental

N was predicted on a Technicon InfraAlyzer 300, an NIR instrument containing positions for a maximum of 10 filters. Of the filters, only five were used to predict N (Table 5). These wavelengths had been chosen by the manufacturers as they were specific points of absorption for fibre, protein, starch, water and particle size.

Sample details:

Samples were taken from plots of Maris Kestrel kale grown at several sites during 1979 and were either harvested as whole plants or divided into leaf and stem fractions. At one site where there were

TABLE 5: Wavelengths used for nitrogen predictions in Technicon InfraAlyzer 300.

Filter position	Wavelength (nm)	Compound or structure absorbed
2	2310	fibre
5	2180	protein
7	2100	starch
8	1940	water
10	1680	particle size

several kale cultivars (Maris Kestrel, Merlin, Vulcan and Canson), leaf samples were further divided into young leaves (approximately one-third expanded) and the older leaves into laminae and petioles. Stems were divided into upper and lower sections. The plants were thus divided into five components. Each sample was chopped, freeze dried and then milled through a 1 mm sieve using a 20 cm "Christy and Norris" mill. Because samples were also required for the analysis of toxins, which are unstable, they were stored at -20°C in a deep freeze for up to nine months prior to being analysed.

Calibration equations were derived on populations of whole plants, entire leaves and entire stems. To check the calibration equations an additional population of each tissue type was selected, plus samples from each of the five plant components. The number of samples, the mean, and range within each calibration and check population are shown in Table 6. In all populations, samples covered the maximum range available and wherever possible, were evenly distributed throughout the range. For all samples, reference N values were obtained using an automated Kjeldahl method (Technicon Industrial method No. 321-74A). All N values are presented as a percentage of freeze dried material (FDM).

TABLE 6: Number, mean and range of samples in Nitrogen prediction populations.

Tissue type	Population	No. of samples	Nitrogen (% FDM*) Mean	Range
1979 populations:				
Whole plants	calibration	40	2.24	1.62 - 3.02
	check	30	2.44	1.25 - 4.25
Leaves	calibration	22	2.27	1.17 - 3.30
	check	18	2.36	1.12 - 3.75
Stems	calibration	26	1.72	0.92 - 2.82
	check	16	1.85	1.07 - 2.85
Young leaves	check	6	3.80	2.87 - 4.25
Laminae	check	6	3.30	2.87 - 3.71
Petioles	check	6	1.60	0.87 - 2.67
Upper stems	check	4	2.26	1.35 - 3.37
Lower stems	check	4	2.10	1.15 - 3.22
1981 check populations:				
Whole plants		20	1.79	1.24 - 2.64
Leaves		30	1.85	0.80 - 2.88
Stems		20	1.82	0.81 - 2.94
Stem pith		5	0.80	0.39 - 1.13
Stem cortex		5	1.20	0.99 - 1.61

* FDM = freeze dried material.

The range of N values in the 1979 calibration populations was limited. In order to increase the range, additional samples from the check populations were subsequently included and new calibration equations derived. Using these modified 1979 equations, N was predicted in 1980 and 1981 samples and extensive checking was carried out with the 1981 samples (Table 6). Some of the stems in 1981 were divided into pith and cortex, hence samples of both fractions were also predicted for N.

Calibration procedure:

Approximately 8.5 cc (1.5 - 2.5 g) of each sample in a calibration population was scanned and the energy absorption value on each of the five filters noted. These energy values plus the reference N values were used to form a mathematical model using linear regression. An equation was used of the form,

$$\text{Predicted N} = K_0 + K_1 \log(F_1) + K_2 \log(F_2) + K_n \log(F_n)$$

where $\log(F_n)$ was the logarithm to the base 10, of the energy reflected at filter position n and K_0 to K_n were regression coefficients which described the input data. Usually three or four filters gave the maximum significant correlation with Kjeldahl N. The Technicon 300 was programmed by inserting the appropriate coefficients into its memory, so that reflectance energy values for the wavelengths were multiplied by the appropriate coefficient when predicting unknown samples.

Statistical details:

When comparing NIR predicted (x) and reference (y) N values for each population, as well as correlation coefficients (r), the standard error of difference (SE) and the coefficient of variation (CV) were also calculated. The equation used for calculating standard errors was,

$$SE = \sqrt{\frac{\Sigma(y-x)^2 - (\Sigma(y-x))^2/n}{n-1}} \qquad (n = \text{number of samples})$$

The correlation coefficient was normally used to compare the relationship between manual and predicted results. However, it is affected by range and number of samples. The coefficient of variation and standard error were independent of range, hence these were used to compare SMCO

predictions in different populations and tissues. Another statistic used was the range error ratio (RER), which related the range and SE. The higher the RER, the more accurate is the test and the better it will be for selecting differences between high and low N results.

The accuracy of the modified Kjeldahl method was ascertained by analysing all the 1981 check samples in duplicate.

Results and discussion

Evaluation of 1979 calibration equations:

The calibration equations formed from the 1979 whole plant, leaf and stem samples (Table 6) were as follows:

Whole plants:

$$Ypred = -0.14 + 78.14 \log(F5) - 64.23 \log(F7) - 2.24 \log(F8) - 2.50 \log(F10)$$

Leaves:

$$Ypred = 1.03 - 17.71 \log(F2) + 105.38 \log(F5) - 77.49 \log(F7)$$

Stems:

$$Ypred = 1.16 + 81.56 \log(F5) - 61.33 \log(F7) - 21.78 \log(F10)$$

(F = filter position - see Table 5)

The main filters used for predicting N were 5 (2180 nm) and 7 (2100 nm) which were specifically for measuring the absorption of protein and starch respectively. Other workers have also found that these wavelengths are useful for measuring N in brassicas (Starr *et al.*, 1981). The starch filter and the other non-protein filters in the calibration equations compensated for the interference effects of other substances with N.

The accuracy by which these equations predicted N in whole plants, leaves and stems can be seen in Table 7. The correlation coefficients in calibration and check populations were very high ($r \geq 0.95$). RER values and coefficients of variation were similar in the check populations

of the three tissue types. The results show that all three calibration equations are suitable for predicting N in the three respective tissues.

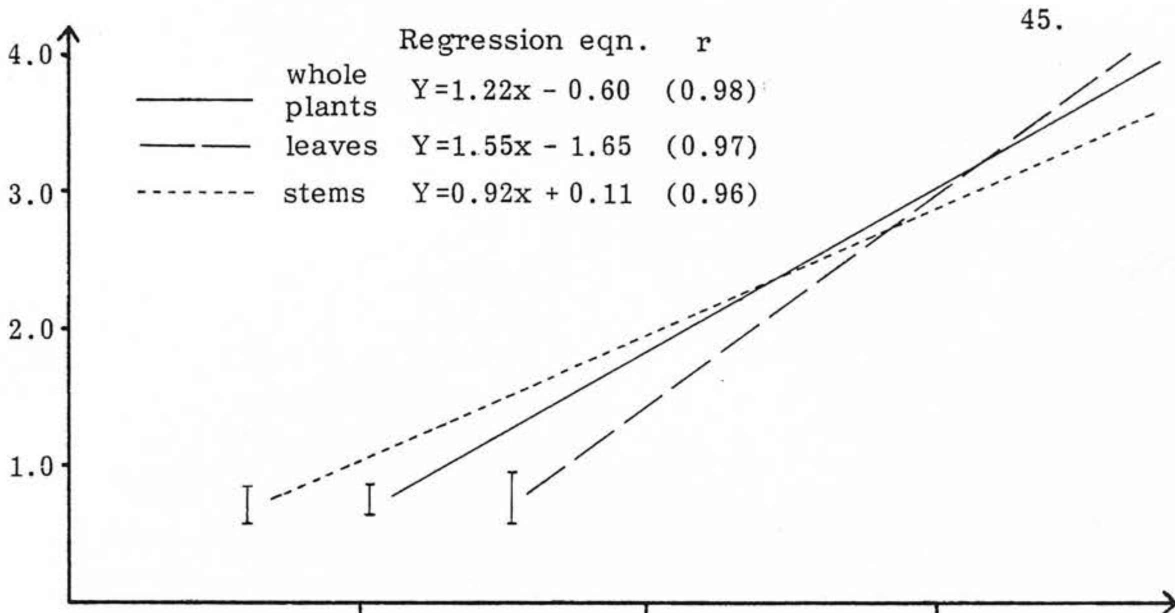
TABLE 7: Evaluation of 1979 nitrogen predictions in kale whole plants, leaves and stems.

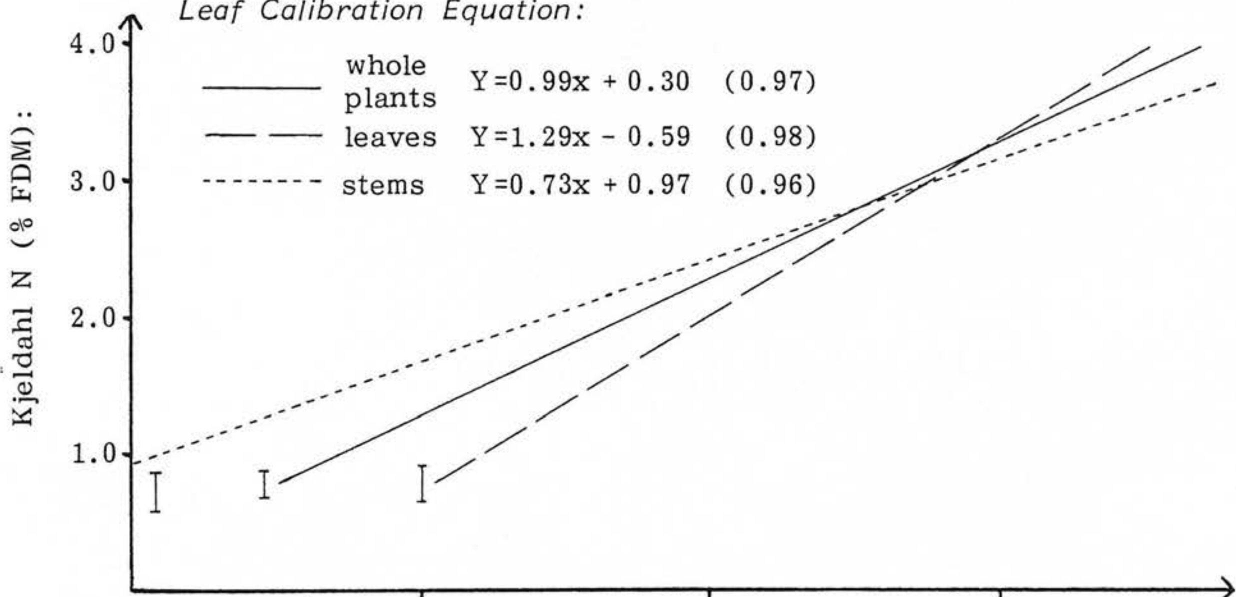
		Nitrogen (% FDM)			
Tissue type	Population	Correlation coefficient (r)	SE	RER	CV%
Whole plants	calibration	0.95	0.12	12.0	5.2
	check	0.98	0.24	12.7	9.7
Leaves	calibration	0.99	0.08	26.3	3.6
	check	0.98	0.26	10.2	11.0
Stems	calibration	0.99	0.08	25.3	4.3
	check	0.96	0.18	9.9	9.7

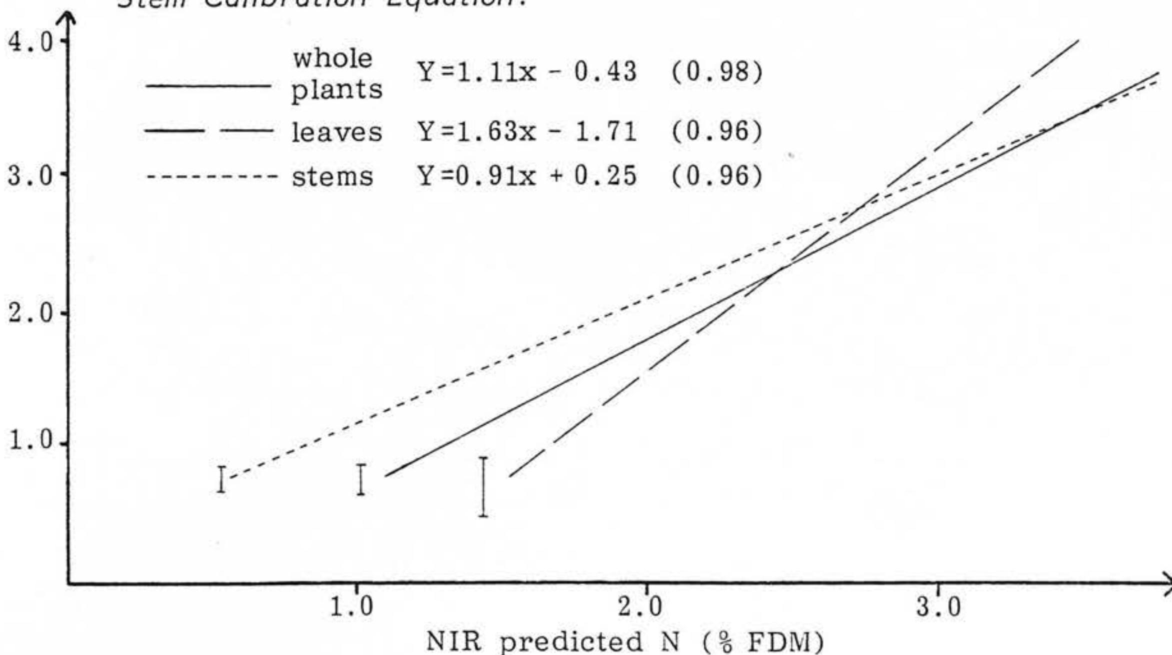
SE = standard error; RER = range error ratio;
CV% = coefficient of variation.

The standard errors for the check populations (0.18 - 0.26) were much higher than those reported previously by Starr *et al.* (1981) in oven dried kale leaves and stems (0.07 - 0.10). This may have been due to there being no duplication of either Kjeldahl or NIR predicted N values for samples in these studies and also fewer samples were used in the check populations.

Figure 1 shows the results of predicting the whole plant, leaf and stem check populations by each of the calibration equations. The whole plant check population was predicted with a high correlation ($r \geq 0.97$) by each calibration equation, even though several values were outside the range of the original calibration equation. The leaf check population was best predicted by its own calibration equation. When predicted by the other calibration equations, higher errors and large skew effects were noted. Stems were accurately predicted by







I standard error of difference between predicted and Kjeldahl N

FIGURE 1: Regression lines of tissue types based on the 1979 calibration equations.

both the stem and whole plant calibration equations, but not by the leaf equation, which caused larger errors and a skew effect.

These results show that no single calibration equation will accurately predict all three components. Whole plant and stem N could be predicted by one equation. However, leaf N needs to be predicted by a separate calibration equation. This may indicate that the whole plant sub-samples are biased by the amount of stem present.

Evaluation of new calibration equations on 1981 samples:

The modified calibration equation formed on the populations with wider ranges, had the following coefficients:

Whole plants (63 samples):

$$Ypred = -0.87 + 90.78 \log(F5) - 71.69 \log(F7) - 4.56 \log(F8) - 3.47 \log(F10)$$

Leaves (35 samples):

$$Ypred = 1.97 - 28.12 \log(F2) + 122.94 \log(F5) - 84.56 \log(F7)$$

Stems (35 samples):

$$Ypred = 0.85 + 83.47 \log(F5) - 58.36 \log(F7) - 29.18 \log(F10)$$

These equations are similar to the original 1979 calibration equations apart from slight changes in regression coefficients. Table 8 shows for each calibration equation, that correlation coefficients between predicted and Kjeldahl N are very high, but standard errors and coefficients of variation are greater than with the original calibration equations. RER values for the leaf and stem calibration populations were reduced, because their standard errors increased and their range of N values remained similar. The RER value for the whole plant population increased however, even though its standard error was higher, due to a large increase in the range of N within its samples.

The accuracy with which these equations predicted N in 1981 check populations is shown in Table 8 and Figure 2. The correlation coefficients between predicted and Kjeldahl N were similar to those obtained with the 1979 check populations using the original calibration equations, being 0.95 or greater with each tissue type. RER values in the 1981 whole plant and leaf check populations were lower than in 1979, although standard errors in all tissue types were similar. Lower RER values were due to a lower range of N values in these tissues in 1981.

One of the main observations with 1981 samples, was a considerable skew in the regression lines for whole plants and leaves. This was not evident in stems. The 1981 leaf population consisted of samples from two trials, each with 15 samples. Regression lines $Y = 0.68x + 0.63$ ($r = 0.91$) and $Y = 0.75x + 0.49$ ($r = 0.97$) were calculated for each trial. This change in slope was therefore consistent in each trial in 1981. For whole plants the regression line was $Y = 0.69x + 0.37$ ($r = 0.95$).

Correcting the 1981 leaf and whole plant predicted N values to compensate for skew effects, reduced the standard errors to levels lower than in 1979 (Table 8). For simplicity, an average regression equation of $Y = 0.73x + 0.54$ ($r = 0.95$) was used to correct all leaf samples in 1981. The corrected RER values and coefficients of variation were at least as good as those obtained in 1979.

In order that all the 1981 samples were comparable, stem samples were also corrected. This took into account the small change of bias in the 1981 stems, but had little effect on the standard error.

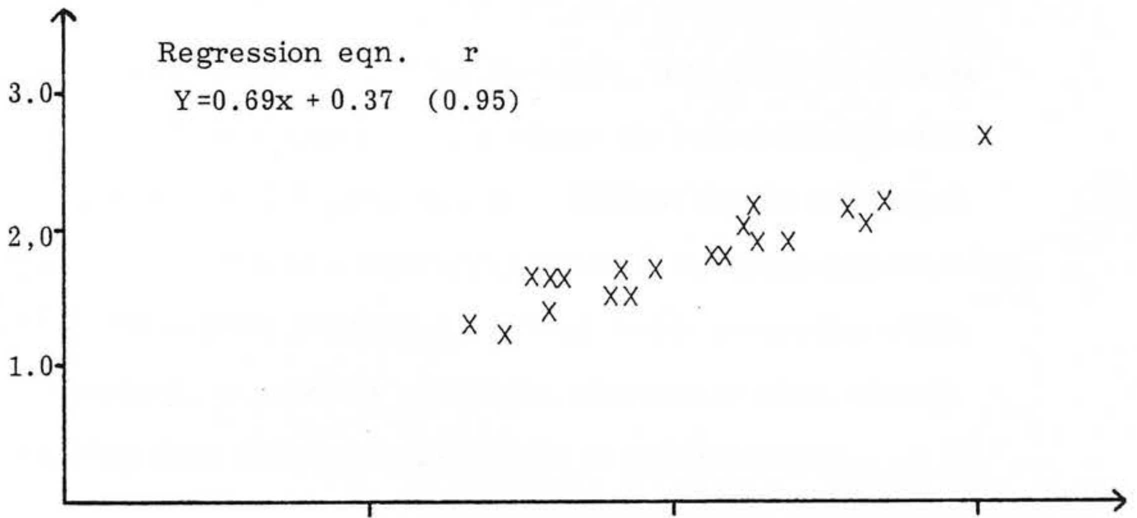
It is not uncommon for skew and bias to be affected using calibration equations for several years or for different locations. Watson *et al.*

TABLE 8: Evaluation of new calibration equations using 1981 kale samples.

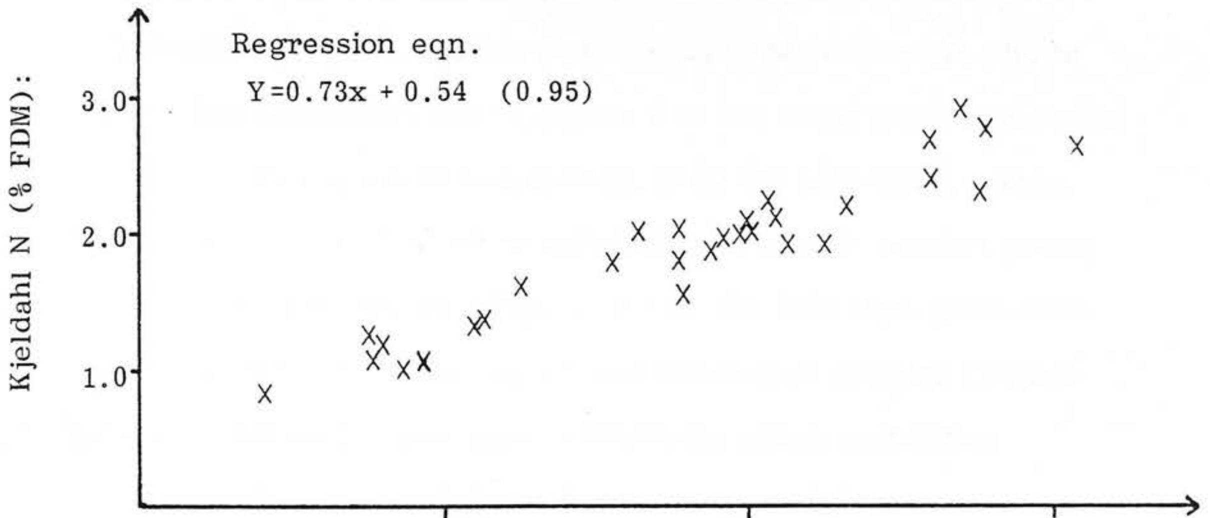
Tissue type	Population	Correlation coefficient	Nitrogen (% FDM) SE	RER	CV%
Whole plants	New caln.	0.97	0.15	20.0	6.3
	Check '81	0.95	0.18 *(0.11)**	7.8 *(12.7)*	10.1 *(6.1)*
Leaves	New caln.	0.97	0.18	14.3	7.6
	Check '81	0.95	0.26 *(0.17)*	8.0 *(12.2)*	14.1 *(9.2)*
Stems	New caln.	0.97	0.12	15.8	6.9
	Check '81	0.97	0.16 *(0.15)*	13.3 *(14.2)*	8.8 *(8.2)*

* Values after predicted N values corrected by regression equations.

Whole plants:



Leaves:



Stems:

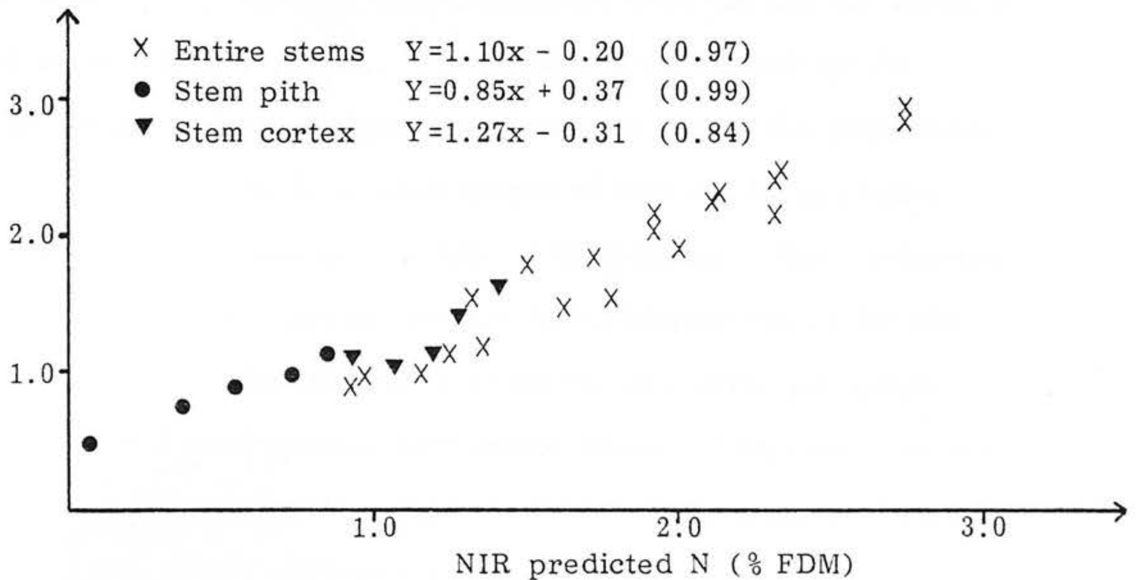


FIGURE 2: NIR predictions of N in 1981 check populations using new calibration equations.

(1977) found when predicting protein in wheat, that different classes of wheat gave different slopes and intercepts for regression equations relating Kjeldahl and NIR predicted N. Williams (1975) also found that season and location of a wheat crop affected the slope and intercept of regression lines when predicting N. This yearly correction which appears necessary, is probably caused by variation in plant mineral levels resulting from differences in climatic or soil conditions.

Prediction of components within leaves and stems:

The samples of all five kale components were predicted using the original 1979 whole plant calibration equation and the results are shown in Figure 3. The regression line in Figure 3 is the whole plant regression line, which for clarity is presented instead of all the individual points. In addition, the 1979 leaf calibration equation was used to predict young leaf, lamina and petiole samples (Figure 4) and the 1979 stem calibration equation used to predict petiole, upper and lower stem samples (Figure 5). The aim of this experiment was to determine which calibration equation was best for the prediction of each component.

High correlations were obtained with all components using each calibration equation, although sample numbers were too few for forming very accurate regression lines. The straight lines formed by the predicted values of each component with at least one of the calibration equations, suggests that N in whole plants of kale can be predicted accurately by dividing plants into several components. When using the whole plant calibration equation (Figure 3), predicted values for the two stem components were less than actual values, whilst predicted values for laminae were greater than actual values. The true N content of samples was predicted less accurately therefore than the ranking order of samples within components.

	Regression eqn.	r
X Young leaves	Y = 0.65x + 1.59	0.97
O Laminae	Y = 0.99x - 0.22	0.90
■ Petioles	Y = 1.10x - 0.19	0.96
▽ Upper stems	Y = 1.13x + 0.13	0.99
● Lower stems	Y = 1.25x + 0.25	0.99
—— Whole plants regression line		0.98

Kjeldahl N (% FDM)

4.0
3.0
2.0
1.0

1.0
2.0
3.0
4.0

NIR predicted N (% FDM)

FIGURE 3: Prediction of N in kale components using 1979 whole plant calibration equation.

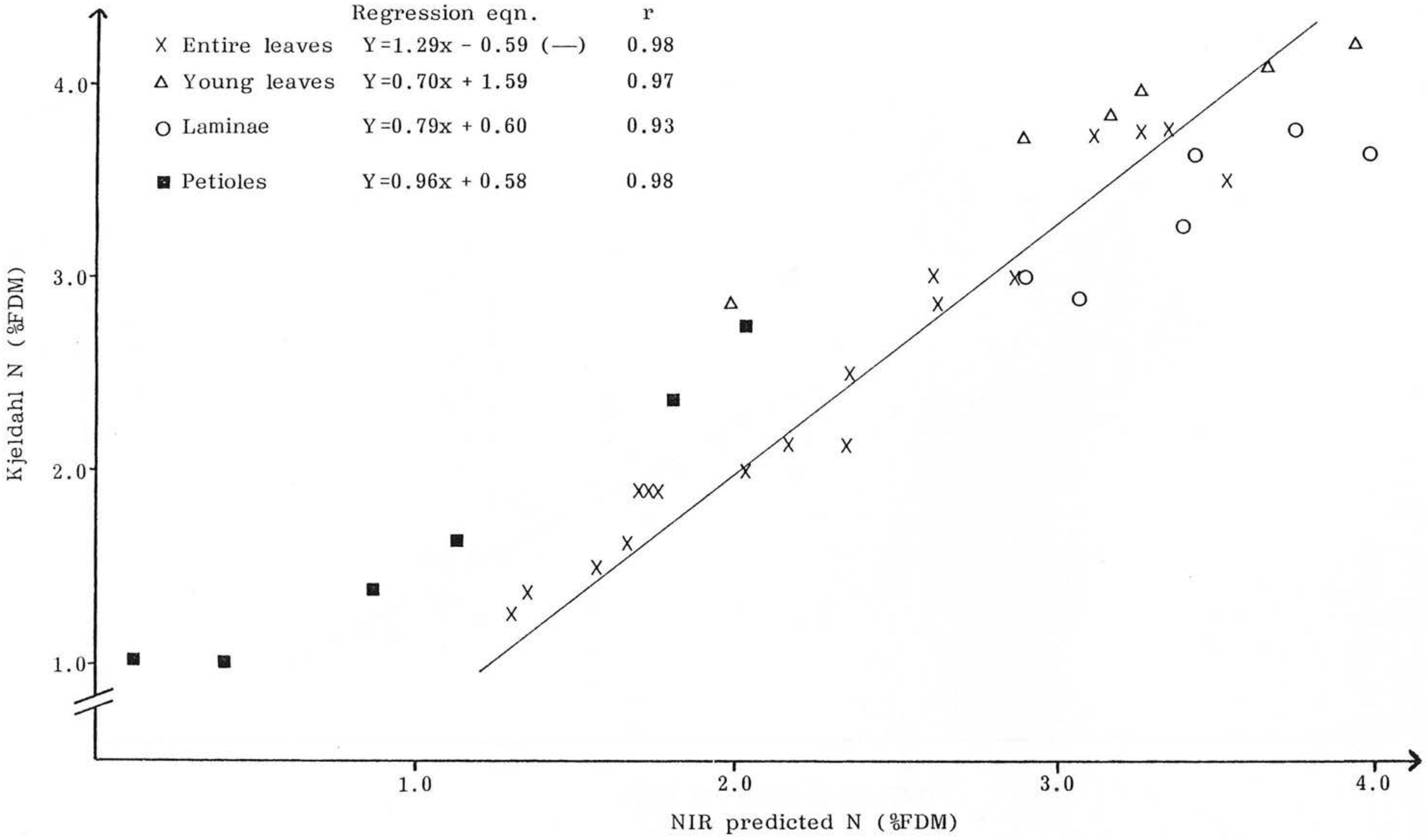


FIGURE 4: Prediction of N in leaf components using 1979 leaf calibration equation.

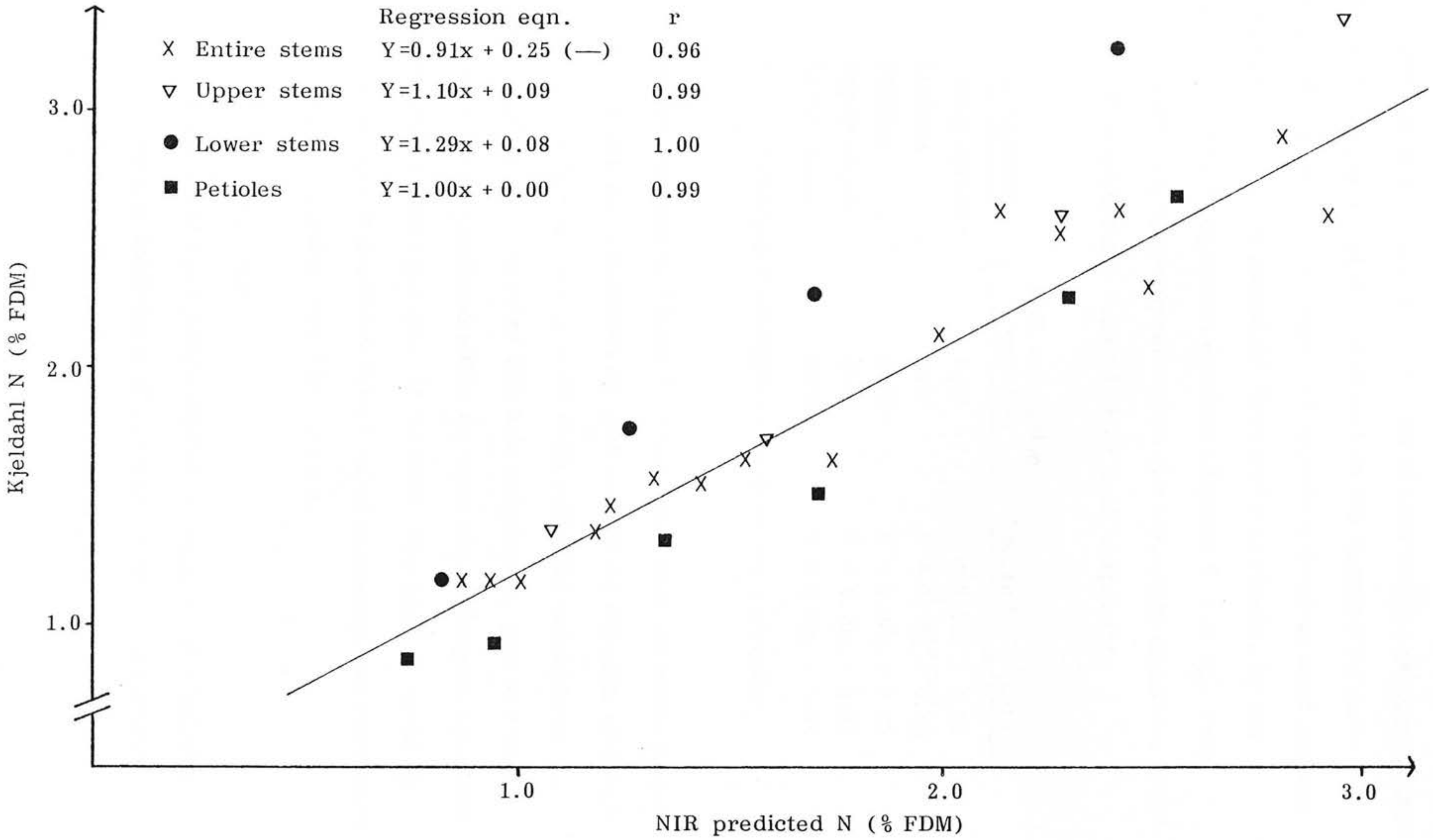


FIGURE 5: Prediction of N in stem components using 1979 stem calibration equation.

For the most accurate predictions of each sample, results suggest that either separate calibration equations are required for each component, or one of the calibration equations could be used and the predicted N values corrected for skew and bias effects, by the appropriate linear regression equation (Figures 3, 4 or 5). From these results, the following calibration and correction equations would give the most accurate N values (Y) for each component.

Component	1979 calibration equation	Correction equation
Young leaves	Leaf	$Y = 0.70x + 1.59$
Laminae	Leaf	$Y = 0.79x + 0.60$
Petioles	Stem	$Y = 1.00x + 0.00$
Upper stems	Stem	$Y = 1.10x + 0.09$
Lower stems	Stem	$Y = 1.29x + 0.08$

x is the predicted nitrogen value from the calibration equation.

For stem samples in Figure 2, it was noted that the trend evident for entire stems was maintained for pith and cortex samples, although there were too few samples to make valid statistical assumptions. It appears that as with the other five kale components, pith and cortex nitrogens could be predicted either by their own calibration equations or by the entire stem equation. If the latter was used, it would be preferable to correct predicted values using an appropriate regression equation similar to those formed in Figure 2.

Accuracy of Kjeldahl method:

The accuracy of the Kjeldahl method is shown by the following standard errors and coefficients of variation formed from differences between duplicate analyses of the 1981 check populations.

	Mean N value (% FDM)	SE	CV%	RER
Whole plants (20 samples)	1.79	0.046	2.6	30.4
Entire leaves (30 samples)	1.85	0.045	2.4	46.2
Entire stems (20 samples)	1.81	0.043	2.4	49.5

Standard errors and coefficients of variation were similar in whole plants, leaves and stems and accounted for approximately 42%, 26% and 29% of the variation produced by corrected NIR nitrogen predictions on the same samples (Table 8). These results show that the Kjeldahl method is a very reproducible technique for analysing N and that the low standard errors and high RER values provide a reasonable basis for the calibration of the NIR InfraAlyzer 300.

In this study, particle size, after milling through a 1 mm sieve, was sufficiently fine to give reasonably accurate NIR predictions in all kale components including stem, which appeared to have the greatest variability in particle size. Starr *et al.* (1981) also found that N in oven dried kale leaves and stems could be predicted satisfactorily by NIR when milled through a 1.25 mm sieve. Winch and Major (1981) showed, however, that N in forages can be more accurately predicted when material is milled though a finer sieve than the 1 mm sieve used here. Recent work by Dr. I. Murray at the North of Scotland College of Agriculture, has shown that particle size effects can be reduced by mathematically correcting spectra to take account of the differences in reflectance path length, caused by sample particle size (pers. comm.). It is possible, therefore, that even better predictions of N could be obtained by NIR analysis, if the kale material was more finely ground.

Moisture levels in the different kale populations were generally between 5 and 15%, although in some populations there were a few values

above 15% and occasionally the range was only about 5 percentage units. It was found that N values predicted by all calibration equations were affected slightly by moisture levels in the samples, even after corrections were made to dry base values. Keeping moisture levels reasonably constant in any group of samples to be compared, should increase the accuracy of NIR predictions of N. Despite moisture variations and its effects, N in all kale samples was predicted satisfactorily by NIR.

The low number of NIR wavelengths available in the instrument used here to predict N, may have limited the degree of accuracy obtained. The wavelengths in the Technicon 300 were chosen, however, as a result of extensive research by the manufacturers and have proved satisfactory for N predictions in several crops. The Technicon Infra-Alyzer 300 used here is a much less expensive instrument than the Neotec 6350 (used to predict SMCO) and is more likely to be used for routine measurements. The Neotec 6350 is a research tool for finding wavelengths, over the full near infrared range, which are most suitable for predicting levels of a particular constituent in any material. Once the best wavelengths have been established, the appropriate wavelength filter can be manufactured and inserted into a routine instrument such as the InfraAlyzer 300.

Conclusions

Calibration equations for predicting N by NIR in freeze dried Maris Kestrel kale, were formed for whole plants, leaves and stems. Check populations of the same tissue types were predicted by their respective calibration equations in each of two years and all populations were predicted with an accuracy of at least $r = 0.95$ between Kjeldahl and predicted N. It was found that kale leaves need to be predicted

using a separate calibration equation from whole plants and stems. The latter two tissue types could be predicted by the same calibration equation.

With kale samples predicted in a different year from when calibration equations were formed, it was necessary to correct predicted N values in order to obtain the best results. Predicted values could be corrected by either using a regression equation relating Kjeldahl and NIR predicted values in a particular year, or by changing the calibration coefficients prior to samples being predicted. With both methods, a population of 20 to 30 samples for each tissue type, would need to be analysed for N yearly, by both Kjeldahl and NIR analysis.

The standard errors for whole plant, leaf and stem check populations, after corrections for year effects, ranged from 0.11 to 0.26, the coefficients of variation from 6.1 to 11.0% and the RER values from 9.9 to 14.2. This degree of accuracy is acceptable for most purposes, especially for screening samples used in plant breeding, where the NIR technique is most beneficial. The accuracy could probably be increased, if the kale material was milled through a finer sieve than 1 mm in order to improve the uniformity of size, or if the moisture levels within a population were kept reasonably constant. Despite these factors, the calibration equations are still very reliable for predicting N in kale.

There appeared to be no increase in the accuracy of NIR predictions when kale was divided into several components. Young leaves, laminae, petioles, upper and lower stems, stem pith and stem cortex were all predicted reasonably accurately by one of the whole plant, leaf or stem calibration equations. For most accurate predictions of components, either a separate calibration equation could be derived for each, or one of the whole plant, leaf or stem calibration equations could be used and

predicted values corrected by a linear regression equation. Best regression equations are outlined for each component plus an appropriate calibration equation, although for future work, more reliable regression equations need to be formed for each component, based on larger populations with wider ranges of values.

2.2.2 SMCO

Introduction

The autoanalysis method for measuring SMCO is a time consuming technique. Due to the large number of samples to be analysed for SMCO in the brassica breeding programme at the SCRI, only a limited number of kale samples in this thesis could be analysed by this technique. It was decided, therefore, to study the accuracy of NIR analysis for measuring (predicting) SMCO in kale.

Experimental

A Neotec 6350 Research Composition Analyzer capable of scanning the near infrared spectrum from 1100 to 2500 nm, was used in this study.

Sample details:

Samples of Maris Kestrel kale were taken from plots grown at several sites during 1980 and 1981 and were either harvested as whole plants or divided into leaf and stem fractions. The samples were from fertiliser and population trials and were harvested at different stages of maturity. Young leaves, approximately one-third expanded, were taken from plots in a trial containing the six kale cultivars, Maris Kestrel, Canson, Merlin, Vulcan, Bittern and Chrysol, and also studied.

Populations of samples, covering a wide and reasonably evenly distributed range of SMCO, were used as calibration and check populations

(Table 9). The young leaf populations, YLC1 and YLC2, were made up respectively of 20 samples from several different kale varieties and 18 Maris Kestrel kale samples.

TABLE 9: Number, mean and range of samples in each SMCO population.

Tissue type	Population	No. of samples	SMCO (g/kg FDM*) Mean	SMCO (g/kg FDM*) Range	Date scanned
Whole plants:	calibration	30	13.9	6.0 - 25.0	Jul '81
	check-WC1	30	13.8	6.0 - 24.0	Jul '81
	check-WC2	22	9.7	4.5 - 15.5	Feb '82
Leaves:	calibration	30	14.0	6.0 - 28.0	Apr '81
	check-LC1	26	11.9	5.0 - 24.0	Apr '81
	check-LC2	20	15.7	5.0 - 24.0	Jul '81
	check-LC3	30	9.0	3.5 - 19.0	Jan '82
	check-LC4	39	7.5	4.0 - 13.0	Feb '82
Stems:	calibration	30	13.0	5.0 - 22.0	Jul '81
	check-SC1	30	13.1	7.0 - 24.0	Jul '81
	check-SC2	30	13.0	7.0 - 22.0	Jul '81
	check-SC3	26	12.0	5.5 - 24.0	Apr '81
	check-SC4	30	11.7	6.0 - 20.0	Apr '81
	check-SC5	30	10.2	5.0 - 16.5	Feb '82
Young leaves:	calibration	26	10.2	2.0 - 18.0	Mar '81
	check-YLC1	20	9.8	4.0 - 17.0	Mar '81
	check-YLC2	18	15.6	5.0 - 29.0	Jul '81

* FDM = freeze dried material.

Each sample was chopped, freeze dried and milled through a 1 mm sieve on a 20 cm 'Christy and Norris' mill. After milling, samples were stored for up to 6 months at -20°C until required for analyses. All samples were analysed for SMCO by the autoanalysis method at approximately the same time as being scanned by the infrared instrument. Table 9 gives details of times populations were analysed. The SMCO results based on this autoanalysis method were used as reference values for calibrating and checking the Neotec 6350. All SMCO values are presented as g SMCO per kg of freeze dried material (FDM).

Calibration procedure:

Approximately 19 cc of material (3-6 g) from each sample was packed in the Neotec sample cup and scanned by the instrument, which was programmed to take 50 readings of the energy absorbed by the sample at each wavelength. These readings were averaged and a typical spectrum is shown in Figure 6. The reference SMCO value for each sample was recorded and stored on computer disk with the spectrum. The sequence of events used in NIR analysis on the 6350 is included in Appendix A. Three methods were used to find wavelength combinations which best correlated with SMCO.

- *a) Forward regression method:*

This is the system supplied with the instrument to identify wavelength combinations which correlated highly with the constituent of interest. In the first stage of forward regression, the wavelength with the highest individual correlation to the dependent variable (reference SMCO) is selected. At each stage the wavelength selected maximises the multiple correlation with those wavelengths already selected. This process is normally continued for up to four regression terms, depending upon whether additional terms significantly increase the multiple correlation. This approach presents a very directional solution because the highest individually correlated wavelength to the dependent variable must be included in the final wavelength combination.

Forward regression indicates the level of correlation that can be formed at each stage of the wavelength selection process in any particular population. It can be used as a target to compare selections by alternative methods.

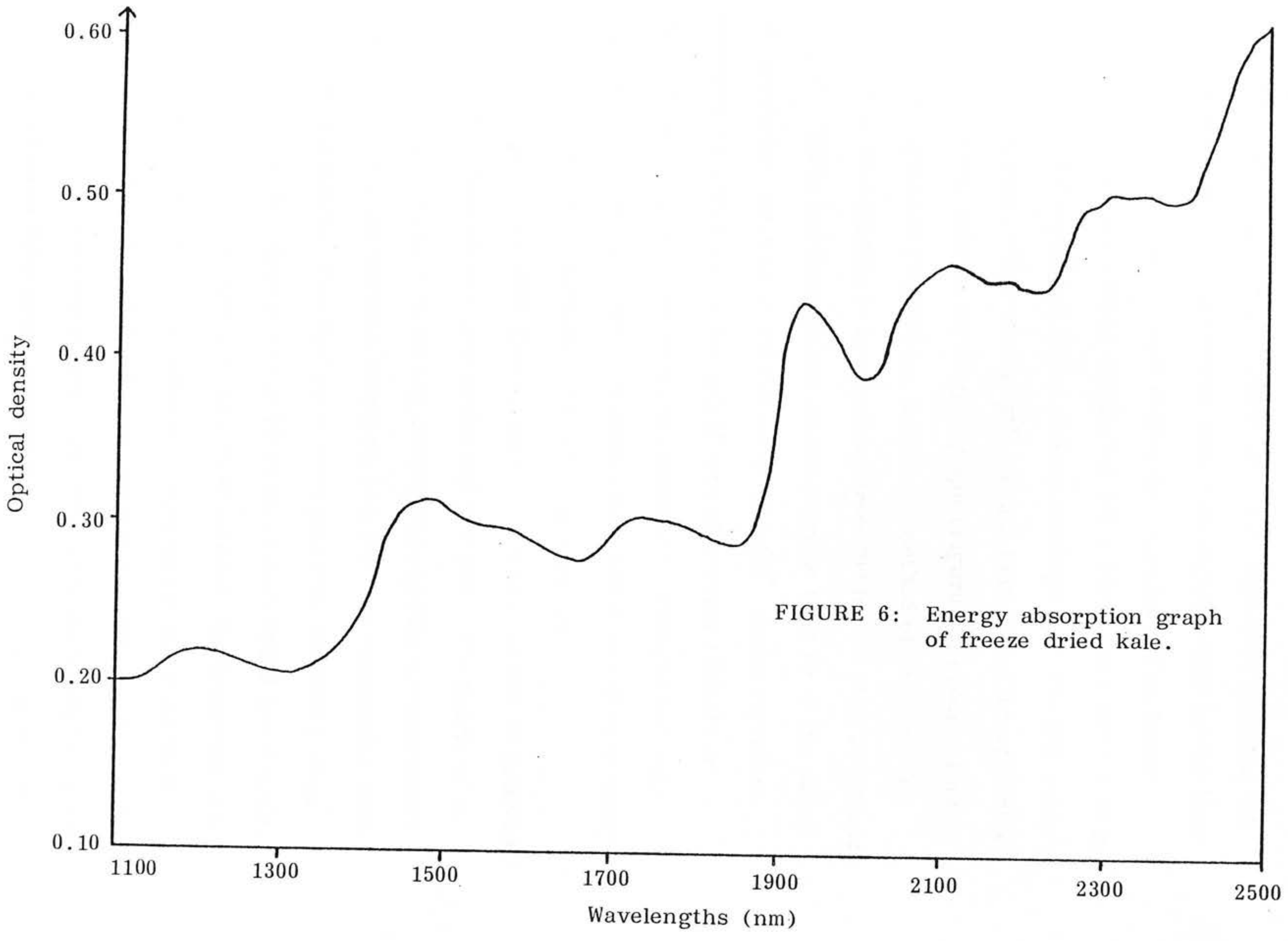


FIGURE 6: Energy absorption graph of freeze dried kale.

- *b) Correlation pair selection:*

The calculation of all possible four term equations from the 700 wavelength energy values stored from each sample would be the ideal method of identifying the best regression equation. This is not practical, as it would entail calculating 700 x 699 x 698 x 697 = 2.38 x 10^{11} regression solutions, where energy readings over the NIR range are made at every other wavelength. It has been noted by workers at SCRI, that the modulus correlation graph (Figure 10) produced by a forward regression programme, provides a method of reducing the number of wavelengths to be examined from 700 to less than 30 for each sample. These wavelengths are chosen because they lie at the apices of correlation peaks or at the bottom of troughs. Peaks represent wavelengths where either the constituent of interest (SMCO) or compounds which correlate to it, are absorbed. Troughs normally represent compounds which have absorptions which interfere at wavelengths where the constituent of interest is absorbed.

From any correlation graph, typically between 12 and 24 individual wavelengths were chosen from peaks and troughs. The matrix of all possible pair combinations for the chosen wavelengths was calculated and the multiple correlations compared with the pair combination from forward regression. The best pairs were tuned by searching other wavelengths within approximately 30-50 nm of each initial wavelength. This ensured that the best combination of wavelengths from each area of the infrared spectrum was chosen. The example in Appendix B (stage 1) shows all pair combinations plus the best tuned pairs in the whole plant calibration population. To form triple combinations, usually 4 to 6 tuned pairs were either combined with each of the chosen wavelengths or were used as initial combinations in forward regression.

When using these pairs as initial terms in forward regression, areas of the spectrum are examined which would not normally be considered by forward regression. The best of these triple combinations were also tuned in a similar manner to that used for pairs (Appendix B, stage 2). For the best three to five triple combinations, a fourth wavelength was found using forward regression. Appendix B (stage 3) shows the best four wavelength combinations for the whole plant calibration population.

- *c) Attributed absorption method:*

One way of defining a subset of NIR wavelengths for an intensive combination search, is to identify wavelengths where the constituent of interest absorbs maximally. Figure 7 shows the absorption spectrum of SMCO crystals prepared by the oxidation of SMC in hydrogen peroxide and water, followed by evaporation of the liquid by air suction and slight heating (<35°C). The data was treated in an identical method to that shown in the correlation pair selection method (Appendix B).

In order to find which of the potential wavelength combinations best predicted SMCO in unknown samples, at least one other population was used, containing different samples of known SMCO values. This check population, usually of a similar number of samples to the calibration population, was scanned by the Neotec. The appropriate wavelength energy values for these samples were multiplied by the regression coefficients for each of the potential wavelength equations to give the amount of predicted SMCO. These values were compared with the reference SMCO values to assess the suitability of any equation for the prediction of SMCO.

The best wavelength combination for each type of sample (leaf, stem, whole plant and young leaf) was used to predict each of the other types, in order to see if a common equation could be identified which would predict all samples.

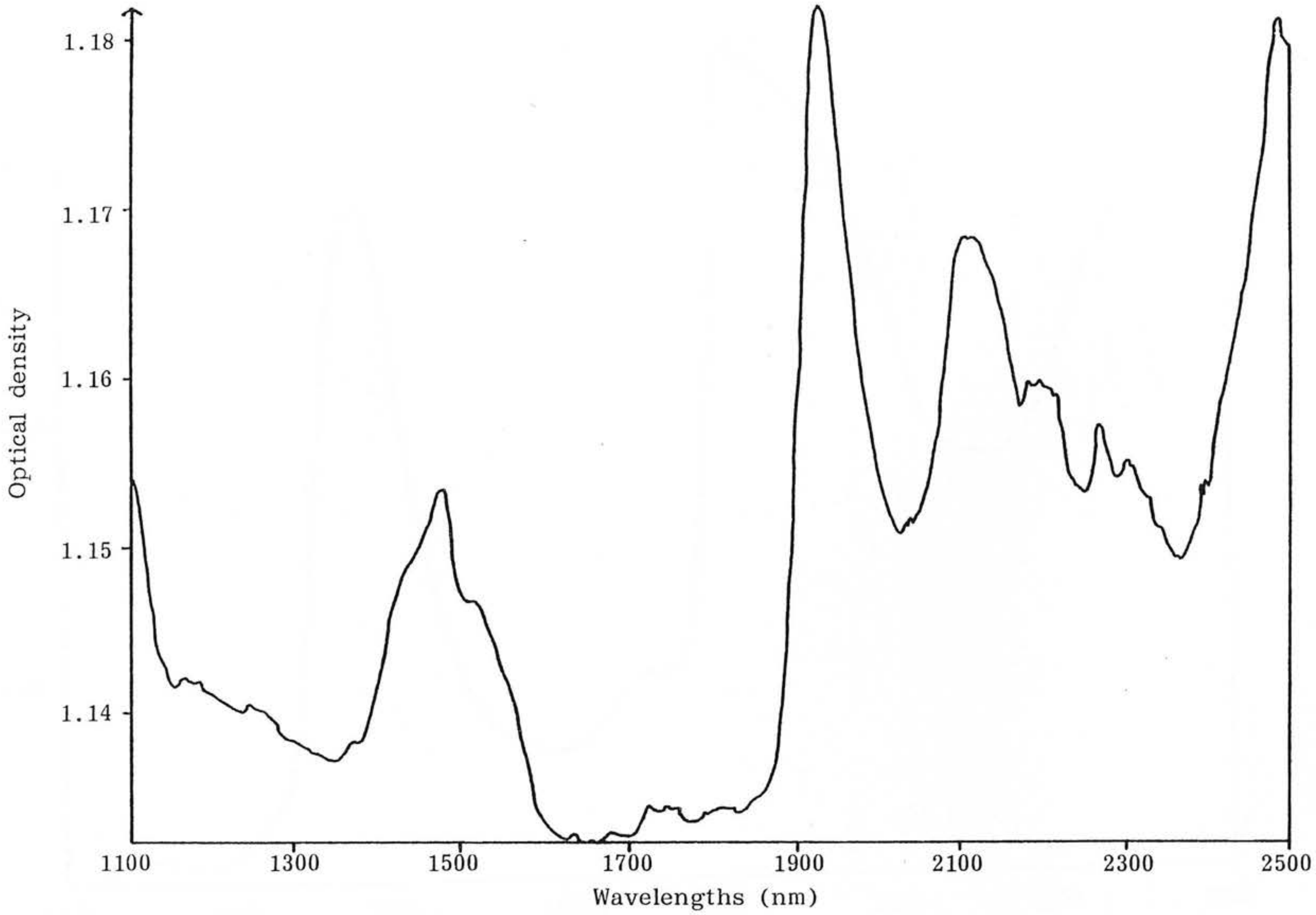


FIGURE 7: Energy absorption graph of SMCO crystals.

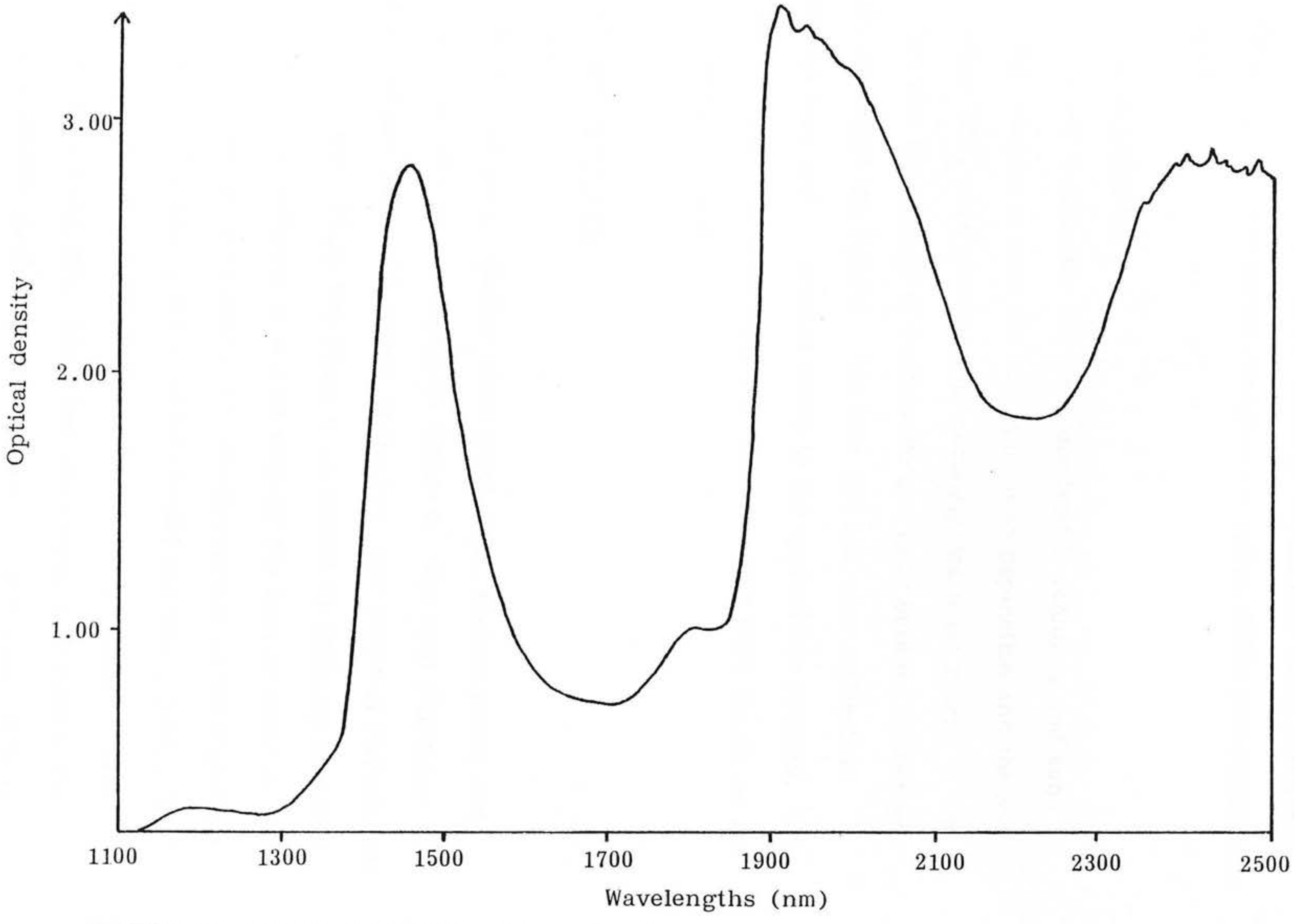


FIGURE 8: Energy absorption graph of water.

Statistical details:

The value of each equation was ascertained, by comparing predicted and reference SMCO values in populations using correlation coefficients, standard errors, range error ratios (RER) and coefficients of variation.

Scanning repeatability:

In order to calculate the error involved in scanning and sub-sampling, samples in both the leaf calibration population and the stem population SC4, were scanned approximately one week apart. It was assumed that the storage of samples for one week between scannings would not affect the results. The best leaf and stem calibration equations were used to predict SMCO in the appropriate samples. The correlation and SE of difference between scannings were calculated for both leaves and stems.

Results and discussion

Spectra:

All kale samples, whether whole plant, leaf, stem or young leaf, had similar spectra to that shown in Figure 6. The main difference between samples was in the height of the two major peaks at wavelengths 1445 and 1940 nm. These two peaks were caused by differing amounts of moisture in the samples, as can be seen by the scan of water in Figure 8. These two wavelengths are widely reported in the literature as being due to the absorption of water (Wetzel and Mark, 1978). The freeze dried brassica samples in these studies had moisture contents ranging between 4 and 15%. The other major peaks in Figure 6 can be attributed to starch, protein or carbohydrates. Wetzel and Mark (*loc.*

cit.) reported that starch is absorbed by NIR at wavelengths 2100, 2270, 2322 and 2330 nm. Law and Tkachuk (1977) reported that protein is absorbed at wavelengths 1460, 1510, 1570 and 2180 nm.

From the spectrum of SMCO crystals (Figure 7), the following wavelengths were chosen as points of maximum absorption: 1162, 1474, 1516, 1678, 1722, 1740, 1810, 2104, 2174, 2262, 2296 and 2388 nm. The biggest peak at 1926 nm caused by moisture in the crystals, was not included. A spectrum of SMC powder (Figure 9) showed some similarities with that of SMCO especially at wavelengths 1722, 2174, 2262 and 2296 nm.

The correlation graphs over the full wavelength range for whole plants, leaves, stems and young leaves are shown in Figures 10, 11, 12 and 13, respectively. There are many common features in each of the correlation graphs. The entire leaf and young leaf correlation graphs in particular have many corresponding peaks and troughs.

Derivation of calibration equations:

For all tissue types, most of the best four wavelength equations were formed using correlation pair selection. Table 10 shows the best calibration equations for each tissue type, using samples from plants grown in 1980. The correlations between predicted and reference values in each 1980 population are also shown.

The combination which consistently gave the highest correlation between predicted and reference SMCO was chosen for each tissue type. These wavelengths, together with their regression coefficients are presented in Table 11. The best equation for leaves used only wavelengths forming absorption peaks for SMCO crystals. For the other tissue types, the best equations were obtained using correlation pair

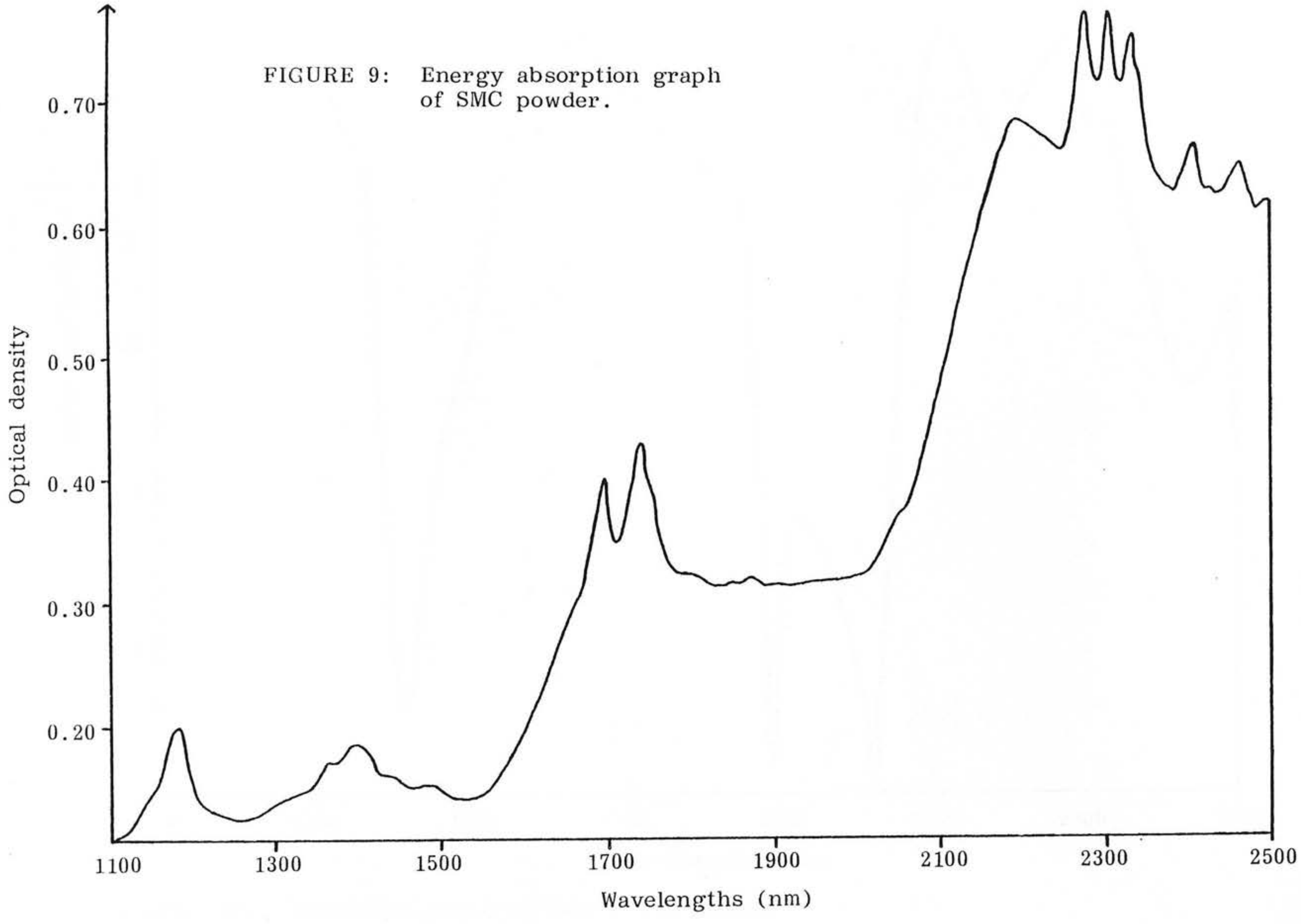


FIGURE 9: Energy absorption graph of SMC powder.

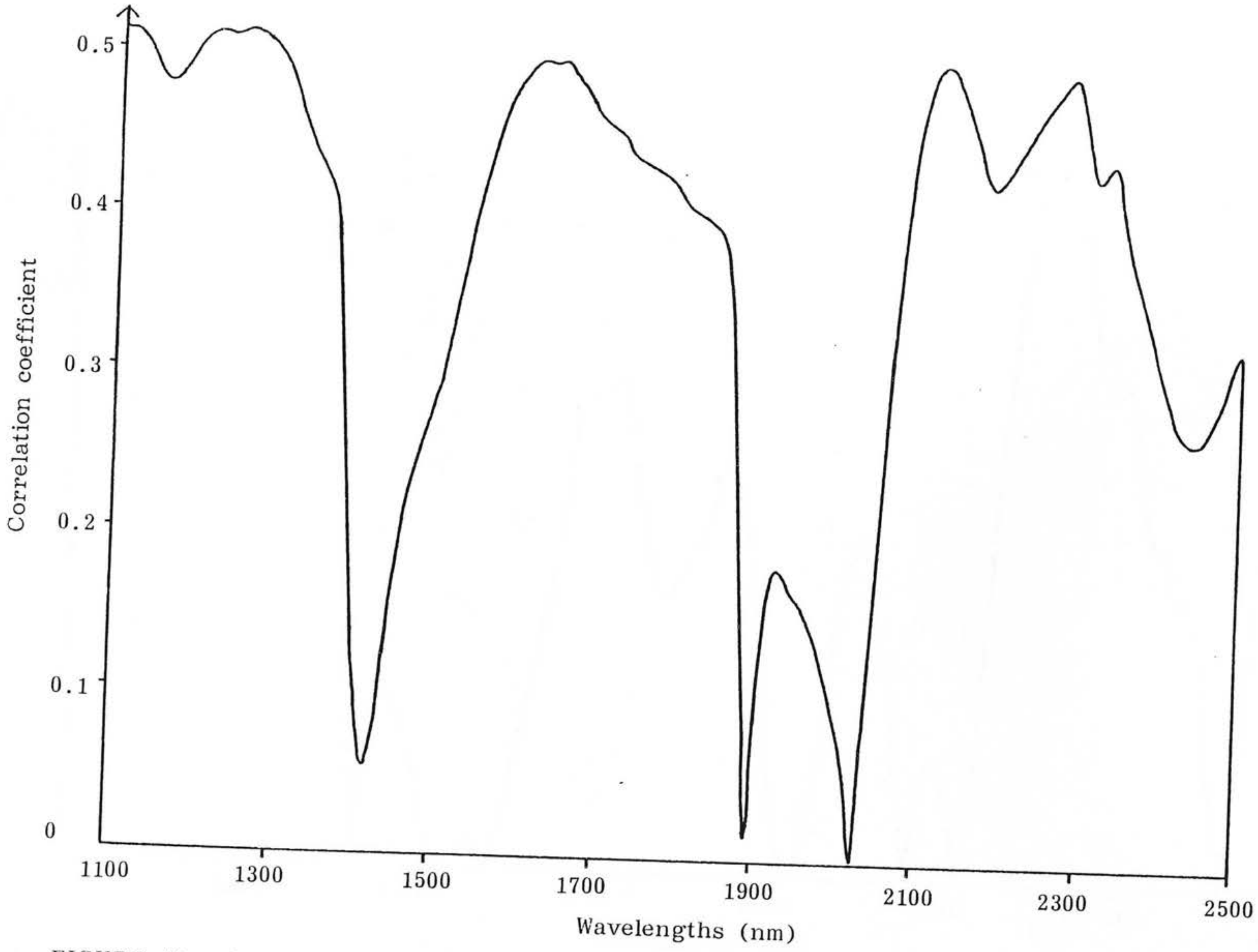


FIGURE 10: Correlation graph for whole kale plants.

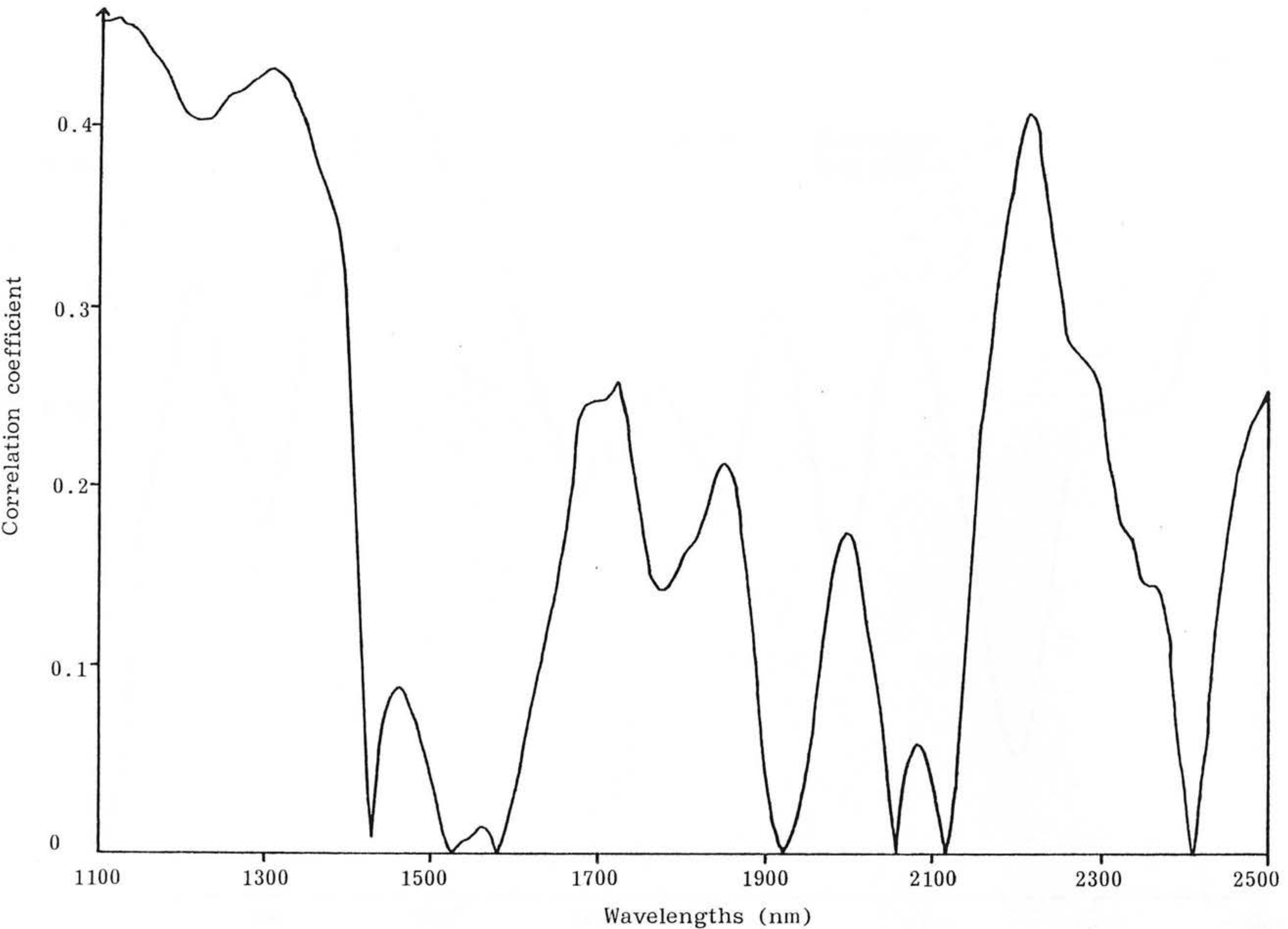


FIGURE 11: Correlation graph for kale leaves.

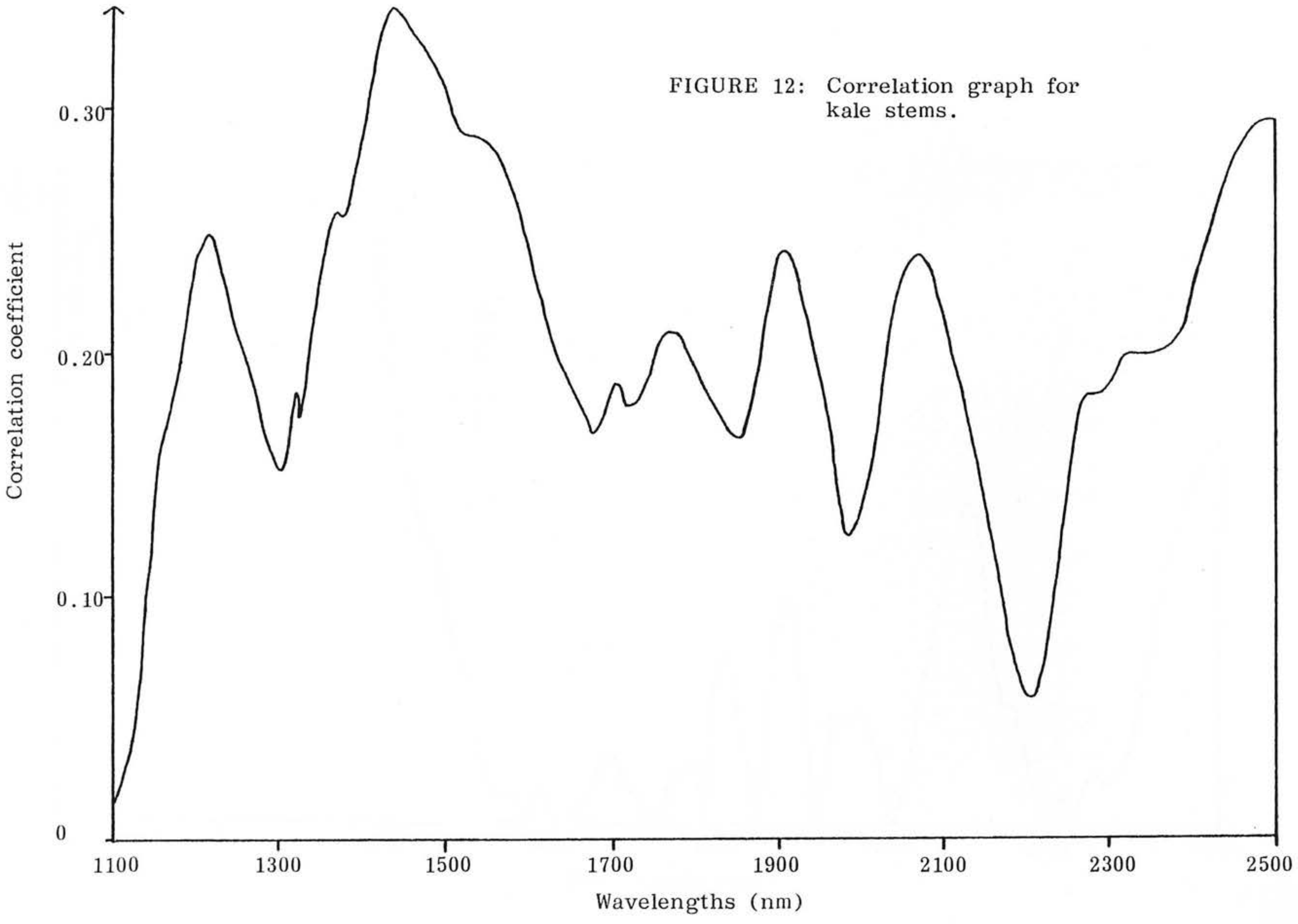


FIGURE 12: Correlation graph for kale stems.

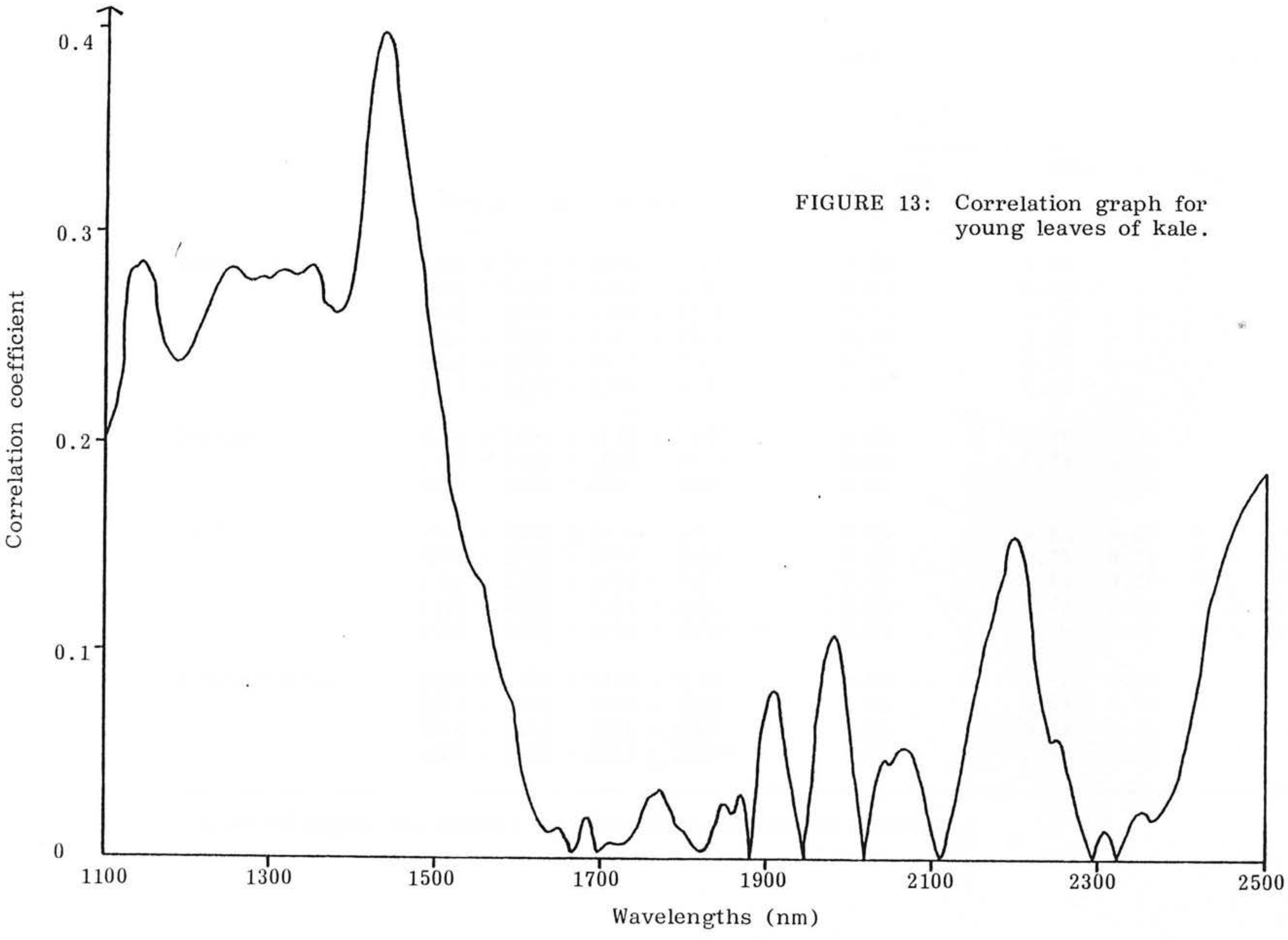


FIGURE 13: Correlation graph for young leaves of kale.

TABLE 10: Correlation coefficients of potential wavelength combinations in calibration and check populations (r).

Tissue type	Potential wavelengths	Calibration population	1980 Check populations			
			1	2	3	4
Whole plants	2184 + 2208 + 2074 + 1808	0.83	0.70			
	2184 + 2208 + 1844 + 2070	0.83	0.69			
	1918 + 1986 + 1430 + 1702	0.79	0.71			
	2260 + 2290 + 2150 + 1702	0.76	0.58			
	1618 + 1820 + 1810 + 1792	0.75	0.27			
	1260 + 1170 + 1268 + 1120*	0.65	0.65			
Leaves	2228 + 2106 + 1744 + 1220†	0.96	0.89	0.91		
	2222 + 2140 + 2294 + 1746*	0.93	0.92	0.84		
	2270 + 2340 + 2386 + 2408	0.92	0.82	0.62		
Stems	1588 + 1618 + 1830 + 2052	0.85	0.82	0.69	0.84	0.74
	1588 + 1618 + 1578 + 2050	0.89	0.73	0.71	0.75	0.67
	2108 + 2190 + 2206 + 2164	0.87	0.83	0.67	0.82	0.61
	2240 + 2358 + 2364 + 2292	0.89	0.73	0.63	0.85	0.65
	1440 + 2022 + 1352 + 1314*	0.80	0.80	0.59	0.84	0.63
Young leaves	2266 + 2292 + 2058 + 2170	0.95	0.87	0.74		
	2264 + 2292 + 1454 + 1526	0.95	0.83	0.76		
	2212 + 2292 + 1862 + 2264	0.95	0.83	0.72		
	1438 + 1550 + 2094 + 1564*	0.93	0.48	0.15		

* Forward regression choice; † From SMCO absorption spectrum.

selection. The equations formed through using forward regressions were poorer in predicting for all tissue types. In each of the best equations (Table 11), some of the chosen wavelengths corresponded to peaks or troughs in the spectrum of SMCO crystals (Figure 7) and some to peaks and troughs in the correlation graphs. There were no common wavelengths which appeared in all the best equations for all tissue types. In each equation there was one wavelength between

TABLE 11: Best wavelength combination for each tissue type.

	Coefficients	Wavelength
Whole plants	-2.590	
	-7111.078	2184
	7094.789	2208
	645.735	2074
	-823.953	1808
Leaves	-30.803	
	866.047	2228
	82.543	2106
	-1679.277	1744
	482.800	1220
Stems	10.273	
	-3063.944	1588
	3432.025	1618
	-342.365	1830
	96.184	2052
Young leaves	10.284	
	3487.069	2266
	-3490.572	2292
	-396.756	2058
	523.460	2170

2052 and 2106 nm and this appeared to be essential. Also three out of four tissue types had at least another wavelength between 2170 and 2228 nm. The wavelength at 1744, with the largest coefficient in the leaf equation, was reported by Whealer (1959) to be approximately

the position (1740) where a sulphur-hydrogen (S-H) bond absorbs. The S-H bond is present in SMCO.

Evaluation of calibration equations on 1980 samples:

Table 12 shows an evaluation of the best equations for each tissue type using the 1980 samples. All populations had significant correlations ($P < 0.01$) between reference and predicted SMCO values, although correlations varied between tissue types.

With whole plants, the best equation only poorly predicted SMCO, having a high standard error and a reasonably low correlation coefficient. The correlation between predicted and reference SMCO, however, was higher than the correlation between N and SMCO, hence SMCO was directly predicted to some degree. There is the danger in measuring one amino acid, such as SMCO, from an amino acid pool, that total N is measured instead. In all tissue types therefore, the correlation between predicted and reference SMCO was compared with the correlation between reference SMCO and N. If these two correlations were always similar, this would indicate that N was being predicted indirectly. With some tissue types, special populations were formed, in which the correlation between SMCO and N was minimised.

In most trials in this thesis, where mature kale was harvested, there was a positive correlation between N and SMCO. The negative correlation in the whole plant populations in 1980 was due to the inclusion of some very young plants which were extremely high in N and low in SMCO. Figure 14a shows predicted SMCO values for samples in the 1980 whole plant check population (WC1). With a correlation of $r = 0.70$, the only practical use for this equation would be in separating the lowest from the highest SMCO samples.

TABLE 12: Evaluation of SMCO calibration equations on 1980 samples.

Tissue type	Population	Correlation coefficients: N v SMCO	Correlation coefficients: Reference SMCO v predicted SMCO	SE	Bias	RER	CV%
Whole plants	calibration	-0.52	0.83	2.99		6.3	21.5
	check-WC1	-0.60	0.70	3.88	1.1	4.6	28.1
Leaves	calibration	0.74	0.96	1.43		15.4	10.2
	check-LC1	0.87	0.89	2.32	-5.5	8.2	19.5
	check-LC2	0.80	0.91	2.28	-1.8	8.3	14.5
Stems	calibration	0.80	0.85	2.38		7.2	18.3
	check-SC1	0.79	0.82	2.91	-0.2	5.8	22.2
	check-SC2	0.53	0.69	3.09	-0.5	4.9	23.8
	check-SC3	0.86	0.84	2.97	-1.7	6.2	24.7
	check-SC4	0.59	0.74	2.68	-3.2	5.2	22.9
Young leaves	calibration	0.61	0.95	1.33		12.1	13.0
	check-YLC1	0.34	0.87	1.70	-0.7	7.6	17.3
	check-YLC2	0.43	0.74	4.76	-1.9	5.0	30.5

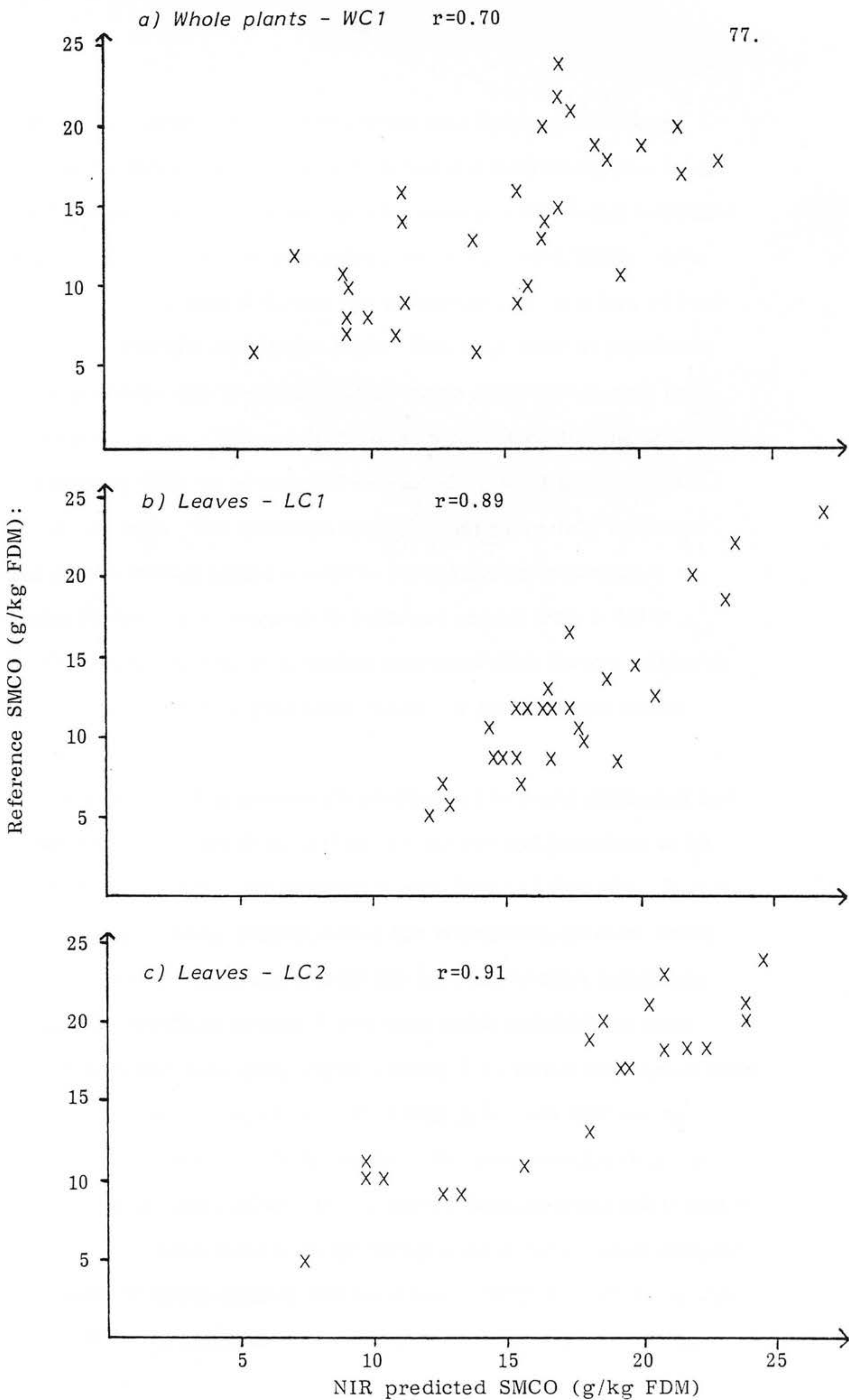


FIGURE 14: NIR prediction of SMCO in 1980 whole plant (WC1) and leaf check populations (LC1 and LC2).

SMCO was predicted better in leaves than in the other tissue types, with all correlation coefficients being r = 0.89 or above. In all 1980 leaf populations, the correlations between predicted and reference SMCO were higher than the correlations between N and SMCO. RER values were greater than 8.0, but the coefficients of variation of some of the leaf populations were quite high. The large bias in population LC1, was probably due to those samples being extracted during autoanalysis by a slightly different technique to that used for the other leaf populations (200 mg of material extracted in 20 ml buffer in LC1, instead of 100 mg). The standard errors of approximately 2.3 incorporated all the manual analysis errors, including harvesting and extraction errors. The increase in standard errors from 0.6/0.9 (Table 4) to 2.3, was due to scanning and prediction errors. Figures 14b and c show individual predicted values for the 1980 leaf check populations.

For kale stems, the correlation coefficients between predicted and reference SMCO were lower than those for leaves and appeared to be dependent on the correlation between N and SMCO (Table 12). Population SC2 was specifically formed with a low correlation between SMCO and N. It incorporated samples from the stem calibration population and SC1. The standard errors in the stem check populations were reasonably constant averaging approximately 2.9, which was higher than with leaf predictions. The results show that it is more difficult to predict SMCO in stems than in leaves and that predictions using the best stem equation were related to the correlations between SMCO and N.

Population comparisons were probably more accurate when samples were analysed at approximately the same time (Table 9). This was due to SMCO levels varying with time. Possible reasons were either loss of

SMCO during storage (see Section 2.3), differences in extraction technique, changes in chemicals or changes in the ion exchange column. Check populations analysed at different times from the calibration population, while less accurate, still gave useful information.

Stem samples ranged from young digestible tissue containing mainly carbohydrates, to older fibrous, lignified tissue. The variation in particle size after milling appeared greater in stems than in leaves. This probably affected the accuracy of predictions, as previous workers have shown that reducing particle size often increases the accuracy of NIR predictions (Winch and Major, 1981). The greater variation in tissue structure in stems than in leaves, may have been partly responsible for the poor predictions of stem SMCO.

Figure 15 shows that the prediction of SMCO in stems by NIR is limited to separating the lowest from the highest SMCO samples. Correlations were reduced by one particular sample in SC1 and SC2 ($x = 18.0$, $y = 10.0$) and by another solitary sample in SC4 ($x = 15.3$, $y = 6.0$). Without these two samples the correlations in SC1, SC2 and SC4 would have been 0.87, 0.74 and 0.78 respectively. These outlying samples may have been due to inaccuracies in the reference method or may have been true values which had interference properties which the calibration equation was unable to predict accurately.

With young leaves, the best wavelength equation was calculated from a population of several kale varieties and this proved satisfactory for predicting SMCO in YLC1, which also contained several kale varieties (Table 12). YLC2, which only contained Maris Kestrel, was not predicted as well. Considering the low correlation between N and SMCO in YLC1 ($r = 0.34$), the correlation coefficient of $r = 0.87$ was very good in terms of specifically measuring SMCO rather than N.

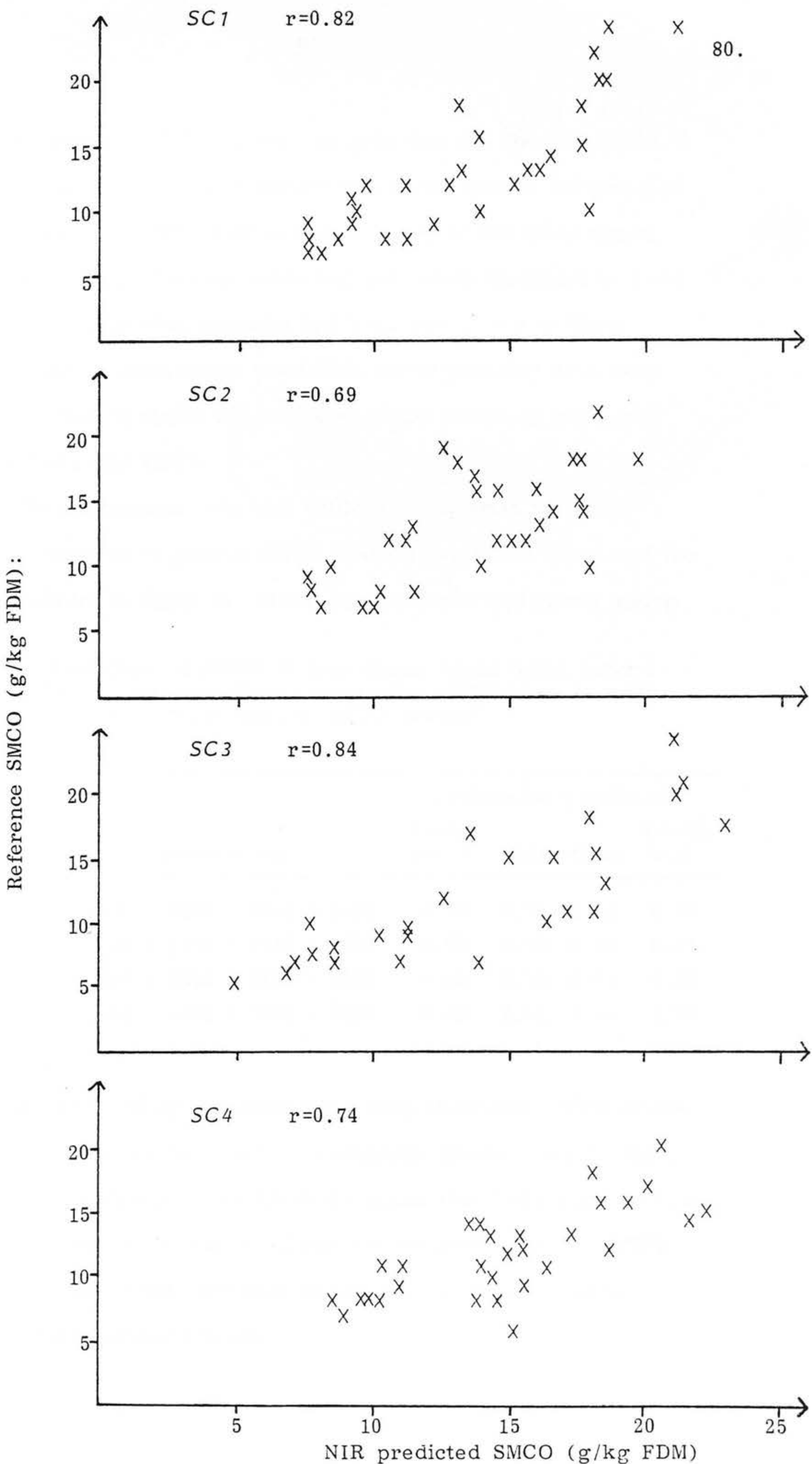


FIGURE 15: NIR prediction of SMCO in 1980 stem check populations.

Although its range of SMCO values was quite low and the RER value (7.6) was not as high as was achieved with entire leaves, its standard error of 1.7 was lower than that achieved in any of the other tissue types. It was surprising that SMCO was not better predicted in YLC2, although if the calibration equation had been based only on Maris Kestrel samples, a much better prediction would probably have been achieved. Figure 16 shows the predicted SMCO values of individual samples in YLC1 and YLC2.

For 1980 populations, the best calibration equation for each tissue type was used to predict SMCO in the other tissue types and the results are shown in Table 13. Whole plants, stems and young leaves

TABLE 13: Prediction of SMCO in kale tissue types using other tissue type equations (correlation coefficients between reference and predicted SMCO values).

		Calibration population			
Tissue type	Wavelengths	Whole plant	Leaf	Stem	Young leaf
Whole plants	2184 + 2208 + 2074 + 1808	0.83	0.81	0.56	0.66
Leaves	2228 + 2106 + 1744 + 1220	0.40	0.95	0.58	0.33
Stems	1588 + 1618 + 1830 + 2052	0.40	0.73	0.85	0.49
Young leaves	2266 + 2292 + 2058 + 2170	0.67	0.71	0.59	0.95

were poorly predicted by equations other than their own. With leaves, all equations predicted SMCO with a correlation greater than $r = 0.70$, although the leaf equation was still much better than the others. These results show that none of the equations will accurately predict SMCO in different tissue types, probably as the amounts of background compounds vary between tissues.

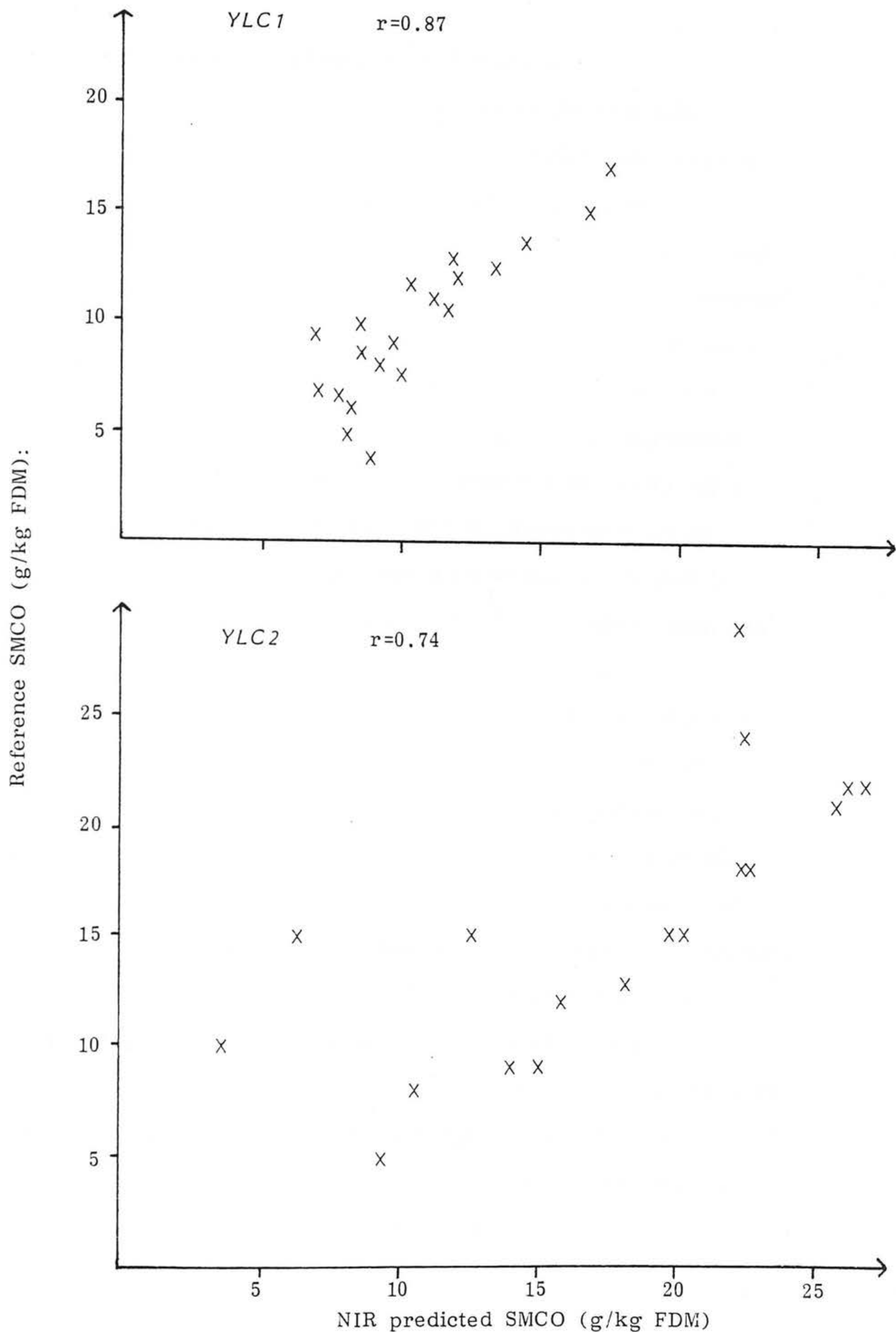


FIGURE 16: NIR prediction of SMCO in young leaf populations (YLC1 and YLC2).

Evaluation of calibration equations on 1981 samples:

The results of predicting 1981 samples using the best 1980 calibration equations for whole plants, leaves and stems are shown in Table 14 and Figure 17. Correlations between predicted and reference SMCO were much less with all tissue types than was achieved with the 1980 check samples. Also correlations between predicted and reference SMCO were less than the correlations between SMCO and N with each tissue type. Standard errors were approximately 40% higher with leaves and stems and 70% higher with whole plants. The regression equations relating predicted SMCO (x) to reference SMCO (y), all had slopes well below the theoretical value of 1.0. There were probably therefore, different levels of background substances in the kale in the two years which affected SMCO predictions. This skew effect was also found when predicting N by NIR in the 1981 samples.

Using the four wavelengths selected for the 1980 leaf calibration equation, new calibration coefficients were calculated for the 1981 population LC3. The correlation coefficient between predicted and reference SMCO values, increased from 0.65 (SE = 3.49) to 0.83 (SE = 2.37). When this 1981 calibration equation with new coefficients was used to predict the other 1981 leaf check population (LC4), the correlation coefficient (Figure 18a) was 0.63 (SE = 2.49), which was higher than that obtained using the 1980 leaf calibration equation (r = 0.41, SE = 3.06). This correlation coefficient of 0.63 was still lower than that obtained with the 1980 populations, although the standard error (2.49) was similar to those obtained in 1980. The correlation coefficient in this population, being dependent on the range of samples, was as a consequence depressed due to the limited range.

TABLE 14: Evaluation of SMCO calibration equations on 1981 samples.

Tissue type	Population	Correlation coefficients: N v SMCO	Correlation coefficients: Reference SMCO v Predicted SMCO	SE	Bias	Regression equation*	RER	CV%
Whole plants	check-WC2	0.61	0.26	6.71	-1.2	Y = 0.11x + 8.78	1.6	69.2
Leaves	check-LC3	0.66	0.65	3.49	-1.1	Y = 0.59x + 4.37	4.4	38.8
	check-LC4	0.85	0.41	3.06	-0.7	Y = 0.36x + 5.04	2.9	40.8
Stems	check-SC5	0.69	0.65	4.19	3.0	Y = 0.39x + 5.10	2.7	41.1

* Relating reference SMCO (y) to predicted SMCO (x).

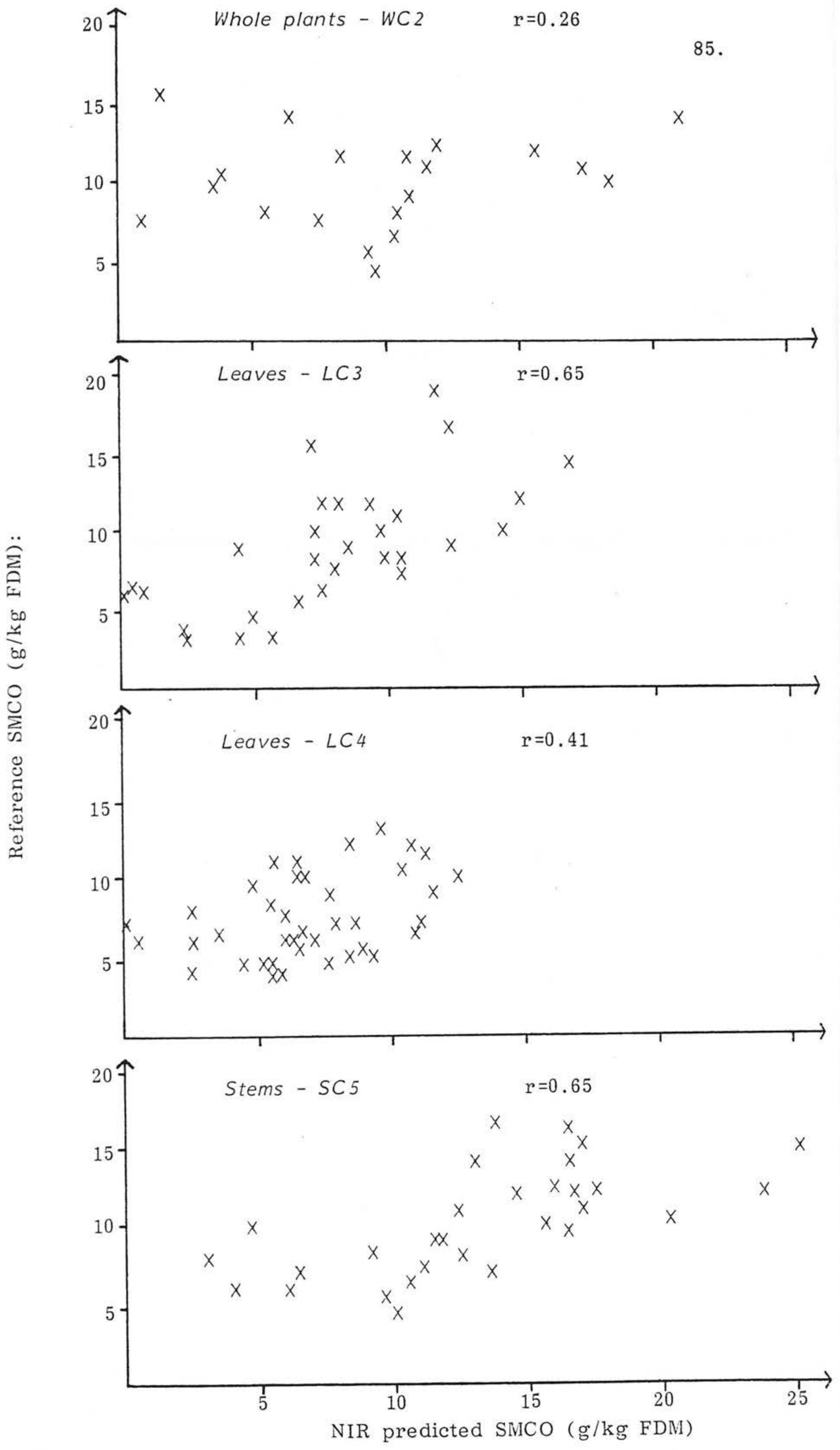


FIGURE 17: NIR prediction of SMCO in 1981 populations using 1980 calibration equations.

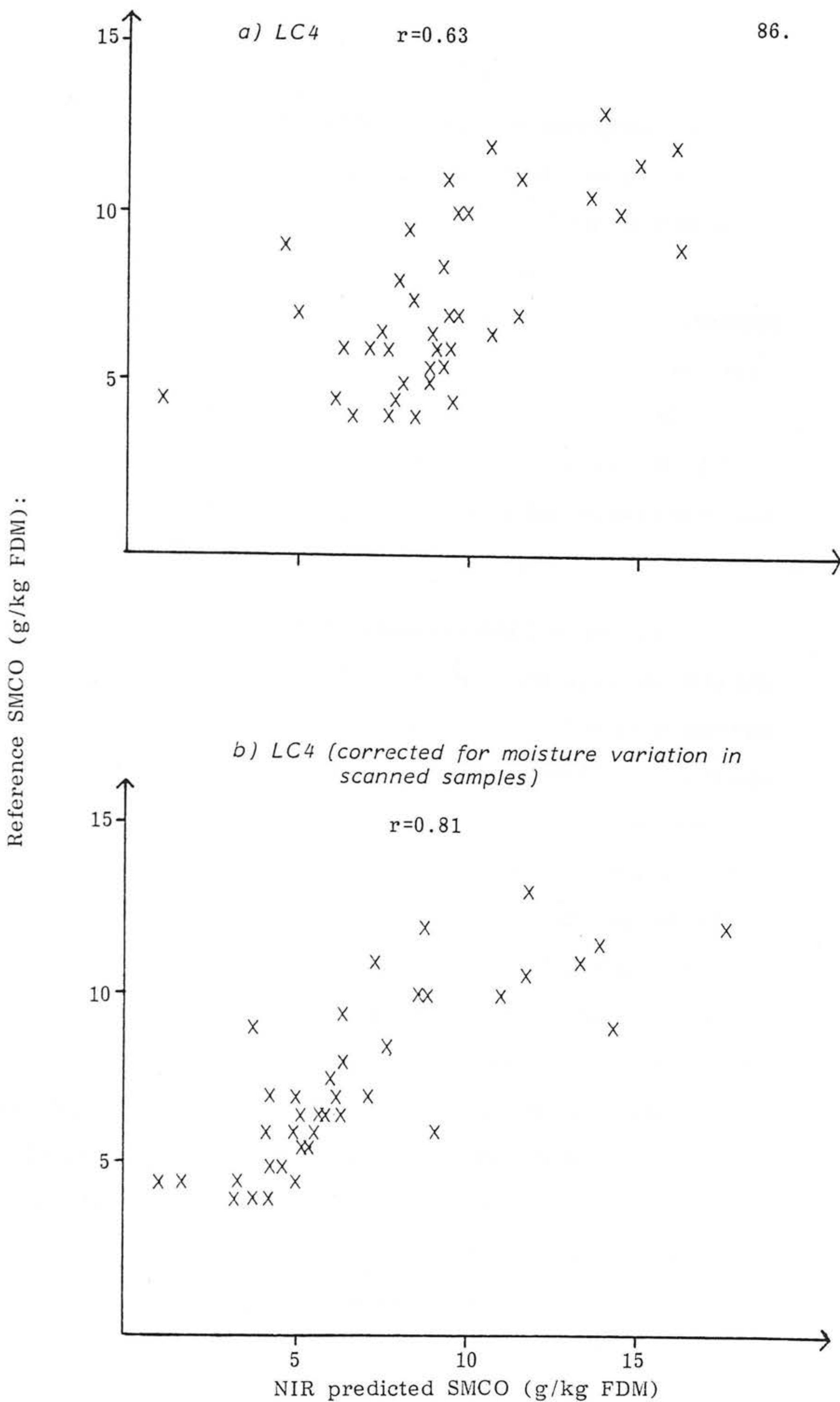


FIGURE 18: NIR prediction of SMCO in 1981 leaf population (LC4) using 1981 calibration equation.

These results suggest that a set of suitable wavelengths have been obtained which will predict leaf SMCO in different years, although new coefficients are required each year. It is not known whether new 1981 coefficients in the stem and whole plant calibration equations would have predicted SMCO in other 1981 samples with a similar accuracy to that obtained in 1980, when both calibration and check samples were from the same year. The large increase in standard error in WC2, may have been related to the whole plant calibration equation in 1980 being based on a population which included young leaf samples with high N contents, which were not present in 1981.

Effect of sample moisture content on predicted SMCO in leaves:

It was noted when using the 1980 leaf calibration equation, that the level of predicted SMCO appeared to be related to the amount of moisture present in the sample at the time of scanning. Increased moisture levels reduced predicted SMCO values, even after corrections to dry base values. Predicted SMCO, averaged over 35 leaf samples from both 1980 and 1981 trials, decreased by an average of 0.94 g SMCO/kg DM (SE = 0.063) for each 1.0% increase in moisture content. Correcting predicted SMCO values to a constant moisture level of 10% by this factor (0.94) only improved the correlation in one of the five leaf populations. In LC4, the correlation between predicted and reference SMCO was increased from 0.41 to 0.74 (SE = 2.14) using the 1980 calibration equation and from 0.63 to 0.81 (SE = 2.21) using the 1981 calibration equation (Figure 18b). The standard error achieved with the leaf population after corrections were made for moisture, was slightly lower than the standard errors in the other leaf populations (Tables 12 and 14).

The range of moisture contents found in each leaf check population was similar to the range in the calibration population. It would appear therefore, that the equation is not compensating adequately for changes in moisture level. For most accurate measurements of leaf SMCO, the range of moisture levels in predicted samples should be kept as low as possible. If there are large variations in moisture levels during scanning, the predicted SMCO values should be corrected to obtain best estimates of SMCO. This moisture effect would cause the most problems if leaf samples were being analysed several times for SMCO over a period of months, when their moisture contents would probably also be increasing (see Section 2.3).

Scanning repeatability:

The leaf calibration population which was scanned twice within a week and predicted using the best leaf calibration equation, had a correlation between scans of r = 0.995, a standard error of 0.56 and a coefficient of variation of 4.0%. With stems, similar figures of r = 0.990, SE = 0.61 and CV = 5.2% were obtained. These were very high correlations and acceptable standard errors, being similar to those obtained when duplicate samples were analysed by the autoanalysis technique (Table 4). Of the NIR prediction errors (coefficients of variation in Table 12), approximately 10 percentage units of the error with leaves and 14 percentage units of the error with stems were due to manual analysis and scanning errors and the rest (5-10 percentage units) were due to prediction errors. None of the populations used to study the accuracy of SMCO predictions by NIR was scanned in duplicate and only LC3 was analysed by the autoanalysis method in duplicate. Errors obtained, therefore, are approximately the same level as would be obtained if NIR analysis were used to analyse routine samples for SMCO.

Conclusion:

With Maris Kestrel kale samples milled through a 1 mm sieve, NIR analysis predicted entire leaf samples more accurately than entire stem or whole plant samples. Young leaf samples from several kale varieties were predicted with a similar accuracy to entire leaves and both sets of predictions were quite adequate for screening samples. With stems and whole plants, only the lowest and highest SMCO samples could be separated. These SMCO predictions were only achieved, however, in samples predicted in the same year as the calibration equations were calculated. With all tissue types tested, calibration equations would only poorly predict SMCO in samples from another year. However, new coefficients improved predictions. It appears, therefore, that new calibration coefficients are required for each tissue type each year.

With check samples predicted in the same year as calibration equations were calculated, correlation coefficients between NIR predicted and reference SMCO ranged from 0.87 to 0.91 for entire leaves and young leaves, whilst standard errors in the two tissue types averaged 2.3 and 1.7 respectively. With whole plants and stems, correlations varied from 0.69 to 0.84 and standard errors averaged 3.9 and 2.9 respectively. Correlations in stems in particular, appeared to be dependent on correlations between N and SMCO. RER values ranged in check populations from 4.6 with whole plants to 8.3 with entire leaves, while coefficients of variation ranged from 28.1 to 14.5 respectively in the same populations.

It appeared that leaf SMCO values predicted by the leaf calibration equation were dependent on the moisture content of the samples. This was probably due to the leaf calibration equation not compensating

adequately for changes in sample moisture levels. For most accurate predictions of leaf SMCO, the range of moisture levels in predicted samples should be kept as small as possible. If large moisture variations exist at scanning, predicted SMCO values may have to be corrected to obtain the best results.

It was found that correlation pair selection was better than forward regression, for calculating wavelength combinations to predict SMCO in populations of kale samples.

Further work is required on predictions of SMCO by NIR analysis, particularly with reference to particle size of milled samples and to the formation of calibration equations based on several kale or other brassica cultivars.

2.2.3 *Moisture*

Introduction

Kale samples analysed for SMCO or nitrogen generally had moisture levels between 5 and 15%. The moisture level was dependent on how long the samples were freeze dried and on the storage time at -20°C before they were analysed. Freeze drying never removed all the moisture and there was usually some variation in moisture levels within a batch of samples after freeze drying. Samples also tended to gain moisture during storage (see Section 2.3). As all SMCO and N levels in this thesis were expressed as a percentage of dry matter, it was important to know the moisture in a sample at the time it was analysed. Measuring moisture levels in samples manually proved to be time consuming, hence the possible use of NIR analysis for measuring moisture in different kale components was studied.

Experimental

Moisture predictions were made on the same NIR instrument as used to predict N, the Technicon InfraAlyzer 300 using five fixed wavelengths. The InfraAlyzer was calibrated with samples of Maris Kestrel kale harvested from several trials at different sites in 1979 and 1980. Calibration equations were derived for whole plants, leaves and stems and were evaluated using both samples harvested in 1980 and samples harvested in 1981. Moistures were also predicted on five kale components (see Section 2.2.1) harvested from a 1980 kale trial containing the six varieties: Maris Kestrel, Canson, Merlin, Vulcan, Bittern and Chrysol. Samples were prepared for analyses as explained previously (see Section 2.2.2). Details on sample numbers, means and ranges for each calibration and check population are shown in Table 15. The instrument was calibrated in a similar manner to that described for N predictions. Calibrations were based on manual (reference) moisture measurements made by drying 0.5 to 1.5 g of freeze dried material in an oven at 90°C for 24 hours.

Results and discussion

Evaluation of calibration equations:

The calibration equations formed for predicting moisture (Y) in whole plants, leaves and stems were as follows:

Whole plants

$$Ypred = 10.69 - 171.87\log(F2) + 154.62\log(F5) + 55.75\log(F8) - 15.38\log(F10)$$

Leaves

$$Ypred = 4.06 - 15.97\log(F2) - 46.05\log(F7) + 79.15\log(F8)$$

Stems

$$Ypred = 4.09 - 135.91\log(F2) + 206.51\log(F5) - 77.02\log(F7) + 44.73\log(F8)$$

TABLE 15: Number, mean and range of samples in moisture prediction populations.

Component	Population	Number of samples	Moisture (%) Mean	Range
Whole plants	calibration	31	13.3	8.5 - 19.6
	check-1980	30	13.1	8.4 - 17.3
	check-1981	20	9.0	7.1 - 12.4
Leaves	calibration	35	10.4	4.3 - 15.5
	check-1980	24	10.2	5.8 - 13.4
	check-1981	25	9.4	5.9 - 13.1
Stems	calibration	29	8.9	4.1 - 15.0
	check-1980	25	10.0	5.7 - 15.5
	check-1981	25	8.7	6.1 - 11.6
Young leaves	check	15	10.9	8.8 - 13.6
Laminae	check	15	12.1	10.1 - 18.7
Petioles	check	15	11.0	7.7 - 17.7
Upper stems	check	15	10.6	5.2 - 16.4
Lower stems	check	15	10.2	6.3 - 14.7

TABLE 16: Evaluation of moisture calibration equations.

Tissue type	Population	Regression equation*	Moisture (%) Correlation coefficient	*SE*	RER	CV%
Whole plants	calibration		0.98	*0.56*	19.8	4.2
	check-1980	Y=1.12x-2.74	0.96	*0.81*	11.0	6.2
	check-1981	Y=0.94x-0.50	0.86	*0.84*	6.3	9.3
Leaves	calibration		0.98	*0.66*	17.0	6.3
	check-1980	Y=1.13x-2.40	0.95	*0.69*	11.0	6.8
	check-1981	Y=0.85x+1.25	0.91	*0.87*	8.3	9.3
Stems	calibration		0.99	*0.33*	33.0	3.7
	check-1980	Y=0.99x+0.20	0.98	*0.54*	18.1	5.4
	check-1981	Y=0.91x+1.50	0.94	*0.59*	9.3	6.8

* Linear regression equation relating reference moisture (Y) and predicted moisture (x).

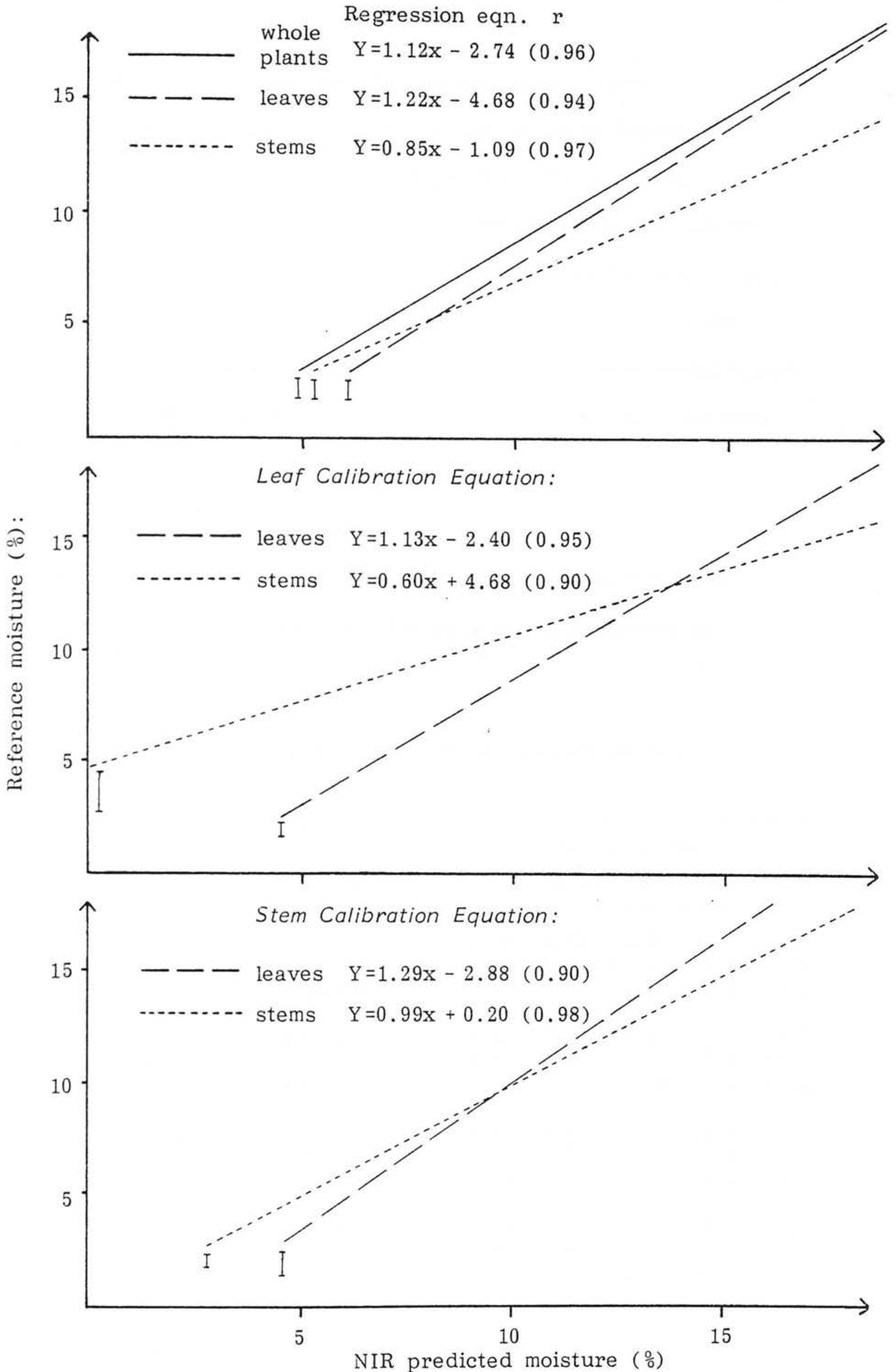
All equations contained filters 2 and 8, which are specific for measuring fibre and moisture respectively.

These calibration equation had very high correlations ($r \geq 0.98$) between NIR predicted and reference moisture values as shown in Table 16. This table also shows the accuracy of these equations in predicting moisture in the appropriate 1980 and 1981 check populations. Correlation coefficients ranged between 0.86 and 0.98. Standard errors were slightly higher in 1981 than in 1980, especially for leaf samples. Also in 1981, correlation coefficients and RER values were lower and coefficients of variation higher with all tissue types. There was a smaller range of moisture levels in 1981, especially in whole plants and stems (Table 15), hence this would have affected correlation coefficients and RER values.

In both 1980 and 1981 moisture was predicted more accurately in stems than in leaves or whole plants, although moisture predictions with all three tissue types in both years were acceptable for most circumstances and especially for correcting SMCO and N to dry base values.

The slopes of the regression equations were slightly lower in 1981 than in 1980, especially with leaves and whole plants. This was similar to that found with N and SMCO predictions in different years, only to a lesser degree, and it was again probably caused by changes in levels of some substances in the 1981 kale. Correcting the 1981 predicted moisture values by their appropriate regression equation would only slightly reduce standard errors. If a greater accuracy was required, a new calibration equation with new coefficients could be formed each year, as was required with SMCO.

In terms of coefficients of variation, NIR predictions of moisture in both years (<9.3%) were at least as good as N predictions (<11%) and considerably better than SMCO predictions (14.5 - 28.1%).



I standard error of difference between predicted and reference moisture.

FIGURE 19: Regression lines of tissue types based on calibration equations.

The 1980 leaf and stem check populations were predicted by all three calibration equations and the results are shown in Figure 19. The leaf and stem populations were best predicted by their own calibration equations. The 1980 whole plant check population was not predicted by the leaf and stem calibration equations which were derived subsequent to the whole plant calibration equation. As for N, no one equation was satisfactory for predicting all three tissue types, hence the necessity of separate calibration equations for each for best predictions.

Prediction of kale components:

Fifteen samples of each of the five components were predicted by the three calibration equations. The results are presented in Table 17.

TABLE 17: Evaluation of NIR predictions of moisture in kale components.

Component	Calibration equation	Regression equation	Moisture (%)				
			r	SE	bias	RER	CV%
Young leaves	whole	Y=1.08x-2.35	0.94	0.45	1.36	10.7	4.1
	leaf	Y=0.55x+4.93	0.61	1.22	-0.13	3.9	11.2
	stem	Y=0.93x+0.98	0.87	0.59	-0.29	8.1	5.4
Laminae	whole	Y=1.40x-4.56	0.89	1.12	-0.21	7.7	9.3
	leaf	Y=1.07x-1.27	0.82	1.21	0.42	7.1	10.0
	stem	Y=1.43x-2.60	0.82	1.32	-1.78	6.5	10.9
Petioles	whole	Y=0.93x-3.68	0.96	0.87	4.77	11.5	7.9
	leaf	Y=0.98x-0.20	0.94	0.94	0.40	10.6	8.5
	stem	Y=1.05x-1.40	0.97	0.89	0.71	11.2	8.1
Upper stems	whole	Y=1.03x-3.26	0.98	0.73	2.84	15.3	6.9
	leaf	Y=0.89x+2.86	0.96	1.28	-1.93	8.8	12.1
	stem	Y=1.08x-0.34	0.99	0.72	-0.46	15.6	6.8
Lower stems	whole	Y=1.05x-3.19	0.99	0.46	2.50	18.3	4.5
	leaf	Y=0.93x+2.32	0.97	1.08	-1.96	7.8	10.6
	stem	Y=1.14x-0.15	0.99	0.47	-1.09	17.9	4.6

Young leaves and laminae were predicted more accurately by the whole plant equation, than by the leaf or stem calibration equations. The young leaf and lamina standard errors, using the whole plant equation, were lower and higher respectively than the standard errors in the whole plant, entire leaf or stem check populations. Correcting the predicted values in the lamina samples, using $Y = 1.40x - 4.56$, reduced the standard error from 1.12 to 0.98 which was only slightly higher than that obtained with entire leaves. For most accurate predictions in laminae, a calibration equation based on lamina samples would be required.

Petioles, and especially upper and lower stems, were predicted very accurately by both the whole plant and stem calibration equations. The stem calibration equation, however, generally gave the most accurate predictions with these three kale components, as each regression line with the whole plant calibration equation had a large bias. This bias could readily be corrected by changing K_o in the whole plant calibration equation by the appropriate bias before predicting each of the three kale components.

For these components, the coefficients of variation when predicted by the whole plant equation ranged from 4.1 to 9.3%. These values were either similar to, or slightly higher than, those obtained with whole plants, leaves and stems in the same year.

Conclusion:

Moisture calibration equations were formed on an InfraAlyzer 300 for whole plant, leaf and stem kale samples combined from two successive years. All calibration equations accurately predicted moisture in check samples harvested in one of these years, giving correlation coefficients between predicted and manual values of at least 0.95 and

coefficients of variation less than 7%. When samples from a third year were predicted, standard errors in comparison with those in the previous year, were similar in whole plants and stems and slightly higher in leaves. Correlation coefficients in the third year were between 0.86 and 0.94 and coefficients of variation between 6.8 and 9.3%. Lower correlations in the third year were partly due to a lower range of moistures in that year and partly due to slightly higher standard errors when predicted samples were harvested in a different year to the calibration samples. It was considered, however, that generally the calibration equations would be adequate for predicting moisture in whole plant, leaf and stem samples harvested in different years, although check samples should be analysed manually each year and if necessary bias and skew corrections made.

Moistures in young leaves, petioles, upper and lower stems were predicted accurately ($r \geq 0.94$) by the whole plant calibration equation, especially if the bias was corrected for each component. Predictions were poorer in laminae ($r=0.89$), which would probably be better predicted by a separate calibration equation. The stem calibration equation could also be used to predict moisture in petioles, upper and lower stems. The results showed that accurate predictions of moisture could be obtained in whole plants and especially in entire leaves and entire stems, without dividing plants into further components.

2.3 Sample Storage

Introduction

Kale samples from trials in this thesis have been stored at -20°C from harvest until analysed for SMCO. Immediately after harvests, samples were stored in plastic bags for a few weeks prior to being

freeze dried and milled, after which they were returned to -20°C and stored in airtight containers. The effect of storage time on SMCO in different Maris Kestrel kale tissue types was studied in two experiments. In one of these, the effect of storage on the moisture content of the samples was also examined.

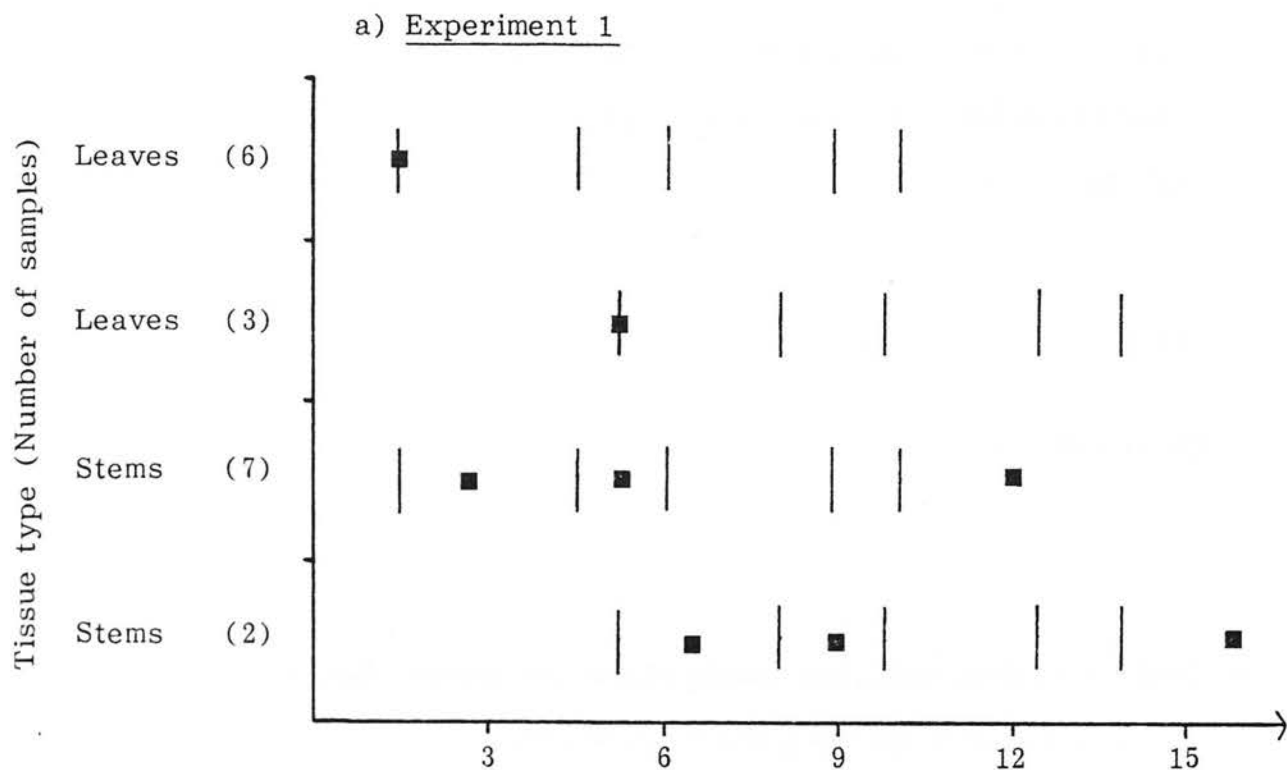
Experimental

In the first experiment, 9 leaf and 9 stem samples from trials harvested in 1980 were each split into two groups and analysed several times for SMCO by both the NIR and the autoanalysis techniques. Figure 20a shows storage times and the number of samples in each group. The division of samples into groups was required because of two different harvest dates.

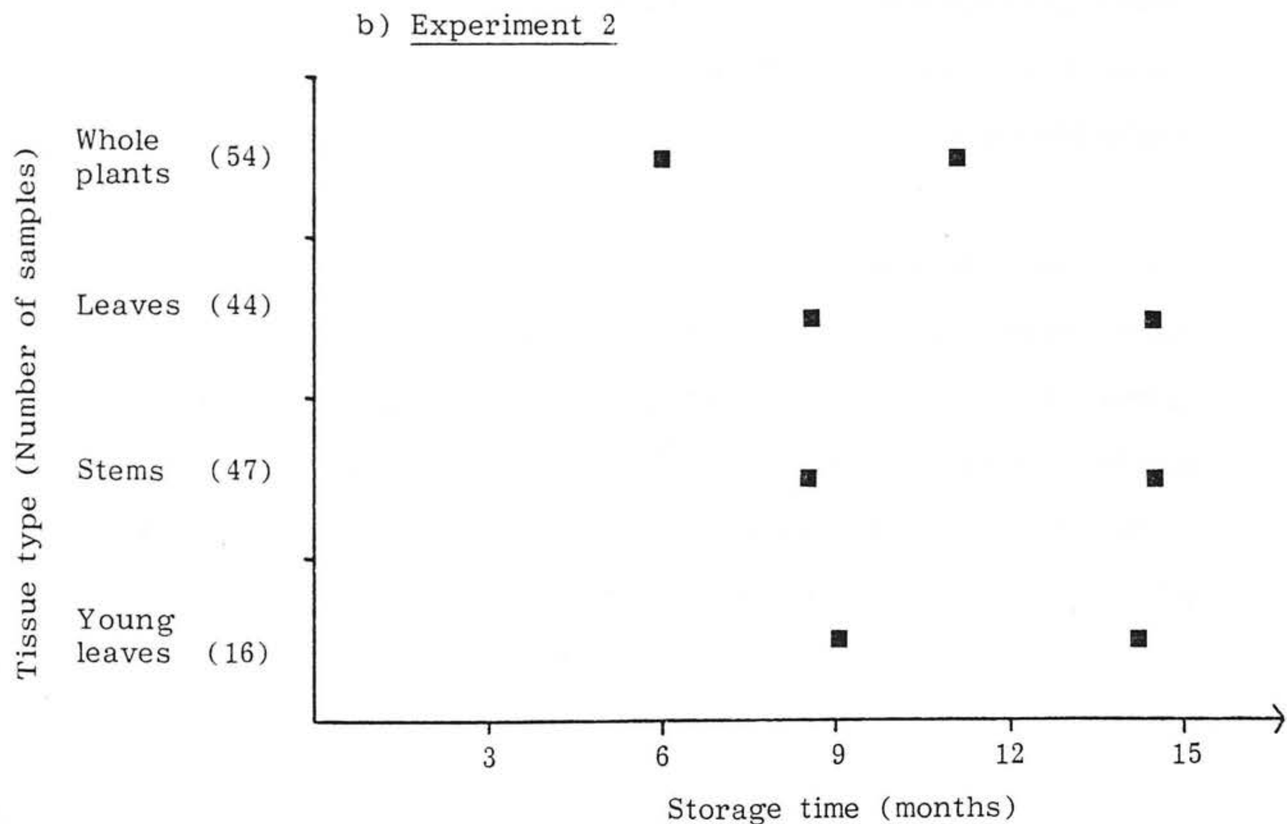
All samples were analysed on five occasions for SMCO, using the Neotec 6350 NIR instrument. The best calibration equations for each tissue type were used, as shown previously in Table 11. Due to the large error in predicting SMCO in stems by NIR (Table 12), all stem samples were analysed three times by autoanalysis. Leaves were analysed only once by autoanalysis, at approximately the same time as first analysed by NIR.

Because of the expected increase in moisture in the samples with storage, it was necessary to correct NIR predicted leaf SMCO values to a constant moisture level. This was required because the particular NIR leaf calibration equation produced dry base SMCO values inversely proportional to moisture levels in the samples (see Section 2.2.2). Correction factors were calculated individually for each of the 9 leaf samples after the final analysis. This was achieved by drying approximately half of each sample in a freeze drier and scanning both the redried and the undried samples for SMCO and moisture. The change

a) Experiment 1



b) Experiment 2



Analysed for SMCO by NIR technique (|) and Autoanalysis technique (■).

FIGURE 20: Storage time and number of samples for each kale tissue type.

in dry base SMCO in relation to the difference in moisture was calculated as the correction factor for each sample and was used to correct predicted SMCO values for each of the two analyses using the following equation:

True SMCO = Predicted SMCO + Correction Factor (% Moisture - 12.0)

All samples were corrected to a moisture level of 12% DM, which was the mean level in the trial samples.

Experiment 2:

In the second experiment, whole plant, leaf, stem and young leaf samples from kale trials in 1979 were all analysed for SMCO on two occasions by the autoanalysis method, as outlined in Figure 20b. Whole plant samples were divided into two groups of 34 samples (Group A) and 20 samples (Group B). Samples in Group A were stored at ambient temperature (approximately 15°C) between the two analyses, instead of at -20°C. These two groups of whole plant samples enabled the effect of storage temperature on SMCO to be also examined.

All samples at the first analysis were analysed once, whereas at the second analysis, samples were analysed in duplicate. Mean values of duplicates were used for comparison with the first analysis values.

In order to examine if changes in SMCO with storage were affected by the level of SMCO in the samples, the whole plant, leaf and stem samples in the second experiment were each divided into low ($\leq$10 g/kg DM) and high (>10 g/kg DM) SMCO samples.

Results and discussion

- *a) SMCO:*

The effects of storage on SMCO levels in the leaf and stem samples in Experiment 1 are shown in Table 18.

TABLE 18: Effect of storage on leaf and stem SMCO (g/kg DM) in Experiment 1.

Tissue type		Analysis 1	2	3	4	5	*SE*
Leaves	6 samples	21.4[a]	21.8[a]	20.8[a]	20.8[a]	19.1[b]	*0.88***
	Months after harvest	1.5	4.5	6.0	9.0	10.5	
	3 samples	12.0[ab]	12.8[a]	11.8[ab]	10.7[b]	9.0[c]	*0.68***
	Months after harvest	5.0	8.0	10.0	12.5	14.0	
Stems	*a) NIR:*						
	7 samples	18.3[ab]	17.2[c]	17.3[c]	17.7[bc]	18.7[a]	*0.88**
	Months after harvest	1.5	4.5	6.0	9.0	10.5	
	2 samples	9.0	7.8	7.6	8.2	9.4	*0.98*
	Months after harvest	5.0	8.0	10.0	12.5	14.0	
	b) Autoanalysis:						
	7 samples	15.5	17.4	13.9			*2.03*
	Months after harvest	2.5	5.5	12.0			
	2 samples	8.2	9.4	10.4			*0.99*
	Months after harvest	6.5	9.0	16.0			

* and ** Significant differences at the 5% and 1% levels respectively.

In the 6 leaf samples, there was no loss of SMCO in the first 9 months storage. At the final analysis after 10.5 months, there was an 11% loss of SMCO compared with the first analysis. This was statistically

significant (P <0.05). Results with the earlier harvested group of 3 leaves were similar, with no significant loss of SMCO up to 10 months, but significant losses at the final two analyses. This data suggests that freeze dried kale leaf samples can be stored in airtight containers in a freezer for approximately 9 months without losing significant amounts of SMCO.

The correction factors relating changes in predicted leaf SMCO (g/kg DM) with moisture, ranged in the 9 leaf samples from 0.39 to 0.95 with a mean of 0.67. If no correction factors had been used, the leaf results based on NIR predictions would have shown an average loss of SMCO between the first and fifth analyses of 27%. For greatest accuracy, correction factors based on individual samples were used, rather than a mean figure based on these or other leaf samples.

At the first analysis, the correlation (r) between NIR predicted leaf SMCO (corrected for moisture) and leaf SMCO measured by autoanalysis, was 0.98 for the 9 samples which ranged from 11.1 to 31.4 g SMCO/kg DM.

In the first experiment, there was no significant (P <0.05) loss of SMCO in stems between the first and last analysis for either group of samples or either analytical technique. With the 7 later harvested stems, however, differences in SMCO between treatments as measured by autoanalysis, were almost significant at the 5% level of significance, mainly through loss of SMCO between 5.5 and 12 months storage. The earlier harvested group of 2 stems, when measured by autoanalysis, had lower levels of SMCO (9.3 g/kg DM) than the 7 stems (15.6 g/kg DM) and showed some tendency to gain SMCO between 6.5 and 16 months storage.

The variation in stem SMCO levels between harvests when measured by NIR, may not have been a real effect, as it was shown previously that predictions of SMCO in kale stems have only a limited accuracy (Table 12). The correlation between mean NIR values and mean autoanalysis values for the 9 stems was $r = 0.81$ ($n=9$), which was similar to previous correlations between the two methods.

In Experiment 2 (Table 19), whole plant and young leaf samples generally lost a greater proportion of SMCO during storage than leaf or stem samples. This was probably due to these tissues having higher average levels of SMCO than the leaf or stem samples. It was shown within each tissue type, that samples with high levels of SMCO (>10) lost a greater proportion of their SMCO than samples with low levels (≤ 10). Leaf and stem samples with low SMCO levels showed no significant SMCO loss between 8.5 and 14.5 months of storage. There is no clear explanation why whole plants with low SMCO levels lost a significant amount of SMCO with storage, whereas low leaf and stem samples lost none, although the whole plant low SMCO samples did have a slightly higher mean SMCO level.

Correlations (r) between the level of SMCO at the first analysis and the loss of SMCO between analyses were 0.75 ($n=54$) for whole plant, 0.89 ($n=44$) for leaf and 0.51 ($n=47$) for stem samples. These correlations, which were all significant ($P < 0.01$), confirm that samples with high levels of SMCO lost more SMCO with storage than samples with low levels. A non-significant correlation of $r = 0.21$ ($n=16$) with young leaves, was probably due to these samples having only a small range of SMCO.

Whole plant samples in both Groups A and B showed similar significant losses in SMCO. This suggests that after six months initial

TABLE 19: Effect of storage on SMCO in whole plants, leaves, stems and young leaves in Experiment 2 (g/kg DM).

Tissue type	Level of SMCO+	No. of samples	First analysis: Mean SMCO (g/kg DM)	First analysis: Months after harvest	Second analysis: Mean SMCO (g/kg DM)	Second analysis: Months after harvest	Loss of SMCO (%)	*SE*
Whole plants:								
A		34	13.6	6.0	9.1	11.0	-33	*1.44***
B		20	11.9	6.0	8.5	11.0	-29	*2.23***
	Total	54	13.0	6.0	8.8	11.0	-32	*1.66***
	low	18	8.2		7.0		-14	*1.50**
	high	36	15.4		9.7		-37	*1.52***
Leaves	Total	44	11.3	8.5	9.7	14.5	-14	*1.78***
	low	20	7.0		7.4		+ 6	*0.94*
	high	24	14.8		11.6		-22	*1.48***
Stems	Total	47	10.3	8.5	9.8	14.5	- 5	*1.92*
	low	23	7.1		7.7		+ 8	*1.46*
	high	24	13.3		11.8		-11	*2.06**
Young leaves	Total	16	19.9	9.0	14.4	14.0	-28	*1.44***

+ low and high = samples with SMCO levels ≤ 10 g/kg DM and >10 g/kg DM respectively at the first analysis.

* and ** Significant at the 5% and 1% levels of significance respectively.

storage at -20°C, storage at room temperature over the next five months causes no additional loss of SMCO from freeze dried samples. It is not known, however, whether temperature is important during the first six months storage, although Gosden (1979, pers. comm.) has suggested that SMCO is not lost from whole kale samples during six months storage at ambient temperature, as long as samples are kept in air tight containers.

Correlations (r) between the first and second analyses were 0.68 for whole plant, 0.91 for leaf, 0.71 for stem and 0.73 for young leaf samples. All correlations were significant ($P < 0.01$), but with the exception of the correlation for leaves, they were not very high, probably mainly due to an erratic loss of SMCO from the samples. This uneven loss is shown by the comparatively low correlations (r) for these components (0.21 - 0.75) between the level of SMCO at the first analysis and SMCO lost.

The results of the two experiments were generally complementary. Experiment 2 confirmed that leaves with high levels of SMCO will lose SMCO after approximately 9 months and that stems with low levels of SMCO show no loss of SMCO up to 14 to 16 months storage. The fact that stems with high levels of SMCO showed significant losses after 8.5 months in Experiment 2, does not conflict with Experiment 1, when the group of 7 high SMCO stems also tended to lose SMCO between 5.5 and 12 months, when analysed by autoanalysis.

- *b) Moisture:*

Moisture levels in leaf and stem samples in Experiment 1 tended to increase with storage, as shown in Table 20. Greatest increases occurred during early storage in samples which were very low in moisture

as shown by the 7 stem samples. Moisture levels stablised with later analyses especially in stems.

TABLE 20: Effect of storage on moisture in Experiment 1 (% DM).

Tissue type	No. of samples	Analysis 1	2	3	4	5	*SE*
Leaves	6	9.1^{c}	11.4^{b}	12.3^{ab}	12.8^{a}	13.4^{a}	*0.98***
	3	11.1^{d}	12.4^{c}	13.0^{b}	13.4^{b}	14.1^{a}	*0.30***
Stems	7	5.1^{d}	9.1^{c}	10.5^{b}	11.9^{a}	12.4^{a}	*1.00***
	2	8.5^{b}	9.8^{ab}	10.3^{a}	11.0^{a}	10.8^{a}	*0.53**

* and ** Significant differences at the 5% and 1% levels of significance respectively.

Conclusion

These trials suggest that freeze dried kale leaf and stem samples can be stored in air tight containers under deep freeze conditions (-20°C) for approximately 9 months without losing significant amounts of SMCO. Storing leaf and stem samples for up to 14.5 months results in SMCO being lost from samples with high levels of SMCO (>10 g/kg DM), but not from samples with SMCO concentrations less than approximately 10 g/kg DM.

Whole plant and young leaf freeze dried samples, after being stored for an initial period of 6 and 9 months respectively, lost approximately 30% of their SMCO during the next 5 months storage. As with leaves and stems, highest SMCO losses occurred in samples with highest SMCO levels. Storage at ambient temperature (approximately 15°C) instead of -20°C, has no additional effect on the rate SMCO is

lost from whole plant freeze dried samples which have previously been stored for six months at -20°C.

Moisture levels in freeze dried leaf and stem samples stored at -20°C increased with time, especially in samples with very low moisture levels.

CHAPTER III

Experiments on Factors Affecting SMCO in Kale

3.1 Inter-plant Variation

Introduction

The variation in SMCO between Maris Kestrel kale plants was examined in a glasshouse trial. The main objectives were to look at the inter-plant variation in SMCO for breeding potential and to obtain an estimate of the number of plants required in a glasshouse trial, in order to achieve an acceptable estimate of SMCO.

Experimental

Maris Kestrel seed sieved to a constant size, was sown in 48 pots (12.5 cm diameter) filled with compost. Pots were arranged on a gravel bed in a glasshouse in a 6 x 8 deisgn, with approximately 15 cm between pots. When plants emerged, they were singled to one per pot (originally 2-3 seeds) and all grown under similar temperature and moisture conditions for 89 days before being harvested. The 24 inner plants, which were surrounded by a guard row of a further 24 plants, were cut to 2 cm above ground level and individually weighed, chopped and stored in a freezer at -20°C. Frozen samples were freeze dried and stored in air tight containers for approximately six months prior to analysis of SMCO content by the autoanalysis technique. At the same time as the analysis, sub-samples were dried in an oven at 90°C to measure the moisture content of the freeze dried material in order to calculate SMCO measurements as a percentage of dry matter.

From the 24 SMCO measurements, a mean and standard deviation were calculated. Using this standard deviation and assuming a standard error based on a least significant difference (LSD) required to give acceptable differences between treatments, an estimate of the number of plants required per treatment (n) was calculated for trials using

the following formula.

$$n = (\text{standard deviation} / \text{standard error})^2$$

Results and discussion

The 24 Maris Kestrel kale plants had SMCO levels ranging from 5.5 to 24.0 g/kg DM with a mean level of 12.9 and a standard deviation of 4.81. This more than four-fold range of SMCO values was very large, although Gosden and Johnston (1977) reported a similar result for SMCO levels in the kale variety Proteor. The fresh weights of individual plants at harvesting varied from 178 to 420 g with a mean of 278 g and a standard deviation of 54.8 g. There was no correlation between SMCO and fresh weight of plants ($r = -0.07$). With environmental conditions probably very similar for each plant and as there was no relationship between yield and SMCO content, the results suggest that there is a large variation in SMCO between individual Maris Kestrel plants. As Maris Kestrel is a triple-cross hybrid, low SMCO plants could not be selected and bred directly. Its individual parent lines could be selected for SMCO, although the variation in SMCO between plants in these varieties is not known. With all kale varieties, there is the problem, as mentioned in the introduction, of low heritability of SMCO.

If it is assumed that for comparing means of treatments in a trial a standard error of 1.0 is required, based on an LSD of approximately 3, the number of plants that would be required per treatment would be $23 = (4.81/1)^2$. If a trial contained four replicates, a sample of 6 plants/plot would be sufficient to give the required accuracy. If a standard error of 0.6 was required based on an LSD of approximately 2, the number of plants required per treatment would be 64. The

relationship between standard error and LSD in a particular trial would depend on the degree of replication of each treatment. Plant numbers calculated are only guides, as plant populations with different average SMCO levels would probably have different standard deviations, hence plant numbers would vary from those calculated here.

These plant numbers per treatment are based on plants grown in a glasshouse, hence possibly more plants would be required per plot in a field trial to give the same degree of accuracy. This would be due to a probable greater variation in SMCO in field grown plants, as a result of a more variable environment especially in the soil.

With most field trials reported in this thesis, plant SMCO was analysed in material from approximately 15 plants, which was usually the maximum number that could practically be carried in bags to the laboratory. When plants were smaller, sometimes up to 25 plants were taken and when plants were very large, as few as eight plants were sometimes sampled. All field trials were factorial designs with usually at least three replicates, hence there were certainly enough plants harvested to give accurate comparisons of treatment main effects. For comparison of interactions, when there were usually only about 45 plants per treatment, the degree of accuracy of SMCO estimates would have been reduced.

In the glasshouse and growth cabinet trials, considerably fewer plants were harvested per treatment than in the field trials. Due to lack of space in growth cabinets, plant numbers in some trials were lower than would have been desired and this may have affected some of the results. The floral initiation trials (G1 and G2), and the 1979 chopping trial (G6) in particular, had reasonably low numbers of plants per treatment (<16), making it more difficult to obtain significant differences between treatments in those trials.

Conclusion

Twenty-four Maris Kestrel kale plants grown under glasshouse conditions had more than a four-fold range of SMCO levels from 5.5 to 24.0 g/kg DM. Considering that the environmental conditions were probably reasonably similar for all plants, these results showed that there is a wide variation in SMCO levels within Maris Kestrel kale which may be of use to breeders, providing heritability is adequate.

Because of the large variation in SMCO levels, it was calculated that in glasshouse trials where SMCO was averaging about 13 g/kg DM, approximately 23 and 64 plants respectively were required per treatment to give standard errors of 1.0 and 0.6 for comparisons of treatments. In field trials an even greater number of plants would probably be required to give the same standard errors.

In field trials described in this thesis, probably sufficient plants were harvested in plots to minimise the effect of inter-plant variation in SMCO on most of the important treatment comparisons. Probably only the accuracy of the several factor interactions was affected. In some of the growth cabinet and glasshouse trials, where plant numbers were less than 16 plants per treatment, the inter-plant variation in SMCO would have made it more difficult to obtain significant differences between treatments.

3.2 Effects of Sulphur, Nitrogen, Population, Harvest and Sowing Date

Introduction

The major objective of these trials was to examine agronomic ways of reducing SMCO in kale. Three of the major factors studied were the effects of sulphur (S), nitrogen (N) and plant population (i.e. plant density). Sulphur levels in the soil have only once been reported to affect SMCO in brassicas and that was in kale in a New Zealand trial (McDonald *et al.*, 1981), hence its effect on kale grown in Scottish soils was examined. It was proposed to look at the level of soil sulphate at which SMCO becomes dependent on available S and to examine any effects of low soil S status on dry matter (DM) yields. The practicability of reducing SMCO by growing three successive kale crops on the same site and thus depleting available sulphate S, was also examined.

The effects of N fertiliser on both glucosinolates (Josefsson, 1970) and SMCO in brassicas have given conflicting results, although generally N has increased SMCO levels within the plant (Anon., 1978b), except for in one trial when S was limiting (McDonald *et al.*, 1981). The effects of N on SMCO in kale, especially in relation to soil sulphate levels, were therefore examined. With N, as with S, it was important to know how any changes in plant SMCO content (or levels) affected DM yield.

The effects of plant population on SMCO in brassicas has never been examined, although Josefsson *et el.* (1972) reported that increasing plant population in both kale and rape increased glucosinolate levels. The effects of population on SMCO and DM yield, both in the presence and absence of S and N fertilisers, were studied. Kale was sampled

at various harvest dates in the trials, in order to examine whether any of the fertiliser or population effects varied as plants matured and also to look at the effect of maturity on SMCO levels in leaves and stems. In one trial, the effect of sowing date on SMCO was also examined. In all trials, N content was measured in order to obtain an estimate of how different treatments affected plant quality in terms of crude protein (CP) content. It was assumed CP = N x 6.25.

Maris Kestrel was used in all trials, since for several years it has been one of the major kale varieties grown in the United Kingdom. In two of the trials another kale variety (Vulcan) was also included, in order to examine whether it was affected by the different treatments in a similar manner to Maris Kestrel. A variety of soils of various fertility and drainage characteristics were used for the trials and differences in effects of fertilisers and populations on SMCO levels in the different soils examined.

Experimental

- *a) Treatments and experimental design:*

The effects on SMCO of some or all of the factors S, N and population, were studied in eight field (F) trials during 1979, 1980 and 1981. Details of treatments in each trial are presented in Table 21. Trial F1 was continued for three years to study if soil sulphate and SMCO levels could be reduced with continual cropping of kale. In this trial, low and high S, N and population treatments were maintained in the same plots in each year, although levels of each factor were changed in some years. Most changes were after the first year, when it was considered that not enough S was being applied in the high S treatment and that a greater difference in intra-row plant spacing would be beneficial. Plant residue in F1 was cut to approximately 5 cm

TABLE 21: Details of sowing date, variety, fertiliser and plant spacing treatments in trials.

Trial	No. of plots (blocks)	Sowing date	Variety	S (kg/ha)	N (kg/ha)	Plant populations: plants/ m^2	inter-row spacing (cm)	intra-row spacing (cm)
F1a (1979)	24 *(3)*	5.7.79	M. Kestrel	1. 0 2. 20	1. 75 2. 150	1. 40 2. 80	25 25	10* 5
F1b (1980)	24 *(3)*	30.5.80	M. Kestrel	1. 0 2. 100	1. 50 2. 150	1. 13 2. 52	25 25	30* 7.5*
F1c (1981)	24 *(3)*	29.4.81	M. Kestrel	1. 0 2. 100	1. 75 2. 250	1. 13 2. 52	25 25	30* 7.5
F2	12 *(2)*	11.6.79	M. Kestrel	40	1. 75 2. 150	1. 4 2. 12 3. 40	50 25 25	50* 33* 10
F3	36 *(3)*	23.6.80	1. M. Kestrel 2. Vulcan	1. 0 2. 100	1. 30 2. 60 3. 150	40	25	10
F4	36 *(3)*	26.6.80	1. M. Kestrel 2. Vulcan	1. 0 2. 100	1. 30 2. 60 3. 150	40	25	10
F5	24 *(3)*	1. 26.6.80 2. 24.7.80	M. Kestrel	-	140	1. 13 2. 26 3. 40 4. 80	25 25 25 25	30* 15* 10* 5*
F6	48 *(3)*	22.5.81	M. Kestrel	1. 0 2. 100	1. 50 2. 150	1. 10 2. 20 3. 40 4. 80	25 25 25 25	40* 20* 10* 5*

* singled to correct intra-row spacing.

above ground level and removed each year prior to ploughing. Most stem bases and roots were buried by ploughing and it was aimed to shift the minimum amount of soil between plots.

Trial F2 was originally sown with 36 plots, including three blocks and two S treatments of 40 and 80 kg S/ha. Due to poor plant establishment, one block was not used and because only a limited number of samples were able to be analysed for SMCO in 1979, due to a shortage of analysing facilities, only samples in the low S plots were analysed. Only these 12 plots were therefore included in DM yield and analytical results. Trials F3 and F4 were carried out at low and medium soil sulphate sites respectively. Vulcan kale was included in these two trials, together with Maris Kestrel, which was used in all trials. Trial F5 included two sowing date treatments as well as four different plant populations.

Trials F1 and F2 were split plot trials with S and population treatments factorially arranged in main plots and N in sub-plots. The other trials had all treatments randomised in a factorial design within blocks.

- *b) Cultural details:*

Method of sowing:

Trials were sown in the Midlothian district at an altitude of between 150 and 200 m above sea level. All trials were sown with a Stanhay precision seeder (5 x 25 cm rows per plot) except trial F2 where a Moore grain drill (8 x 25 cm rows per plot) was used. Theoretical plant spacings are shown in Table 21, together with details on which population treatments were sown to a stand and which were singled to the appropriate intra-row seed spacing. Details

for each trial on soil group, soil series and drainage characteristics, plus the number of previous crops after pasture, are shown in Table 22.

Fertiliser application:

Information on basal fertiliser rates, plot sizes, weed control and harvest dates are shown in Table 23. All fertilisers were applied evenly by hand immediately before drilling. Sulphur was applied in all trials, in the form of calcium sulphate - gypsum (18.6% S). Nitrogen was applied as NPK (22.11.11) in F1a and F2 and as NPK (20 : 10 : 10) in F5. In the other trials, N was applied as "Nitrotop", which contained 16.7 and 16.8% N as nitrate and ammonia respectively. Basal dressings of phosphate (P) and potassium (K) at the rates outlined in Table 23, were applied as double season PK (0 : 20 : 20) in trials where "Nitrotop" was used. In F1a and F2, P and K were applied at different rates in proportion to applied N. In all other trials, P and K were each at a constant rate over the trial area.

Herbicide and insecticide application:

A herbicide, Treflan (48% trifluralin), was applied to each trial except F5 (Table 23), at approximately 2.4 l/ha in 280 l water/ha. The Treflan was rotary hoed into the soil during seed bed preparations. No post emergent herbicide was used in any trial, although plots in most trials were weeded by hand. In 1980, a granular insecticide "Sapotec", was spread over the kale in F1b, in order to protect plants from cabbage root fly. In F4, which was sown immediately after pasture, slug pellets ("Draza") were applied a few days after sowing. Pigeons consumed some of the upper leaves in F6 during snow in December 1981, hence percentage leaf estimates measured at the final harvest were probably slightly underestimated.

TABLE 22: Soil characteristics for fertiliser and population field trials.

Trial	Major soil group	Soil series	Drainage	Previous crops since pasture
F1 a b c	Brown earth	Darvel	Freely drained gravel and sands	5
F2	Brown earth	Easter Bush	Imperfectly drained gravel and sands	4
F3	Brown earth	Darvel	Freely drained gravel and sands	5
F4	Brown earth or Gley	Macmerry	Imperfectly drained clay-loam	0
F5	Brown earth and Gley	Sourhope and Winton	Freely drained drifts and Imperfectly drained clay-loam	5
F6		Alf	Alluvial fans	3

TABLE 23: Cultural and harvest details in field trials.

Trial	Basal fertiliser: P (kg P/ha)	K (kg K/ha)	Plot size (m)	Weed control (date applied)	Sample size (m^2)	Harvest date (days after sowing)
F1 a	16 + 32	31 + 62	7.5 x 2	Treflan (4.7.79)	1.14	25.10.79 *(112)* 13.12.79 *(161)* 25. 2.80 *(235)*
F1 b	48	91	7.5 x 2	Treflan (28.5.80)	1.14	17.10.80 *(140)* 16.12.80 *(200)* 12. 2.81 *(258)*
F1 c	48	91	7.5 x 2	Treflan (27.4.81)	1.14	14. 8.81 *(107)* 13.10.81 *(167)+* 9.12.81 *(224)*
F2	16 + 32	31 + 62	9 x 2	Treflan (10.6.79)	2.03	10.12.79 *(182)*
F3	48	91	20 x 2	Treflan (15.5.80)	2.29	1.10.80 *(100)* 4.12.80 *(164)* 3. 2.81 *(225)*
F4	48	91	20 x 2	Treflan (26.6.80)	2.29	3.12.80 *(160)*
F5	31	58	24 x 2		1.86	1. 9.80 *(39, 67)* 22.10.80 *(90, 118)* 17.11.80 *(116, 144)*
F6	48	91	16 x 2	Treflan (14.5.81)	2.29	20. 8.81 *(100)* 23.10.81 *(164)+* 18.12.81 *(220)**

+ samples harvested for dry matter yield only; * samples harvested for SMCO, but no dry matter yield measured.

Soil sampling:

Bulk soil samples were taken from each trial for soil sulphate measurements. Trials F1a, F2 and F3 were sampled to a depth of 30 cm while all other trials were sampled to 15 to 20 cm. Usually samples were taken prior to sowing and/or at the first harvest. In 1981 (F1c and F6), samples were also taken at the second harvest and in both trials, soil samples from each harvest were analysed for pH, P, K and magnesium (Mg), as well as soil sulphate. With samples prior to sowing, there was usually one bulk sample per block made up of approximately 12 cores. When sampled at the first and second harvests, two bulk samples were taken from each block, one in plots which had received no S and one in plots which had received S. An exception was in F6 at the first harvest, when only one bulk sample per block was taken.

- *c) Harvesting details:*

Harvest dates and sample sizes for each trial, are shown in Table 23. At each harvest, kale plants within a sampling area were cut to approximately 5 cm above ground level, counted and weighed. From each plot, a sub-sample of between 8 and 26 plants, depending on their size, was taken to the laboratory where they were either divided into leaf (lamina and petiole) and stem components, or kept whole. If split into leaf and stem, the total weight of each component was recorded. At the December harvest in F1c, stems were separated into outer (cortex) and inner (pith) components. All stems were chopped into segments 2-3 cm long and leaves into pieces approximately 10 cm square. From chopped material from either whole plants, leaves or stems, two subsamples were taken for storage in a freezer at -20°C

prior to chemical analysis and for drying in an oven at 90°C for dry matter content determinations. From fresh weights and dry matter percentages, DM yields were calculated.

At the December harvest in F6, samples were taken for chemical analyses, but no total plant weights were recorded for DM yield determinations. In order to calculate the total production (kg/ha) of SMCO and N at this harvest, it was assumed that the total DM yield was the same as in October and this was divided into leaf and stem yield based on the percentage of each component measured in December. The same yield in October and December was assumed, as in the other 1981 field trial (F1c), there was a similar DM yield at these two harvests.

The samples stored at -20°C were freeze dried within a few weeks of being harvested. Approximately 150 g of fresh material from each sample was freeze dried and stored (-20°C) in an airtight plastic carton until required for analysis.

- *d) Chemical determinations:*

Plant:

SMCO was measured by two different methods. The autoanalysis technique (see Section 2.1) was used for all whole plant and stem samples, plus the leaf samples in 1979 (F1a and F2) and 1981 (F1c and F6). For all leaf samples in 1980 (F1b, F3 and F5), NIR analysis was used. Table 24 outlines for each trial the kale component analysed, the SMCO method of analysis and the storage time of samples between harvest and SMCO analysis.

In all samples analysed for SMCO, nitrogen and moisture in the freeze dried material were also measured by NIR analysis. The best NIR wavelength equations for measuring SMCO, N and moisture in the different kale components have been outlined previously (see Section 2.2).

TABLE 24: SMCO analytical technique and sample storage time in field trials.

Trial	Harvest date	Component analysed	SMCO determination method (NIR or AA)*	Months between harvest and SMCO analysis
F1 a	25.10.79	Leaf	AA	10.5
		Stem	AA	10.5
	13.12.79	Leaf	AA	8
		Stem	AA	8
	25. 2.80	Leaf	AA	6
		Stem	AA	6
F1 b	17.10.80	Leaf	NIR	5
		Stem	AA	10
	16.12.80	Leaf	NIR	3
		Stem	AA	8
	12. 2.81	Leaf	NIR	1.5
		Stem	AA	6
F1 c	16. 8.81	Leaf	AA	7
		Stem	AA	6
	9.12.81	Leaf	AA	3
		Stem-pith	AA	2
		Stem-cortex	AA	2
F2	10.12.79	Leaf	AA	9
		Stem	AA	9
F3	1.10.81	Whole	AA	9
	4.12.81	Whole	AA	7
	3. 2.81	Leaf	NIR	2
		Stem	AA	7
F4	3.12.80	Whole	AA	7
F5	1. 9.80	Whole	AA	10
	22.10.80	Whole	AA	9
	17.11.80	Leaf	NIR	4
		Stem	AA	8
F6	20. 8.81	Leaf	AA	6
		Stem	AA	6
	18.12.81	Leaf	AA	3
		Stem	AA	2

* AA = autoanalysis technique; NIR = near infrared reflectance analysis.

In stem samples from the February harvest in F1b and the August and December harvests in F1c, nitrate N and protein N were measured. For nitrate N, samples in F1b and F1c were extracted by the methods outlined by Orion (1978) and Milham *et al*. (1970) respectively. Extracted N in both trials was estimated on an Orion Research Ionalyser/901, using Orion electrodes. For protein N measurements, the material in both years was precipitated with copper sulphate and sodium hydroxide as described by the Association of Official Analytical Chemists (1965) and the precipitated N measured by the Kjeldahl method. In F1b (February harvest), total N in stems was measured by the Kjeldahl method at the same time as nitrate N and protein N measurements were made, whereas in the stems in F1c, total N was measured by NIR.

Soil:

Soil sulphate was extracted from air dried soil samples using calcium dihydrogen orthophosphate and determined on an autoanalyser (Sinclair, 1973). pH was determined following an equalibration with calcium chloride (Schofield and Wormald Taylor, 1955) and extractable phosphate determined using a modification of the Peech and English (1944) method. Potassium and magnesium were determined by the flame emission and atomic absorption techniques respectively.

- *e) Statistical methods:*

In each trial, treatments at a particular harvest were compared by an analysis of variance (ANOVA). Standard errors presented for the results of trials F3, F4, F5 and F6 were calculated by taking the square root of the error mean square in a particular analysis. In split plot trials F1 and F2, the standard errors are either based on

main plots for comparisons of treatments which do not include N, or on sub-plots when N is one of the factors being compared. Main plot standard errors were calculated by dividing the square root of the main plot error mean square by the square root of the number of plots in each main plot. Sub-plot standard errors were calculated by taking the square root of the sub-plot error mean square.

In Figures 21, 22, 23 and 24, mean values for trials are presented together with standard errors of means, calculated by dividing the square roots of error mean squares by the square roots of the number of plots in the trials. Small case letters are used in some tables to compare means by the least significant difference test at the 5% level of significance. Means with different letters are significantly different.

Results and discussion

3.2.1 Effects of sulphur fertiliser(S)

S/soil sulphate availability:

Levels of available soil sulphate (ppm) in the topsoil (15-30 cm) at each field trial site are shown in Table 25. In each of the six trials where S fertiliser was a treatment, soil sulphate levels at at least one harvest date are presented for plots which received and did not receive S.

In trials F1a, F1b, F1c and F3, there were either only small or no differences in available sulphate between S fertiliser treatments in October. In all four trials, soil sulphate in plots which had received S fertiliser was less than 10 ppm. In F4 and F6, there were slightly larger differences between S treatments in October, although in F4 the soil sulphate level was quite high (12.5 ppm) even when no S was applied.

TABLE 25: Soil sample results.

Trial	Sampling date	pH	P (ppm)	K (ppm)	Mg (ppm)	Sulphate-S (ppm)	
F1 a	25.10.79					-S	4.5 *(0.87)**
						+S	4.2 *(0.25)*
F1 b	30. 5.80						9.4 *(1.87)*
	14.10.80					-S	3.6 *(0.53)*
						+S	5.4 *(0.52)*
F1 c	29. 4.81	6.0 *(0.02)*	6.5 *(0.09)*	184 *(41.5)*	142 *(4.7)*	-S	9.1 *(1.44)*
						+S	12.1 *(1.86)*
	17. 8.81	5.8 *(0.03)*	9.0 *(0.67)*	123 *(21.6)*	134 *(8.0)*	-S	13.1 *(1.66)*
						+S	49.3 *(9.21)*
	15.10.81	6.1 *(0.02)*	6.1 *(0.16)*	64 *(4.1)*	158 *(12.4)*	-S	4.1 *(0.07)*
						+S	9.6 *(0.46)*
F2	2.11.79						8.6 *(0.72)*
F3	15. 5.80						8.1 *(2.16)*
	14.10.80					-S	<1.0 *(0.53)*
						+S	4.5 *(0.53)*
F4	26. 6.80						11.9 *(1.24)*
	14.10.80					-S	12.5 *(0.88)*
						+S	25.1 *(10.28)*
F5	14.10.80						<0.5 *(0.10)*
F6	20. 5.81	6.1 *(0.09)*	5.4 *(0.23)*	81 *(4.6)*	209 *(8.7)*		12.0 *(0.64)*
	20. 8.81	6.1 *(0.06)*	6.6 *(0.31)*	80 *(3.5)*	227 *(6.2)*		29.0 *(4.81)*
	23.10.81	6.2 *(0.07)*	4.6 *(0.23)*	54 *(5.3)*	243 *(8.7)*	-S	7.0 *(0.59)*
						+S	14.7 *(0.21)*

( )* standard error of mean

The major reason for most trials having reasonably low soil sulphate levels in October in plots which had received S fertiliser was probably because five of the six trials were on free draining gravels and sands. Only in F4, which had the highest levels of available sulphate, was the soil a less well drained clay-loam (Table 22). On the free draining soils, leaching probably removed most of the applied gypsum by October each year, although some of the applied S would have also been taken up by the plants.

Soil samples taken in August 1981 in F1c and F6 showed that available sulphate was still high at that time. In F1c there were large differences between S treatments in August, although even on plots which had received no S available sulphate was relatively high. Unfortunately in F6, available sulphate was not measured in the different S treatments in August.

The higher sulphate levels in the low S plots in F1 during April and August 1981, compared with levels in October 1979 and October 1980, were surprising. Possible explanations are that there was a breakdown of small amounts of organic S especially from kale roots and stem bases, or sulphate has some mobility within the soil, or there may have been small amounts of S transferred between plots either during cultivation or less likely by water movement. The breakdown of organic matter probably caused some of the increase in sulphate levels, as levels at sowings in 1980 and 1981 were higher than those in the preceding Octobers, suggesting that there had been an increase in available sulphate prior to sowing. This probably occurred during the two or three months the soil lay fallow after being ploughed in February or March each year.

In F2 and F5, in which the effect of S fertiliser on SMCO was not studied, mean soil sulphate levels were relatively low. The levels of pH, P, K and Mg in F1c and F6 were normal except for K levels, which were reasonably low especially at October harvests (Table 25).

S/dry matter yield:

The main effect of S fertiliser on total dry matter (DM) yield (t/ha) averaged over all harvests, was not significant ($P < 0.05$) in any trial. DM yields averaged 5.30, 5.40, 6.85, 6.61, 6.54 and 8.06 t/ha in trials F1a, F1b, F1c, F3, F4 and F6 respectively. The only significant effect of S at individual harvests was in February 1981 in F3, when leaf DM yield was increased ($P < 0.05$) from 2.39 to 2.69 (SE = 0.409).

In each year in F1, there was a tendency for S to increase both stem and total DM yields at the low rate of N fertiliser but not at the high rate. This S x N interaction was only significant at some harvests, however, as shown in Table 26. In other trials this trend did not occur.

S/SMCO content:

The main effect of S on whole plant SMCO was significant ($P < 0.05$) at only one harvest date in one of the six trials. In F6 at the August harvest, whole plant SMCO was increased ($P < 0.01$) by S from 6.3 to 7.2 g/kg DM (SE = 1.14). In another trial, F4, S increased whole plant SMCO in December from 19.9 to 21.4 g/kg DM (SE = 2.53), which was significant at the 10% level of significance.

With stems in F6, there was a significant ($P < 0.05$) interaction between S and plant population at the August harvest. S tended to increase SMCO at the highest population (80 plants/m^2) from 8.7 to 11.0 g/kg DM and reduce SMCO at the lowest population (10 plants/m^2) from 16.5 to 14.8 g/kg DM (SE = 2.25).

TABLE 26: Effect of sulphur x nitrogen interaction, on DM yield in trial F1 (t/ha).

Trial (No. plots)	Harvest date	N applied (kg/ha)	Leaves: -S	Leaves: +S	Leaves: *SE*	Stems: -S	Stems: +S	Stems: *SE*	Whole plants: -S	Whole plants: +S	Whole plants: *SE*
F1 a (24)	Oct	75	2.82	3.21	*0.419*	2.08	2.32	*0.449*	4.90	5.54	*0.789*
		150	3.25	3.09		2.96	2.58		6.20	5.67	
	Dec	75	2.05	2.42	*0.338*	2.21	2.70	*0.291***	4.26	5.12	*0.375***
		150	2.50	2.46		3.42	3.02		5.91	5.48	
	Feb	75	1.91	2.21	*0.296*	2.32	2.84	*0.547*	4.23	5.05	*0.772*
		150	2.17	2.43		3.36	3.21		5.53	5.65	
	<u>Mean</u>	75	2.26	2.61	*0.281*	2.20	2.62	*0.263***	4.46	5.24	*0.437**
		150	2.64	2.66		3.24	2.94		5.88	5.60	
F1 b (24)	Oct	50	2.47	3.28	*1.270*	1.14	1.45	*0.728*	3.61	4.73	*1.900*
		150	4.48	4.05		3.50	3.03		7.98	7.08	
	Dec	50	2.02	2.58	*0.757*	1.52	1.91	*0.888*	3.54	4.49	*1.604*
		150	3.23	3.19		3.88	3.28		7.11	6.46	
	Feb	50	1.71	1.84	*0.443*	1.26	1.34	*0.503**	2.98	3.18	*0.769*
		150	3.10	3.05		4.26	3.30		7.36	6.35	
	<u>Mean</u>	50	2.07	2.56	*0.729*	1.31	1.57	*0.577*	3.38	4.13	*1.273*
		150	3.60	3.43		3.88	3.20		7.48	6.63	
F1 c (24)	Aug	75	2.87	2.67	*0.271*	0.82	0.79	*0.212*	3.69	3.46	*0.380*
		250	4.62	4.50		2.23	1.83		6.86	6.33	
	Oct	75	2.90	2.83	*1.031*	2.03	1.79	*1.323*	4.93	4.62	*2.264*
		250	6.14	5.21		5.36	4.16		11.50	9.37	
	Dec	75	2.40	2.84	*0.858*	2.15	2.91	*1.074*	4.56	5.75	*1.732*
		250	5.48	4.85		5.75	5.06		11.23	9.91	
	<u>Mean</u>	75	2.73	2.78	*0.437*	1.67	1.83	*0.524*	4.39	4.61	*0.854*
		750	5.41	4.85		4.44	3.68		9.86	8.54	

* and ** SxN interaction significant at 5% and 1% levels of significance respectively.

The effects of the interactions between S and N fertilisers on SMCO in each of the six trials are shown in Tables 27 and 28. At the October harvest in F3, S significantly ($P < 0.05$) increased whole plant SMCO at the lowest N level but not at the higher levels.

The small differences in available soil sulphate between S treatments in most trials in October (Table 25) may have affected SMCO responses to S fertiliser. In F1c in August, when there was a large difference in available sulphate between S treatments, S had no effect on SMCO, probably because available sulphate on the low S plots was quite high (13 ppm). It has previously been suggested that S is more likely to increase SMCO levels in kale grown on soils with less than 10 ppm of soil sulphate (McDonald *et al.*, 1981). In the trial (F6) where S increased whole plant SMCO in August, there were probably large differences in available sulphate between plots receiving and not receiving S. Differences were not measured, although the mean value for all plots was 29 ppm and the plots which had received no S were unlikely to have had a much higher sulphate level than their 12 ppm at sowing and were possibly lower since their level had decreased to 7 ppm by October.

In the three trials where S tended to increase whole plant SMCO, the effects were obtained at the first harvest in either August or October in F3 and F6 and in the other trial (F4), the soil was not as free draining as the others (Table 22). It would appear therefore, that in free draining soils reasonably low in available sulphate (≤ 12 ppm) at sowing, SMCO levels will not be affected by S applied due to leaching, except at reasonably early stages of growth. Sulphur would probably have more effect on SMCO in kale grown on poorer drained soils low in available sulphate. The effect of S on SMCO in the trial on the poorly

TABLE 27: Effect of sulphur x nitrogen interaction on SMCO content in trial F1 (g/kg DM).

Trial (No. plots)	Harvest date	N applied (kg/ha)	Leaves: -S	Leaves: +S	Leaves: SE	Stems: -S	Stems: +S	Stems: SE	Whole plants: -S	Whole plants: +S	Whole plants: SE
F1 a (24)	Oct	75	8.1	8.8	*2.42*	8.2	9.0	*2.52*	8.2	8.9	*2.25*
		150	7.7	7.9		8.2	7.9		7.9	7.9	
	Dec	75	12.7	11.8	*2.94*	12.5	12.0	*2.09*	12.6	11.9	*1.90*
		150	15.5	12.9		14.4	13.7		14.7	13.3	
	Feb	75	15.9	16.5	*3.71*	11.8	11.4	*2.43*	13.6	13.5	*2.12*
		150	19.7	17.7		13.2	12.0		15.6	14.4	
	Mean	75	12.3	12.4	*1.70*	10.8	10.8	*1.26*	11.5	11.4	*1.20*
		150	14.3	12.8		12.0	11.2		12.8	11.9	
F1 b (24)	Oct	50	8.1	8.1	*0.78**	9.0	9.9	*3.43*	8.2	8.6	*1.30*
		150	11.6	9.9		11.5	12.9		11.1	11.0	
	Dec	50	11.4	12.3	*1.42***	13.1	12.6	*4.44*	12.0	12.5	*2.25*
		150	18.4	14.1		13.8	13.1		15.7	13.5	
	Feb	50	14.6	16.1	*2.86*	13.6	13.7	*3.86*	14.1	15.0	*1.86*
		150	19.2	16.9		13.8	14.8		15.9	15.5	
	Mean	50	11.4	12.1	*1.32**	11.9	12.1	*2.37*	11.4	12.0	*1.23*
		150	16.4	13.7		13.0	13.6		14.2	13.4	
F1 c (24)	Aug	75	3.9	3.6	*0.87*	8.0	7.1	*2.13*	4.8	4.4	*1.10*
		250	6.5	7.8		11.3	14.2		8.0	9.6	
	Dec	75	5.3	5.5	*2.54*	5.2	5.2	*0.71*	5.2	5.4	*1.30*
		250	7.9	9.1		7.1	7.9		7.4	8.5	
	Mean	75	4.6	4.6	*1.63*	6.6	6.2	*1.24*	5.0	4.9	*1.06*
		250	7.2	8.4		9.2	11.0		7.7	9.0	

* and ** SxN interaction significant at 5% and 1% levels of significance respectively.

TABLE 28: Effect of sulphur x nitrogen interaction on SMCO content in trials F3, 4 and 6 (g/kg DM).

Trial (No. plots)	Harvest date	N applied (kg/ha)	Leaves: -S	Leaves: +S	Leaves: *SE*	Stems: -S	Stems: +S	Stems: *SE*	Whole plants: -S	Whole plants: +S	Whole plants: *SE*
F3 (36)	Oct	30							11.4	13.8	
		60							14.5	13.6	*1.65**
		150							14.8	14.2	
	Dec	30							16.8	17.6	
		60							18.8	19.0	*2.07*
		150							18.8	21.1	
	Feb	30	20.5	21.1		14.3	15.3		16.6	17.4	
		60	20.6	21.0	*3.03*	14.1	16.8	*4.42*	16.3	18.2	*3.18*
		150	22.4	22.7		15.6	14.9		17.7	17.6	
	<u>Mean</u>	30							15.0	16.3	
		60							16.6	16.9	*1.45*
		150							17.1	17.7	
F4 (36)	Dec	30							19.9	21.5	
		60							20.1	21.2	*2.53*
		150							19.8	21.4	
F6 (48)	Aug	50	3.8	4.2	*1.48*	11.2	12.8	*2.25*	5.4	6.0	*1.14*
		150	5.0	6.3		13.4	14.3		7.2	8.4	
	Dec	50	9.0	9.7	*1.98*	8.9	8.9	*1.79*	8.9	9.2	*1.52*
		150	9.9	10.3		10.1	10.1		9.8	10.1	
	<u>Mean</u>	50	6.4	6.9	*1.26*	10.0	10.8	*1.42*	7.2	7.6	*0.86*
		150	7.4	8.3		11.8	12.2		8.5	9.2	

* SxN interaction significant at the 5% level of significance.

drained soil (Trial F4) was small, mainly because it was reasonably high in available sulphate and was not harvested before December.

There were few interactions between S and N and in none of the trials, at any harvests or with any of the components, did N tend to reduce SMCO levels when available soil sulphate was low. These findings conflict with those reported previously by McDonald *et al.* (1981). In the significant S x N interaction in F3, in both leaves and whole plants, N tended to increase SMCO more when available soil sulphate was low.

S/SMCO production:

In general, S fertiliser either as a main effect or in interactions with other factors, had no effect on SMCO production within the plant (kg/ha). Only in F3 in December was whole plant SMCO production significantly ($P < 0.05$) increased by S from 132 to 148 kg/ha (SE = 19.9). SMCO production, averaged over all harvests, was 63 (F1a), 70 (F1b), 46 (F1c), 112 (F3), 136 (F4) and 49 kg/ha (F6).

S/nitrogen content:

The main effect of S on the N content in whole kale plants was not significant in five of the six trials. In F1a, the mean N content over all harvests was significantly ($P < 0.05$) reduced by S from 2.38 to 2.29% DM (SE = 0.192). In F1b at the December harvest, S reduced the N content at the high N treatment from 2.16 to 1.81% (SE = 0.179), compared with N values at the low N treatment of 1.72 (-S) and 1.75 (+S) % DM. These results showed that S fertiliser may have had some disadvantageous effect on N content in some trials, but generally effects were small and unimportant.

3.2.2 *Effects of nitrogen fertiliser (N)*

N/dry matter yield:

The effect of N fertiliser on kale DM yield was examined in seven field trials (Table 29). In all trials, except F4, N increased DM yields, although differences were not always significant with all components at all harvests. Generally stem yields were increased more than leaf DM yields. The mean percentage increases in total DM yield between the lowest and highest N treatments were 18, 88, 104, 22, 25, 4 and 22% respectively for trials F1a, F1b, F1c, F2, F3, F4 and F6. The kale in F4 was not affected by N, probably due to a plentiful supply of available N in the soil, as this trial was sown immediately after pasture, whereas the other trials were sown after at least three previous crops since pasture (Table 22). The large increases in DM yield in F1b and F1c, were probably due mainly to low levels of available N on the low N plots in those trials.

In trials where there were both N and population treatments (F1a, F1b, F1c, F2 and F6), there was usually a tendency for N to increase stem DM yields more at higher plant populations than at lower populations, as shown in Table 30. In F1c, the N x population interaction was significant ($P < 0.05$) in both stem pith and stem cortex in December. This interaction generally did not occur with leaves.

In the three years of the F1 trials, there were sometimes significant S x N interactions in stems and whole plants, with a tendency for N to increase total DM yield more on plots which had received no S, than on plots which had received S (Table 26). These S x N interactions were not significant in the other trials.

TABLE 29: Effect of nitrogen on dry matter yield in field trials (t/ha).

Trial (No. plots)		Leaves N applied			*SE*	Stems N applied			*SE*	Whole plants N applied			*SE*
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F1 a (24)	Oct	3.02	3.17		*0.419*	2.20	2.77		*0.449**	5.22	5.94		*0.789*
	Dec	2.23	2.48		*0.338*	2.46	3.22		*0.291***	4.69	5.69		*0.375***
	Feb	2.06	2.30		*0.296*	2.58	3.29		*0.547**	4.64	5.59		*0.772**
	Mean	2.44	2.65		*0.281*	2.41	3.09		*0.263***	4.85	5.74		*0.437***
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F1 b (24)	Oct	2.88	4.27		*1.270**	1.30	3.26		*0.728***	4.17	7.53		*1.900***
	Dec	2.30	3.21		*0.757**	1.72	3.58		*0.888***	4.01	6.78		*1.604***
	Feb	1.77	3.08		*0.443***	1.30	3.78		*0.503***	3.08	6.86		*0.769***
	Mean	2.32	3.52		*0.729***	1.44	3.54		*0.577***	3.75	7.06		*1.273***
		N_{75}	N_{250}			N_{75}	N_{250}			N_{75}	N_{250}		
F1 c (24)	Aug	2.77	4.56		*0.271***	0.80	2.03		*0.212***	3.57	6.59		*0.380***
	Oct	2.87	5.67		*1.031***	1.91	4.76		*1.323***	4.78	10.43		*2.264***
	Dec	2.62	5.16		*0.858***	2.53	5.41		*1.074***	5.15	10.57		*1.732***
	Mean	2.75	5.13		*0.516***	1.75	4.06		*0.690***	4.50	9.20		*1.137***
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F2 (12)	Dec	3.24	3.22		*0.727*	2.68	3.99		*0.411**	5.92	7.22		*1.136*
		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}	
F3 (36)	Oct									4.58	5.56	6.18	*0.767***
	Dec									6.52	7.62	8.26	*0.855***
	Feb	2.44	2.55	2.64	*0.410*	3.92	4.35	4.81	*0.566***	6.35	6.90	7.45	*0.707***
	Mean									5.82	6.69	7.30	*0.590***
										N_{30}	N_{60}	N_{150}	
F4 (36)	Dec									6.29	6.79	6.53	*0.866*
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F6 (48)	Aug	4.33	5.07		*0.485***	1.35	2.01		*0.295***	5.68	7.08		*0.710***
	Oct	4.62	5.65		*0.921***	4.24	4.97		*0.823***	8.86	10.62		*1.444***
	Mean	4.47	5.36		*0.550***	2.80	3.49		*0.501***	7.27	8.85		*0.912***

* and ** Main effect of N significant at 5% and 1% levels of significance respectively.

TABLE 30: Effect of nitrogen x population interaction on DM yield in trials F1, 2 and 6 (t/ha).

Trial (No. plots)	Harvest date	Population (plants/m²)	Leaves Low N	Leaves High N	Leaves *SE*	Stems Low N	Stems High N	Stems *SE*	Whole plants Low N	Whole plants High N	Whole plants *SE*
F1 a (24)	Oct	40	3.21	3.35	*0.419*	2.06	2.52	*0.449*	5.27	5.87	*0.789*
		80	2.82	2.98		2.35	3.02		5.17	6.00	
	Dec	40	2.10	2.80	*0.338**	2.06	2.93	*0.291*	4.16	5.73	*0.375***
		80	2.36	2.15		2.85	3.51		5.22	5.66	
	Feb	40	1.82	2.30	*0.296*	2.05	3.08	*0.547*	3.87	5.38	*0.772*
		80	2.30	2.30		3.10	3.50		5.41	5.80	
F1 b (24)	Oct	13	3.04	4.11	*1.270*	1.26	2.17	*0.728***	4.31	6.28	*1.900*
		52	2.71	4.42		1.32	4.36		4.03	8.78	
	Dec	13	2.61	3.34	*0.757*	1.72	2.58	*0.888**	4.33	5.92	*1.604*
		52	1.99	3.08		1.71	4.57		3.70	7.65	
	Feb	13	1.75	3.01	*0.443*	1.14	3.09	*0.503**	2.89	6.11	*0.769*
		52	1.79	3.14		1.47	4.47		3.26	7.61	
F1 c (24)	Aug	13	2.27	4.07	*0.271*	0.52	1.43	*0.212***	2.79	5.50	*0.380*
		52	3.28	5.05		1.08	2.63		4.35	7.69	
	Oct	13	2.78	5.90	*1.031*	1.79	4.43	*1.323*	4.58	10.32	*2.264*
		52	2.95	5.45		2.03	5.09		4.98	10.54	
	Dec	13	2.91	5.44	*0.858*	2.78	4.43	*1.074**	5.69	9.88	*1.732*
		52	2.33	4.89		2.28	6.38		4.61	11.27	
F2 (12)	Dec	4	3.98	3.90	*0.727*	2.20	2.58	*0.411*	6.17	6.47	*1.136*
		12	2.97	2.91		2.79	4.32		5.76	7.23	
		40	2.78	2.85		3.06	5.08		5.84	7.94	
F6 (48)	Aug	10	4.65	5.00	*0.485**	0.99	1.25	*0.295***	5.64	6.25	*0.710**
		20	4.69	5.36		1.46	1.79		6.15	7.15	
		40	3.66	5.15		1.23	2.24		4.89	7.39	
		80	4.30	4.76		1.74	2.76		6.04	7.52	
	Oct	10	5.78	6.26	*0.921*	3.85	3.96	*0.823*	9.63	10.22	*1.444*
		20	4.99	6.09		4.55	4.96		9.53	11.05	
		40	3.85	5.17		4.05	4.84		7.90	10.01	
		80	3.86	5.10		4.53	6.10		8.39	11.21	

* and ** N x population interaction significant at 5% and 1% levels of significance respectively.

N/SMCO content:

N fertiliser significantly ($P < 0.05$) increased SMCO content in whole plants in four out of seven trials (Table 31). In two other trials, F1a and F2, N tended to increase SMCO content, although differences were not significant. The increases in whole plant SMCO between the lowest and highest N treatments, when averaged over all harvests, were 8, 18, 68, 14, 12, 0 and 20% for trials F1a, F1b, F1c, F2, F3, F4 and F6 respectively. The largest SMCO increase was in F1c, where there was a very high application of N (250 kg/ha) at the high N treatment due to the soil being very depleted in available N after at least seven years of previous crops since pasture. In F4, N had no effect on SMCO or DM yield, due to high levels of available N in the soil. Where N increased SMCO content, the effect usually occurred in both leaves and stems and was not affected by harvest date (Table 31).

With N addition, the percentage increase in DM yield was either similar or slightly higher than the percentage increase in SMCO. In F3, where there were three rates of N fertiliser, approximately two-thirds of the increases in both DM yield and SMCO, at the October and December harvests, occurred when the N rate was increased from 30 to 60 kg N/ha. It is probably therefore not possible to add a small amount of N in order to obtain a near maximum yield, without also increasing the SMCO content. This means that probably a high yielding kale crop cannot be obtained by adding N, without also making it potentially more toxic to animals grazing the crop.

Whereas N tended to increase stem DM yields more at high populations than low populations, this trend did not occur with SMCO, and in F1c at the December harvest N increased stem SMCO more at the low

TABLE 31: Effect of nitrogen on SMCO content in field trials (g/kg DM).

Trial (No. plots)	Harvest date	Leaves N applied			*SE*	Stems N applied			*SE*	Whole plants N applied			*SE*
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F1 a (24)	Oct	8.5	7.8		*2.42*	8.6	8.1		*2.52*	8.5	7.9		*2.26*
	Dec	12.3	14.2		*2.94*	12.3	14.0		*2.09*	12.3	14.0		*1.90*
	Feb	16.2	18.7		*3.71*	11.6	12.6		*2.43*	13.6	15.0		*2.12*
	Mean	12.3	13.6		*1.70*	10.8	11.6		*1.26*	11.4	12.3		*1.20*
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F1 b (24)	Oct	8.1	10.8		*0.78***	9.4	12.2		*3.43*	8.4	11.0		*1.30***
	Dec	11.8	16.3		*1.42***	12.9	13.5		*4.44*	12.3	14.6		*2.25**
	Feb	15.3	18.1		*2.86**	13.6	14.3		*3.86*	14.5	15.7		*1.86*
	Mean	11.8	15.0		*1.32***	12.0	13.3		*2.37*	11.7	13.8		*1.23***
		N_{75}	N_{250}			N_{75}	N_{250}			N_{75}	N_{250}		
F1 c (24)	Aug	3.8	7.2		*0.87***	7.5	12.7		*2.13***	4.6	8.8		*1.10***
	Dec	5.4	8.5		*2.54**	5.2	7.5		*0.71***	5.3	8.0		*1.30***
	Mean	4.6	7.8		*1.63***	6.4	10.1		*1.24***	5.0	8.4		*1.06***
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F2 (12)	Dec	8.3	9.8		*2.50*	11.7	12.5		*1.50*	9.7	11.1		*1.76*
		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}	
F3 (36)	Oct									12.6	14.1	14.5	*1.65**
	Dec									17.2	18.9	20.0	*2.07**
	Feb	20.8	20.8	22.6	*3.03*	14.8	15.4	15.2	*4.42*	17.0	17.2	17.7	*3.18*
	Mean									15.6	16.7	17.4	*1.45**
										N_{30}	N_{60}	N_{150}	
F4 (36)	Dec									20.7	20.6	20.6	*2.53*
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F6 (48)	Aug	4.0	5.6		*1.48***	12.0	13.8		*2.25***	5.7	7.8		*1.14***
	Dec	9.4	10.1		*1.98*	8.9	10.1		*1.79**	9.0	9.9		*1.52*
	Mean	6.7	7.8		*1.26***	10.4	12.0		*1.42***	7.4	8.9		*0.86***

* and ** Main effect of N significant at 5% and 1% levels of significance respectively.

TABLE 32: Effect of nitrogen x population interaction on SMCO content in trials F1, 2 and 6 (g/kg DM).

Trial (No. plots)	Harvest date	Population (plants/m²)	Leaves Low N	Leaves High N	Leaves *SE*	Stems Low N	Stems High N	Stems *SE*	Whole plants Low N	Whole plants High N	Whole plants *SE*
F1 a (24)	Oct	40	9.3	8.1	*2.42*	9.4	8.8	*2.52*	9.4	8.4	*2.26*
		80	7.6	7.5		7.7	7.3		7.7	7.4	
	Dec	40	13.5	13.3	*2.94*	13.0	15.2	*2.09*	13.2	14.3	*1.90*
		80	11.0	15.2		11.5	12.9		11.3	13.8	
	Feb	40	15.4	18.7	*3.71*	13.6	13.7	*2.43*	14.4	15.8	*2.12*
		80	17.0	18.7		9.5	11.5		12.7	14.2	
F1 b (24)	Oct	13	8.4	9.6	*0.78***	11.4	14.8	*3.43*	9.3	11.4	*1.30*
		52	7.7	11.9		7.5	9.6		7.6	10.7	
	Dec	13	12.1	15.2	*1.42**	15.1	14.2	*4.44*	13.3	14.7	*2.25*
		52	11.6	17.4		10.6	12.7		11.2	14.6	
	Feb	13	15.8	17.0	*2.86*	16.2	17.3	*3.86*	16.0	17.0	*1.86*
		52	14.8	19.2		11.0	11.3		13.1	14.4	
F1 c (24)	Aug	13	3.8	7.3	*0.87*	8.1	13.9	*2.13*	4.6	9.0	*1.10*
		52	3.8	7.0		7.0	11.5		4.6	8.5	
Pith	Dec	13				6.5	11.4	*1.25***			
		52				5.1	6.4				
Cortex	Dec	13				5.3	7.5	*0.91*			
		52				4.2	5.6				
	Dec	13	5.5	8.0	*2.54*	5.9	9.1	*0.71***	5.7	8.5	*1.30*
		52	5.3	9.0		4.6	5.9		5.0	7.4	
F2 (12)	Dec	4	8.0	10.8	*2.50*	14.8	16.0	*1.50*	10.5	12.9	*1.76*
		12	10.3	9.8		14.2	11.8		12.2	10.9	
		40	6.6	8.9		6.1	9.8		6.4	9.5	
F6 (48)	Aug	10	3.3	5.8	*1.48*	15.2	16.1	*2.25*	5.4	7.8	*1.14*
		20	4.0	5.7		13.9	14.5		6.3	7.9	
		40	3.8	5.3		10.0	13.8		5.3	7.8	
		80	4.8	5.7		8.7	10.9		5.9	7.6	
	Dec	10	9.1	9.4	*1.98*	11.4	14.0	*1.79*	10.4	11.7	*1.52*
		20	8.9	9.2		9.4	10.6		9.2	10.0	
		40	10.2	10.7		7.9	8.7		9.0	9.4	
		80	9.2	11.1		6.8	7.2		7.6	8.6	

* and ** N x population interaction significant at 5% and 1% levels of significance respectively.

than the high plant population (Table 32). This interaction was mainly in the pith of stems. In most trials there were no N x population interactions, especially for whole plants.

Generally, the effect of N on SMCO was not affected by whether S was applied or not (Tables 27 and 28). In trials on soils low in available sulphate, N tended to increase SMCO even when no S was applied. This result is contrary to that reported by McDonald *et al.* (1981), who found that when S was limiting, N tended to reduce SMCO concentrations, especially in kale stems. In the present study, the effect of N increasing SMCO when S was limiting, occurred in several trials at different harvest dates. It may be concluded that it is not beneficial to use N in the absence of S in order to reduce kale SMCO content on the reasonably free draining soils studied here.

N/SMCO production:

N fertiliser increased total SMCO production (kg/ha) in all field trials except F4, where neither DM yield nor SMCO content was affected (Table 33). The increased SMCO production occurred in both leaves and stems.

There were no significant interactions between N and plant population on whole plant SMCO production in any of the trials (Table 34). At harvests in two of the trials (F2 and F6) N increased SMCO production in stems more at high than low plant populations, mainly through the effect of N on DM yields.

N/nitrogen content:

In all trials, except F4, nitrogen levels in leaves and stems tended to be increased with additional N fertiliser, although increases were not significant ($P < 0.05$) in F2 and at some of the harvests in the

TABLE 33: Effect of nitrogen on SMCO production in field trials (kg/ha).

Trial (No. plots)	Harvest date	Leaves N applied			*SE*	Stems N applied			*SE*	Whole plants N applied			*SE*
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F1 a (24)	Oct	26	25		*8.8*	19	22		*8.6*	45	47		*15.7*
	Dec	27	35		*7.7**	30	44		*7.0***	57	80		*9.7***
	Feb	34	43		*9.2**	29	41		*11.3**	63	84		*18.8**
	Mean	29	34		*5.1**	26	36		*5.5***	55	70		*10.1***
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F1 b (24)	Oct	23	46		*12.0***	13	37		*13.4***	36	83		*22.6***
	Dec	28	52		*12.6***	23	47		*17.7**	51	99		*28.4***
	Feb	27	55		*11.4***	17	52		*11.4***	45	107		*19.6***
	Mean	26	51		*10.7***	18	46		*10.8***	44	97		*20.3***
		N_{75}	N_{250}			N_{75}	N_{250}			N_{75}	N_{250}		
F1 c (24)	Aug	11	33		*4.2***	6	24		*3.3***	17	57		*7.0***
	Dec	14	44		*13.8***	14	39		*6.7***	28	82		*14.8***
	Mean	12	38		*7.7***	10	32		*2.6***	22	70		*7.6***
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F2 (12)	Dec	27	32		*2.6*	30	47		*1.6**	57	79		*4.0**
		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}	
F3 (36)	Oct									59	79	89	*12.6***
	Dec									112	145	164	*19.8***
	Feb	50	53	59	*10.9*	58	66	71	*19.8*	109	119	130	*24.2*
	Mean									93	114	128	*14.0***
										N_{30}	N_{60}	N_{150}	
F4 (36)	Dec									131	140	136	*29.6*
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F6 (48)	Aug	17	28		*8.6***	16	27		*5.1***	33	55		*10.6***
	Dec	34	43		*11.6***	47	62		*15.4***	80	105		*22.4***
	Mean	25	36		*7.6***	31	44		*8.5***	57	80		*12.9***

* and ** Main effect of N significant at 5% and 1% levels of significance respectively.

TABLE 35: Effect of nitrogen on kale nitrogen content in field trials (% DM).

Trial (No. plots)	Harvest date	Leaves N applied			*SE*	Stems N applied			*SE*	Whole plants N applied			*SE*
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F1 a (24)	Oct	2.38	2.82		*0.388**	1.68	1.92		*0.376*	2.08	2.40		*0.357*
	Dec	2.61	3.07		*0.272***	1.56	2.15		*0.311***	2.07	2.54		*0.269***
	Feb	3.05	3.54		*0.289***	1.61	2.07		*0.346**	2.26	2.67		*0.261***
	Mean	2.68	3.14		*0.177***	1.62	2.05		*0.232***	2.14	2.54		*0.192***
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F1 b (24)	Oct	1.77	2.16		*0.498*	1.29	1.40		*0.120*	1.62	1.81		*0.377*
	Dec	2.01	2.58		*0.249***	1.38	1.44		*0.238*	1.74	1.98		*0.178***
	Feb	2.45	2.62		*0.392*	1.62	1.64		*0.184*	2.10	2.08		*0.229*
	Mean	2.08	2.45		*0.226***	1.43	1.49		*0.152*	1.82	1.96		*0.178*
		N_{75}	N_{250}			N_{75}	N_{250}			N_{75}	N_{250}		
F1 c (24)	Aug	1.20	2.21		*0.299***	1.18	2.02		*0.137***	1.19	2.15		*0.227***
	Dec	1.42	1.82		*0.221***	1.02	1.20		*0.143***	1.23	1.50		*0.140***
	Mean	1.31	2.01		*0.191***	1.10	1.61		*0.080***	1.21	1.82		*0.117***
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F2 (12)	Dec	2.06	2.27		*0.421*	1.98	2.02		*0.624*	1.99	2.11		*0.146*
		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}	
F3 (36)	Oct									2.78	2.98	3.03	*0.166***
	Dec									2.87	3.01	2.84	*0.233*
	Feb	3.92	3.95	4.03	*0.323*	2.11	2.12	2.35	*0.215**	2.79	2.79	2.93	*0.228*
	Mean									2.81	2.93	2.93	*0.154*
										N_{30}	N_{60}	N_{150}	
F4 (36)	Dec									2.96	2.93	2.90	*0.179*
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F6 (48)	Aug	2.21	2.66		*0.232***	2.13	2.55		*0.274***	2.19	2.61		*0.204***
	Dec	2.24	2.36		*0.355*	1.48	1.83		*0.345***	1.77	2.04		*0.297***
	Mean	2.23	2.51		*0.206***	1.80	2.19		*0.216***	1.98	2.32		*0.179***

other trials (Table 35). In comparison to S fertiliser, the application of N appears usually to have a beneficial effect on nitrogen and crude protein levels in kale.

N/dry matter content:

The effects of N on the dry matter content of leaves, stems and whole plants in each trial are shown for all harvest dates in Table 36. In all trials, application of N tended to result in lower plant dry matter contents, although differences were not significant in F4. Generally, the dry matter content of both leaves and stems was affected. Although the changes in dry matter content with N were often significant, the average reduction was fairly small and would probably have had no effect on animal performance if the crops had been grazed. The dry matter content in kale is usually higher than that in most other brassicas and there have been no reports that animal performance on kale has been affected by changing the dry matter content of the crop.

3.2.3 Effects of plant polpulation (pop)

Pop/plant numbers:

Plant numbers counted in the different plant population treatments at each harvest are outlined for trials F1a, F1b, F1c, F2 and F6 in Table 37. Plant numbers at harvests are not available for F5. At higher populations, plant numbers were often less at later harvests than earlier harvests, probably due to mortality as a result of increased competition as plants matured. Generally plant numbers, averaged over all harvests, were similar to the theoretical plant populations aimed at after sowing and singling.

TABLE 36: Effect of nitrogen on dry matter content in field trials (% DM).

Trial (No. plots)	Harvest date	Leaves N applied			*SE*	Stems N applied			*SE*	Whole plants N applied			*SE*
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F1 a (24)	Oct	12.8	11.5		*1.01**	14.6	12.8		*2.42*	13.6	12.1		*1.60*
	Dec	13.1	12.7		*0.72*	15.4	14.0		*1.44**	14.3	13.5		*1.07*
	Feb	12.2	11.6		*0.40***	14.6	13.1		*0.95***	13.5	12.5		*0.64***
	Mean	12.7	11.9		*0.54***	14.9	13.3		*1.47**	13.8	12.7		*0.93**
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F1 b (24)	Oct	14.2	13.0		*0.77***	17.1	14.5		*1.73***	15.0	13.7		*1.03**
	Dec	14.4	13.5		*0.53***	18.4	15.6		*2.07***	16.2	14.6		*1.21***
	Feb	13.9	13.7		*0.78*	16.0	14.6		*1.59*	14.8	14.2		*0.87***
	Mean	14.2	13.4		*0.42***	17.2	14.9		*1.35***	15.4	14.1		*0.76***
		N_{75}	N_{250}			N_{75}	N_{250}			N_{75}	N_{250}		
F1 c (24)	Aug	16.0	11.9		*1.04***	15.1	10.8		*1.25***	15.8	11.6		*1.03***
	Oct	17.8	14.2		*1.35***	20.5	16.2		*2.16***	18.9	15.2		*1.38***
	Dec	16.0	15.0		*0.78***	19.9	16.8		*1.04***	17.9	15.9		*0.82***
	Mean	16.0	13.4		*0.69***	17.7	14.1		*0.76***	17.0	13.9		*0.62***
		N_{75}	N_{150}			N_{75}	N_{150}			N_{75}	N_{150}		
F2 (12)	Dec	12.5	11.8		*0.69*	15.0	14.0		*0.42***	13.6	13.0		*0.48**
		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}		N_{30}	N_{60}	N_{150}	
F3 (36)	Oct									11.4	10.9	9.9	*0.80***
	Dec									13.9	13.9	13.3	*0.95*
	Feb	11.6	11.6	12.4	*1.65*	12.3	12.4	11.3	*1.18*	12.0	12.0	11.7	*1.15*
	Mean									12.4	12.2	11.6	*0.58***
										N_{30}	N_{60}	N_{150}	
F4 (36)	Dec									13.1	13.1	12.8	*0.45*
		N_{50}	N_{150}			N_{50}	N_{150}			N_{50}	N_{150}		
F6 (48)	Aug	11.5	10.5		*0.98***	10.4	9.3		*1.44**	11.3	10.2		*1.04***
	Oct	14.2	13.3		*1.11**	16.4	14.4		*1.40***	15.3	13.9		*1.07***
	Dec	14.6	15.0		*0.98*	14.9	14.6		*1.61*	14.8	14.8		*1.21*
	Mean	13.4	13.0		*0.68**	13.9	12.8		*0.94***	13.8	13.0		*0.76***

TABLE 37: Plant densities at harvests in trials F1a, 1b, 1c, 2 and 6 (plants/m²).

Trial (No. plots)	Harvest date	Plant population (plants/m²) Theoretical population				*SE*
		P_{40}	P_{80}			
F1 a (24)	Oct	44	90			*4.9*
	Dec	44	84			*12.4*
	Feb	43	74			*8.4*
	Mean	44	83			*7.0*
		P_{13}	P_{52}			
F1 b (24)	Oct	14	45			*5.0*
	Dec	14	44			*4.6*
	Feb	14	48			*2.0*
	Mean	14	45			*3.6*
		P_{13}	P_{52}			
F1 c (24)	Aug	18	50			*2.4*
	Oct	18	46			*1.8*
	Dec	18	47			*1.3*
	Mean	18	48			*1.2*
		P_{4}	P_{12}	P_{40}		
F2 (12)	Dec	4	12	40		*5.4*
		P_{10}	P_{20}	P_{40}	P_{80}	
F6 (48)	Aug	11	22	44	86	*6.1*
	Oct	11	21	39	71	*4.2*
	Mean	11	21	41	79	*4.8*

Pop/dry matter yield:

The effects of plant population on DM yield in trials F1a, F1b, F1c, F2, F5 and F6 are shown in Table 38. Total DM yields tended to be increased by higher plant densities at some harvests in all trials. In the two 1981 trials, F1c and F6, which were sown in April and May respectively, total DM yields were significantly increased by higher populations at harvests in August but not at later harvests in October and December. Trial F5 in 1980 had a similar range of populations to F6, but at later harvests plant population tended to have a greater effect on DM yield in the earlier trial (F5). This was probably because F5 was sown later in June and July and therefore was more affected by varying plant populations than the more mature kale in F6.

In all trials, stem DM yield was increased by higher plant populations. The effect of population on leaf DM yield was more variable. In two trials increased population significantly increased leaf yields at harvests in August (F1c) and September (F5), whereas at later harvests in the same trials and especially at some harvests in two other trials, F1a and F6, higher populations tended to reduce leaf DM yields. In all trials, the percentage of total DM yield as leaf was reduced with increased plant population. The photograph overleaf shows typical Maris Kestrel plants, grown at different plant densities between 10 and 80 plants/m^2. The plants were from plots of kale yielding between 5 and 8 t DM/ha in trial F6.

These results of higher plant populations increasing DM yield in kale, confirm previous reports by Hunter-Smith and Rhys Williams (1927), Holliday (1960) and Tamás (1976).

The effect of higher populations increasing stem DM yields was greater with high as opposed to low N treatments (Table 30). This

TABLE 38: Effect of plant population on DM yield in field trials (t/ha).

Trial (No. plots)	Harvest date (Sowing date)	Leaves				SE	Stems				SE	Whole plants				SE
		P_{40}	P_{80}				P_{40}	P_{80}				P_{40}	P_{80}			
F1 a (24)	Oct	3.28	2.90			*0.174***	2.29	2.68			*0.229**	5.57	5.59			*0.336*
	Dec	2.45	2.26			*0.165*	2.50	3.18			*0.371**	4.95	5.44			*0.474*
	Feb	2.06	2.30			*0.285*	2.56	3.30			*0.355**	4.63	5.60			*0.589**
	Mean	2.60	2.49			*0.163*	2.45	3.06			*0.228***	5.05	5.54			*0.362*
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 b (24)	Oct	3.58	3.56			*0.247*	1.72	2.84			*0.229***	5.29	6.41			*0.346***
	Dec	2.97	2.53			*0.346*	2.15	3.14			*0.265***	5.12	5.68			*0.456*
	Feb	2.38	2.47			*0.374*	2.12	2.97			*0.379***	4.50	5.44			*0.601**
	Mean	2.98	2.85			*0.224*	1.99	2.98			*0.157***	4.97	5.84			*0.314***
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 c (24)	Aug	3.17	4.16			*0.621**	0.98	1.85			*0.285***	4.15	6.02			*0.869***
	Oct	4.34	4.20			*0.426*	3.11	3.56			*0.504*	7.45	7.76			*0.809*
	Dec	4.18	3.61			*0.419*	3.61	4.33			*0.896*	7.78	7.94			*1.224*
	Mean	3.90	3.99			*0.319*	2.56	3.25			*0.343**	6.46	7.24			*0.603*
		P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}		
F2 (12)	Dec	3.93	2.94	2.82		*0.394*	2.39	3.55	4.07		*0.170**	6.32	6.49	6.89		*0.256*
		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}	
F5 (24)	Sep (Jun)	2.05	2.42	2.95	3.61		0.30	0.53	0.71	1.28		2.34	2.95	3.67	4.90	
	(Jul)	0.13	0.22	0.36	0.69		0.01	0.02	0.03	0.05		0.14	0.24	0.39	0.74	
	(Mean)	1.09	1.32	1.66	2.15		0.16	0.27	0.37	0.67		1.24	1.60	2.03	2.82	
	SE					*0.260***					*0.110***					*0.348***
	Oct (Jun)	4.30	4.67	5.13	4.54		2.15	3.22	3.36	4.62		6.44	7.89	8.50	9.16	
	(Jul)	2.76	2.80	3.16	3.31		0.55	0.82	1.07	1.44		3.31	3.62	4.22	4.75	
	(Mean)	3.53	3.73	4.14	3.92		1.35	2.02	2.22	3.03		4.88	5.75	6.36	6.95	
	SE					*0.512*					*0.353***					*0.615***
	Nov (Jun)	4.51	3.68	3.60	3.86		3.42	4.15	3.70	5.66		7.93	7.83	7.29	9.52	
	(Jul)	3.25	3.27	3.41	3.38		1.15	1.13	1.39	2.14		4.40	4.40	4.80	5.52	
	(Mean)	3.88	3.48	3.50	3.62		2.28	2.64	2.54	3.90		6.16	6.12	6.05	7.52	
	SE					*0.497*					*0.295***					*0.704***
	Mean (Jun)	3.62	3.59	3.89	4.00		1.95	2.63	2.59	3.85		5.57	6.22	6.49	7.86	
	(Jul)	2.05	2.10	2.31	2.46		0.57	0.65	0.83	1.21		2.62	2.75	3.14	3.67	
	(Mean)	2.83	2.84	3.10	3.23		1.26	1.64	1.71	2.53		4.09	4.49	4.81	5.76	
	SE					*0.281*					*0.167***					*0.339***
		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}	
F6 (48)	Aug	4.83	5.03	4.40	4.53		1.12	1.62	1.73	2.25		5.95	6.65	6.14	6.78	
	SE					*0.485**					*0.295***					*0.710**
	Oct	6.02	5.54	4.51	4.48		3.91	4.75	4.45	5.32		9.92	10.29	8.96	9.80	
	SE					*0.921***					*0.823***					*1.444*
	Mean	5.42	5.28	4.46	4.51		2.51	3.19	3.09	3.78		7.94	8.47	7.55	8.29	
	SE					*0.550***					*0.501***					*0.912*

* and **, Main effect of plant population significant at 5% and 1% levels of significance respectively.

same trend generally occurred with total DM yields, although the interaction was usually not significant.

In F1c at the December harvest, kale stems consisted on average of 42.5% pith and 57.5% cortex. Increasing the plant population from 18 to 47 plants/m^2 only slightly increased ($P < 0.10$) the percentage of stem yield as cortex from 56.6 to 58.4%.

Pop/SMCO content:

The effects of plant population on SMCO content in each tissue type are shown in Table 39. Increasing the plant population reduced SMCO levels in stems and whole plants, but generally had no effect on leaf SMCO. The effects of population on whole plant SMCO were greater at later harvests, as at harvests prior to November population had no significant ($P < 0.05$) effect in any of the trials, although at most harvests the same trend occurred. At later harvests, between November

TABLE 39: Effect of plant population on SMCO content in field trials (g/kg DM).

Trial (No. plots)	Harvest date (Sowing date)	Leaves				*SE*	Stems				*SE*	Whole plants				*SE*
		P_{40}	P_{80}				P_{40}	P_{80}				P_{40}	P_{80}			
F1 a (24)	Oct	8.7	7.5			*1.64*	9.1	7.5			*1.61*	8.9	7.5			*1.42*
	Dec	13.4	13.1			*1.05*	14.1	12.2			*1.99*	13.8	12.5			*1.15*
	Feb	17.1	17.9			*2.04*	13.7	10.5			*2.19**	15.1	13.4			*0.90**
	Mean	13.1	12.8			*1.00*	12.3	10.1			*1.32**	12.6	11.2			*0.82**
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 b (24)	Oct	9.0	9.8			*1.54*	13.1	8.6			*1.30***	10.3	9.1			*1.14*
	Dec	13.6	14.5			*1.25*	14.7	11.7			*3.68*	14.0	12.9			*1.54*
	Feb	16.4	17.0			*1.62*	16.8	11.1			*1.90***	16.5	13.8			*1.24***
	Mean	13.0	13.8			*1.24*	14.8	10.4			*1.71***	13.6	11.9			*1.15**
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 c (24)	Aug	5.5	5.4			*0.61*	11.0	9.2			*1.20**	6.8	6.6			*0.72*
	Dec - *Pith*						9.0	5.7			*1.79**					
	Cortex						6.4	4.9			*0.26***					
		6.7	7.2			*1.70*	7.5	5.2			*0.75***	7.1	6.1			*0.80*
	Mean	6.1	6.3			*0.89*	9.2	7.2			*0.82***	7.0	6.4			*0.59*
		P_4	P_{12}	P_{40}			P_4	P_{12}	P_{40}			P_4	P_{12}	P_{40}		
F2 (12)	Dec	9.4	10.0	7.8		*1.73*	15.4 a	13.0 a	8.0 b		*0.82**	11.7	11.5	8.0		*0.90*
		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}	
F5 (24)	Sep (Jun)											12.2	10.0	12.9	11.5	
	(Jul)											7.7	10.3	9.9	8.0	
	(Mean)											10.0	10.1	11.4	9.8	
	SE															*1.41*
	Oct (Jun)											10.6	8.6	9.4	10.2	
	(Jul)											10.9	12.7	9.4	10.1	
	(Mean)											10.7	10.6	9.4	10.1	
	SE															*1.55*
	Nov (Jun)	15.1	16.5	14.0	16.9		19.0	14.9	16.1	12.3		16.7 a	15.6 abc	15.0 abc	14.2 c	
	(Jul)	12.5	14.2	12.9	15.2		20.5	22.8	18.9	18.4		14.4 b	16.4 ab	14.6 abc	16.4 ab	
	(Mean)	13.8	15.4	13.4	16.0		19.8	18.9	17.5	15.4		15.6	16.0	14.8	15.3	
	SE					*1.41**					*2.74*					*1.23+*
	Mean (Jun)											13.2	11.4	12.5	12.0	
	(Jul)											11.0	13.1	11.3	11.5	
	(Mean)											12.1	12.3	11.9	11.7	
	SE															*0.99+*
		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}	
F6 (48)	Aug	4.5	4.9	4.6	5.2		15.6 a	14.2 a	11.9 b	9.8 c		6.6	7.1	6.6	6.8	
	SE					*1.48*					*2.25***					*1.14*
	Dec	9.3	9.0	10.4	10.2		12.7 a	10.0 b	8.3 c	7.0 c		11.0 a	9.6 b	9.2 bc	8.1 c	
	SE					*1.98*					*1.79***					*1.52***
	Mean	6.9	7.0	7.5	7.7		14.2 a	12.1 b	10.1 c	8.4 d		8.8 a	8.4 ab	7.9 bc	7.4 c	
	SE					*1.26*					*1.42***					*0.857***

* and ** Main effect of plant population significant at 5% and 1% levels of significance respectively.

\+ Plant population x sowing date interaction significant at 5% level of significance.

and February, whole plant SMCO was significantly ($P < 0.05$) reduced by increased population in the three trials, F1a, F1b and F6. In two other trials (F1c and F2) the effects were significant at the 10% level of significance and in a sixth trial, F5, increased population significantly reduced SMCO in June sown kale but not in July sown kale, which was lower yielding. The effects of plant population on SMCO levels are greater therefore on mature kale, either as a result of later harvests or earlier sowings.

Stem SMCO levels were reduced by higher populations at some early harvests as well as at later harvests. In F1c, stem SMCO (g/kg DM) was higher in pith (7.4) than cortex tissue (5.6) and both were significantly reduced by increasing plant population (Table 39). The effect of population on stem SMCO was not due to stems at higher populations having a much greater proportion of low SMCO cortex, as the proportion of the two stem tissues changed only slightly and SMCO in both components was affected by population. Also the effect of population on whole plants was not the result of an increase in the proportion of stems in plants, since stems often had higher SMCO levels than leaves.

The percentage changes in whole plant and stem SMCO contents between different plant populations were averaged over all trials and the data are shown for different harvest dates in Table 40. Data for the late July sown kale in F5 were not included as the crop was sown approximately three weeks later than any other trial and was thus very low yielding and unlikely to be grazed at any of the times harvested. If data from the late sowing had been included, this would have reduced the accuracy of the information from the earlier sown crops.

TABLE 40: Percentage reductions in SMCO content with higher plant populations in field trials.

Harvests	Increases in theoretical plant populations (plants/m^2)				
	4 to 12	10/13 to 20/26	10/13 to 40/52	20/26 to 40	40 to 80
Whole plants:					
Aug - Oct		5 (2)	4 (4)	-6 (2)	5 (3)
Nov - Dec	2 (1)*	10 (2)	16 (5)	4 (2)	9 (3)
Feb			16 (1)		11 (1)
Mean +	2 (1)	9 (2)	13 (5)	-2 (2)	7 (3)
Stems:					
Aug - Oct		9 (1)	25 (3)	16 (1)	18 (2)
Nov - Dec	16 (1)	21 (2)	28 (5)	4 (2)	18 (3)
Feb			34 (1)		23 (1)
Mean +	16 (1)	18 (2)	27 (5)	4 (2)	19 (3)

( )* Number of trials used to calculate percentage reductions.

The late July sown plots in trial F5 were not included in calculations.

+ Based on plot SMCO values meaned over all harvests.

Table 40 shows the greater effect of plant population on stems than whole plants and also its greater effect on whole plant SMCO at later harvests. SMCO was reduced by increasing plant population from approximately 10 to 80 plants/m^2, although there was little effect between 20 and 40 plants/m^2 in the two trials where this comparison was made. The results show that if kale is to be grazed after October it will be increasingly less toxic to animals if it is sown to give a population at grazing of up to 80 plants/m^2.

The recommended sowing rate for kale in Scotland is 4 to 5 kg/ha when sown with a precision drill, modified grain drill or root drill (Scottish Agricultural Colleges, 1981). Under very dry conditions, or when broad-cast or direct drilled, a rate of up to 6.5 kg/ha is advised. A survey in South-east Scotland on 14 kale crops, which were mainly direct drilled at seeding rates similar to those recommended, showed that plant densities at harvests ranged from 11 to 57 plants/m^2, with a mean of 39 plants/m^2 (Lambert, 1981).

Kales in the breeding programme at the Scottish Crop Research Institute are sown in 50 cm rows with usually a 7.5 cm spacing between plants. This would give a density at harvests of less than 25 plants/m^2. Holliday (1960) reported that the optimum density for maximum DM production was about 15 plants/m^2 for marrow-stem kale varieties. ADAS (1976) in one trial, reported that a plant spacing of 18 x 18 cm (= 31 plants/m^2) gave optimum kale DM yields. A closer spacing of 5 cm between seeds at sowing was recommended by Toosey (1972) with precision drilled marrow-stem kale in 3.5 cm rows (1.7 kg/ha, 57 plants/m^2).

Plant densities at harvests in many breeding and scientific trials and on many farms are therefore fairly low, often being less than 40

plants/m². There is therefore potential for reducing SMCO levels, by increasing seeding rates at sowing.

There were no significant interactions in any of the trials, between population and S or population and N, on whole plant SMCO content. In stems in F1c, the population x N interaction was significant ($P < 0.01$). SMCO, especially within pith tissue, was highest in the high N, low population treatment (Table 32). In F6, at the August harvest, the population x S interaction was significant ($P < 0.05$) in stems, as mentioned previously. In this trial, increased population reduced stem SMCO more when S was limiting (see Section 3.2.1).

The effect of population on whole plant SMCO generally occurred when low or high levels of S and N were applied, both on free draining soils, such as in F1 and F6, and on a poorly drained soil as in F2. Unlike the effects of S fertiliser, the effects of population on SMCO in kale appear therefore to be independent of soil fertility and drainage characteristics. These population trials, however, were on soils which were reasonably low in available sulphate. Even when S fertiliser was applied, sulphate in the soils at harvests was still generally low with the exception of August 1981 (F1c and F6), when soil sulphate was higher. In one of these trials (F6) on the higher sulphate soil, the reduction in SMCO with increased population was slightly less.

The effect of higher plant densities reducing SMCO content, is the opposite to the effect of population on kale glucosinolate levels reported by Josefsson *et al.* (1972). They found that thinner stemmed plants (20 mm thick) contained higher levels of glucosinolates than thicker stemmed plants (35 mm). Unfortunately, glucosinolate levels were not measured in any of the trials here. Whenever S fertiliser has affected these two non-protein sulphur compounds, it has been to

increase the levels of each. In the present study, N application clearly increased plant SMCO levels, however, with glucosinolates there have been reports of N both increasing and reducing levels in kale (Josefsson, 1970). The variable effects of N on glucosinolates have not been explained, just as the effects of higher populations increasing glucosinolate levels have not been elucidated.

Pop/SMCO production:

The effects of plant population on SMCO produced (kg/ha) in each trial, are shown in Table 41. In stems and whole plants, population had less effect on SMCO production than on its two constituents DM yield and SMCO content, which were increased and decreased respectively by higher populations.

SMCO produced in whole plants was not affected by plant density in three trials (F1a, F1b and F2), while in F1c and F5, it was increased by higher populations, although only at early harvests in August and September respectively. In F6, SMCO production at a December harvest was significantly ($P < 0.01$) reduced by increasing the plant population. The effects of population on whole plant SMCO production were mainly the result of effects in stems, as levels in leaves were generally not affected by population.

There were no significant interactions between population and S or population and N with whole plant SMCO production.

As a result of population generally having little effect on SMCO production in comparison to its effect on DM yield and SMCO levels, it could be considered that the higher DM yields with higher populations may have caused a dilution of the minerals such as S or N, hence reducing SMCO levels. The reduction in SMCO levels with higher populations, however, occurred when high levels of S and N were

TABLE 41: Effect of plant population on SMCO production in field trials (kg/ha).

Trial (No. plots)	Harvest date (Sowing date)	Leaves				*SE*	Stems				*SE*	Whole plants				*SE*
		P_{40}	P_{80}				P_{40}	P_{80}				P_{40}	P_{80}			
F1 a (24)	Oct	29	22			*5.1**	21	20			*4.6*	50	42			*9.0*
	Dec	33	29			*2.9*	35	39			*4.0*	68	68			*4.7*
	Feb	36	41			*5.4*	35	35			*7.5*	71	76			*9.3*
	Mean	33	31			*3.3*	31	31			*2.8*	63	62			*4.8*
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 b (24)	Oct	33	37			*4.7*	24	26			*3.0*	57	63			*4.4*
	Dec	41	38			*4.9*	33	38			*6.7*	74	76			*8.6*
	Feb	40	43			*7.4*	36	33			*9.3*	76	76			*14.0*
	Mean	38	39			*4.7*	31	32			*4.6*	69	72			*8.2*
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 c (24)	Aug	19	24			*4.3*	12	18			*3.0**	31	42			*7.1**
	Dec - *Pith*						15	11			*5.5*					
	Cortex						14	13			*2.4*					
		30	28			*7.6*	29	24			*7.8*	59	51			*5.8*
	Mean	25	26			*3.9*	20	21			*4.9*	45	47			*5.6*
		P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}		
F2 (12)	Dec	37	28	22		*2.8*	37	45	34		*5.2*	74	74	56		*9.2*
		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}	
F5 (24)	Sep (Jun)											28	30	48	56	
	(Jul)											1	2	4	6	
	(Mean)											15	16	26	31	
	SE															*6.5***
	Oct (Jun)											67	66	81	92	
	(Jul)											35	45	39	48	
	(Mean)											51	56	60	70	
	SE															*15.0*
	Nov (Jun)	68	60	50	66		65	62	60	70		133	123	110	136	
	(Jul)	41	47	44	51		23	26	26	40		64	72	70	91	
	(Mean)	55	54	47	59		44	44	43	55		98	98	90	114	
	SE					*10.7*					*10.3*					*17.5*
	Mean (Jun)											76	73	80	95	
	(Jul)											33	40	38	48	
	(Mean)											55	56	58	72	
	SE															*8.3**
		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}	
F6 (48)	Aug	22	25	21	24		17	23	22	23		39	48	42	46	
	SE					*8.6*					*5.1**					*10.6*
	Dec	42	39	38	36		69	60	44	44		111	99	82	80	
	SE					*11.6*					*15.4***					*22.4***
	Mean	32	32	29	30		43	42	33	33		75	73	62	63	
	SE					*7.6*					*8.5***					*12.9***

* and ** Main effects of plant population significant at 5% and 1% levels of significance respectively.

applied, although S fertiliser did not necessarily increase the availability of S for many months after sowing. In F6, moreover, the effect of population in reducing SMCO content was greater than any changes in DM yield on the same plots, hence total SMCO production was significantly ($P < 0.01$) reduced. The effect of higher population reducing SMCO content was probably therefore not caused by available N being diluted by increased DM yield. Competition for available S, however, may have had some importance, especially as in the only trial where SMCO was significantly increased by S (F6 - August harvest), there was a significant population x S interaction for stems. Although even in this interaction, SMCO was reduced by increased population when high levels of S were available, therefore other factors are also associated with the population effect on SMCO.

Pop/nitrogen:

Increasing the plant population tended to result in reduced N levels in stems and whole plants as shown in Table 42. In leaves, higher populations increased N in two of the trials, although effects were small compared with the N reductions in stems. These results confirm previous findings by Willey (1964) and Frame and Robinson (1966 a,b) who also found that crude protein was reduced by a higher plant population.

The reduction in N and, therefore, crude protein content in whole plants between the lowest and highest population treatments was small, averaging only 5% in the six trials. This reduction would only have a minor effect on the quality of the kale crop and could surely be tolerated when compared with the beneficial effect of lower SMCO levels. The crude protein level and *in vitro* digestibility (75-90%) of

TABLE 42: Effect of plant population on kale nitrogen content in field trials (% DM).

Trial (No. plots)	Harvest date (Sowing date)	Leaves				SE	Stems				SE	Whole plants				SE
		P_{40}	P_{80}				P_{40}	P_{80}				P_{40}	P_{80}			
F1 a (24)	Oct	2.58	2.62			*0.227*	1.90	1.70			*0.237*	2.30	2.18			*0.202*
	Dec	2.91	2.78			*0.257*	1.95	1.76			*0.264*	2.43	2.18			*0.104***
	Feb	3.23	3.36			*0.182*	1.86	1.82			*0.196*	2.48	2.45			*0.118*
	Mean	2.91	2.92			*0.085*	1.90	1.76			*0.089**	2.40	2.27			*0.046***
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 b (24)	Oct	1.90	2.03			*0.183*	1.48	1.21			*0.066***	1.76	1.67			*0.119*
	Dec	2.26	2.33			*0.189*	1.46	1.36			*0.096*	1.93	1.79			*0.133*
	Feb	2.49	2.58			*0.222*	1.68	1.58			*0.189*	2.12	2.05			*0.178*
	Mean	2.22	2.31			*0.101*	1.54	1.38			*0.080**	1.94	1.84			*0.086*
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 c (24)	Aug	1.70	1.71			*0.182*	1.73	1.47			*0.119**	1.70	1.64			*0.130*
	Dec - *Pith*						1.00	0.88			*0.088*					
	Cortex						1.30	1.17			*0.114*					
		1.58	1.66			*0.174*	1.17	1.05			*0.086*	1.39	1.34			*0.107*
	Mean	1.64	1.69			*0.166*	1.45	1.26			*0.065***	1.54	1.49			*0.103*
		P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}		
F2 (12)	Dec	2.03	2.09	2.36		*0.185*	2.21	1.96	1.83		*0.380*	2.10	2.04	2.03		*0.322*
		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}	
F5 (24)	Sep (Jun)											3.86	3.31	3.39	3.29	
	(Jul)											4.58	4.42	4.38	4.50	
	(Mean)											4.22	3.86	3.88	3.90	
	SE															*0.185**
	Oct (Jun)											3.32	3.26	3.10	2.93	
	(Jul)											3.67	3.62	3.51	3.35	
	(Mean)											3.49	3.44	3.30	3.14	
	SE															*0.167**
	Nov (Jun)	3.12	3.40	3.10	3.49		2.35	1.97	2.08	1.98		2.79	2.64	2.58	2.59	
	(Jul)	3.02	3.22	3.29	3.56		2.45	2.33	2.28	2.42		2.86	2.99	3.00	3.12	
	(Mean)	3.07	3.31	3.20	3.52		2.40	2.15	2.18	2.20		2.83	2.81	2.79	2.85	
	SE					*0.214**					*0.218*					*0.180*
	Mean (Jun)											3.32	3.07	3.02	2.94	
	(Jul)											3.70	3.68	3.63	3.66	
	(Mean)											3.51	3.37	3.33	3.30	
	SE															*0.100**
		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}	
F6 (48)	Aug	2.47	2.44	2.32	2.50		2.56	2.38	2.25	2.16		2.49	2.42	2.30	2.38	
	SE					*0.232*					*0.274***					*0.204*
	Dec	2.31	2.10	2.27	2.51		1.94	1.58	1.61	1.50		2.10	1.80	1.86	1.85	
	SE					*0.355*					*0.345**					*0.297*
	Mean	2.39	2.27	2.30	2.51		2.25	1.98	1.93	1.83		2.30	2.11	2.08	2.12	
	SE					*0.206**					*0.216***					*0.179**

* and ** Main effects of plant population significant at 5% and 1% levels of significance respectively.

kale are normally adequate for good animal performance and compare favourably with levels in pasture.

In order to understand more about the reasons for higher plant populations reducing stem SMCO levels, the N components, protein and nitrate, were measured together with SMCO and the effects of population on each examined.

Pop/SMCO N (% total N):

The effects of higher populations on SMCO N (% total N) in the six trials are shown in Table 43. The proportion of total N as SMCO in stems was significantly reduced in four of the trials. The trend also occurred in F2, where the small number of plots made it difficult to obtain a significant difference and in F5 with the June sown kale. With whole plants, trends were similar to those in stems, although differences were only significant in two trials.

Averaged over all six trials, the mean percentages of total N as SMCO at the lowest and highest populations were 6.9 and 5.5% respectively for stems and 4.6 and 4.2% respectively for whole plants. With stems, therefore, approximately one-fifth less N formed SMCO when the plant population was increased and the reduction was greater at later than earlier harvests.

Table 44 shows the effects of population at low and high N levels on SMCO N (% total N), protein N (% total N) and nitrate N (% total N) in kale stems in F1b and F1c. In both trials, SMCO N (% total N) was reduced ($P < 0.05$) by increasing plant population, although only at the December harvest in F1c and the reduction was usually greater in the presence of high levels of N.

In the low N treatments, the reduction in the proportion of total N forming SMCO with increased plant population was associated with

TABLE 43: Effect of plant population on SMCO N (% total N) in field trials.

Trial (No. plots)	Harvest date (Sowing date)	Leaves				*SE*	Stems				*SE*	Whole plants				*SE*
		P_{40}	P_{80}				P_{40}	P_{80}				P_{40}	P_{80}			
F1 a (24)	Oct	3.2	2.7			*0.49*	4.4	4.2			*0.70*	3.6	3.2			*0.47*
	Dec	4.3	4.4			*0.44*	6.8	6.6			*0.70*	5.3	5.4			*0.51*
	Feb	4.9	5.0			*0.54*	6.9	5.5			*0.94**	5.7	5.1			*0.41*
	Mean	4.2	4.1			*0.24*	6.1	5.4			*0.44**	4.9	4.6			*0.28*
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 b (24)	Oct	4.5	4.5			*0.56*	8.2	6.6			*0.93**	5.5	5.1			*0.37*
	Dec	5.6	5.7			*0.37*	9.3	8.1			*2.41*	6.8	6.7			*1.01*
	Feb	6.1	6.1			*0.34*	9.3	6.5			*1.22***	7.3	6.2			*0.60**
	Mean	5.4	5.4			*0.34*	8.9	7.0			*1.22**	6.5	6.0			*0.46*
		P_{13}	P_{52}				P_{13}	P_{52}				P_{13}	P_{52}			
F1 c (24)	Aug	3.0	2.9			*0.48*	5.9	5.9			*0.29*	3.7	3.7			*0.42*
	Dec - *Pith*						8.2	6.1			*1.36**					
	Cortex						4.6	3.9			*0.40**					
		4.0	4.1			*0.14*	5.9	4.6			*0.64**	4.7	4.2			*0.47*
	Mean	3.5	3.5			*0.66*	5.9	5.3			*0.42**	4.2	4.0			*0.37*
		P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}			P_{4}	P_{12}	P_{40}		
F2 (12)	Dec	4.3	4.8	3.0		*0.38*	6.5	6.5	4.4		*1.06*	5.2	5.5	3.8		*0.54*
		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}		P_{13}	P_{26}	P_{40}	P_{80}	
F5 (24)	Sep (Jun)											2.9	2.8	3.5	3.2	
	(Jul)											1.6	2.2	2.1	1.7	
	(Mean)											2.2	2.5	2.8	2.5	
	SE															*0.35*
	Oct (Jun)											2.9	2.4	2.8	3.2	
	(Jul)											2.8	3.2	2.5	2.8	
	(Mean)											2.8	2.8	2.7	3.0	
	SE															*0.50*
	Nov (Jun)	4.5	4.5	4.2	4.5		7.6	7.0	7.2	5.8		5.6	5.5	5.4	5.1	
	(Jul)	3.8	4.1	3.7	4.0		7.8	9.1	7.7	7.1		4.7	5.1	4.5	4.9	
	(Mean)	4.2	4.3	3.9	4.2		7.7	8.0	7.4	6.5		5.1	5.3	5.0	5.0	
	SE					*0.32*					*1.12*					*0.45*
	Mean (Jun)											3.8	3.6	3.9	3.8	
	(Jul)											3.0	3.5	3.0	3.1	
	(Mean)											3.4	3.5	3.5	3.5	
	SE															*0.29*
		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}		P_{10}	P_{20}	P_{40}	P_{80}	
F6 (48)	Aug	1.7	1.8	1.8	1.9		5.8	5.6	4.9	4.2		2.4	2.7	2.6	2.6	
	SE					*0.53*					*0.85***					*0.39*
	Dec	3.7	4.0	4.4	3.8		6.3	6.0	4.9	4.4		4.9	5.0	4.6	4.1	
	SE					*1.05*					*0.90***					*0.66**
	Mean	2.7	2.9	3.1	2.9		6.0	5.8	4.9	4.3		3.7	3.8	3.6	3.4	
	SE					*0.61*					*0.72***					*0.37**

* and ** Main effects of plant population significant at 5% and 1% levels of significance respectively.

TABLE 44: Effect of population x nitrogen interaction on SMCO N, protein N and nitrate N in stems in trials F1b and F1c (% total N).

Trial (No. plots)	Harvest date	Population (plants/m^2)	SMCO N (% total N)		Protein N (% total N)		Nitrate N (% total N)	
			N_{50}	N_{150}	N_{50}	N_{150}	N_{50}	N_{150}
F1 b (24)	Feb	13	10.2ab	10.7a	60.8b	57.4b	1.8b	1.4b
		52	8.2ab	7.7b	69.1a	57.7b	1.0b	5.3a
		sign. effects	Pop.*		N*		Pop.*; N*; Pop.xN*	
		SE (MP)$^+$	1.31		3.83		1.06	
		SE (SP)	2.53		6.96		1.86	
			N_{75}	N_{250}	N_{75}	N_{250}	N_{75}	N_{250}
F1 c (24)	Aug	13	5.8a	6.0a	63.4	53.1	1.0b	12.2a
		52	6.1a	5.6a	67.2	52.4	1.2b	11.8a
		sign. effects	S*		N**		S*; N**	
		SE (MP)	0.29		1.39		0.99	
		SE (SP)	0.96		3.86		2.27	
	Dec (pith)	13	6.9b	9.5a	53.8b	58.3ab	0.9a	0.9a
		52	5.9b	6.4b	64.0a	58.0ab	1.1a	1.7a
		sign. effects	Pop.*; N*		Pop.*			
		SE (MP)	1.36		3.11		0.49	
		SE (SP)	1.22		8.02		0.68	
	(cortex)	13	4.1b	5.0a	60.7a	61.2a	0.7a	0.7a
		52	3.7b	4.1b	61.8a	60.9a	0.9a	1.3a
		sign. effects	Pop.*; N*					
		SE (MP)	0.40		2.85		0.34	
		SE (SP)	0.68		4.66		0.47	
	(entire)	13	5.1b	6.6a	58.2a	60.1a	0.8a	0.8a
		52	4.4b	4.9b	62.6a	59.8a	0.9a	1.4a
		sign. effects	Pop.*; N**					
		SE (MP)	0.64		2.70		0.38	
		SE (SP)	0.64		4.40		0.54	

SE (MP)$^+$ and SE (SP) are standard errors for main plot comparison of population main effect means and sub-plot comparison of population x nitrogen interaction means respectively.

Small case letters show comparison of population x nitrogen interaction means at the 5% level of significance according to the LSD test.

a greater proportion of N being incorporated into protein N. This preference, at the low N treatments, for N in plants to form protein instead of SMCO when plant density was increased, mainly occurred in stem pith tissue. When there were high levels of available N, protein N (% total N) remained constant when plant populations were increased, however nitrate N (% total N) tended to increase, although only in F1b were differences significant. There were larger increases in stem DM yields at the high N treatments (Table 30), hence competition for available S, as mentioned previously, may have also been a factor in the population effect together with the increase in nitrate N (% total N).

At the August harvest in F1c, SMCO N (% total N) was increased ($P < 0.05$) by S from 5.6 to 6.1 (SE = 0.29), while nitrate N (% total N) was reduced ($P < 0.05$) by S from 7.5 to 5.6 (SE = 0.99). There were no significant interactions between population and S.

In F1c, nitrate levels were higher in August (mean = 0.13% DM, SE = 0.053) than December (mean = 0.01% DM, SE = 0.007), while SMCO N (% total N) in all trials tended to increase with later harvests (Table 43). In stems, the highest total N and protein N levels were in cortex tissue, whereas the highest SMCO levels were in pith tissue.

The slightly different SMCO N (% total N) results for stems in F1b in Tables 43 and 44 were due to total N levels in the samples being analysed by different techniques at different times.

Pop/dry matter content:

Increasing plant population, generally increased the dry matter content in stems and whole plants in each trial. The mean DM contents over all trials for the low and high population treatments were 14.3 and 15.2% DM respectively for stems and 13.7 and 14.3% DM respectively

for whole plants. This increase with higher plant populations is similar to previous findings by Willey (1964) and Frame and Robinson (1966a,b).

3.2.4 Effects of harvest date (HD)

HD/dry matter yield:

Total, leaf and stem DM yields in trials with more than one harvest date are shown in Figure 21. Total yield increased up to the October harvest in all trials. In the three years of F1, there were no significant changes in total DM yield after October, whereas in the 1980 trials F3 and F5 (July sowing), yield significantly increased ($P < 0.01$) up to December and November respectively. Between December and February, there was a decrease ($P < 0.05$) in total yield in F3. Maximum total DM yield was reached therefore between October and December depending on the site and the year.

Leaf DM yields increased to October and then tended to decrease throughout the winter. Stem yields also increased to October and then either increased slightly up to December or remained reasonably constant through to February.

The effect of leaf yields reaching a peak in October and then decreasing was almost certainly due to older mature leaves abscising during cold winter conditions. Stephen (1976) found a similar trend with leaf DM yields of marrow-stem kale grown at various sowing dates in New Zealand.

HD/SMCO content:

The effects of harvest date on SMCO contents in the different tissue types are shown in Figure 22. In all trials except F1c, whole plant SMCO increased between the first and last harvests. In the three

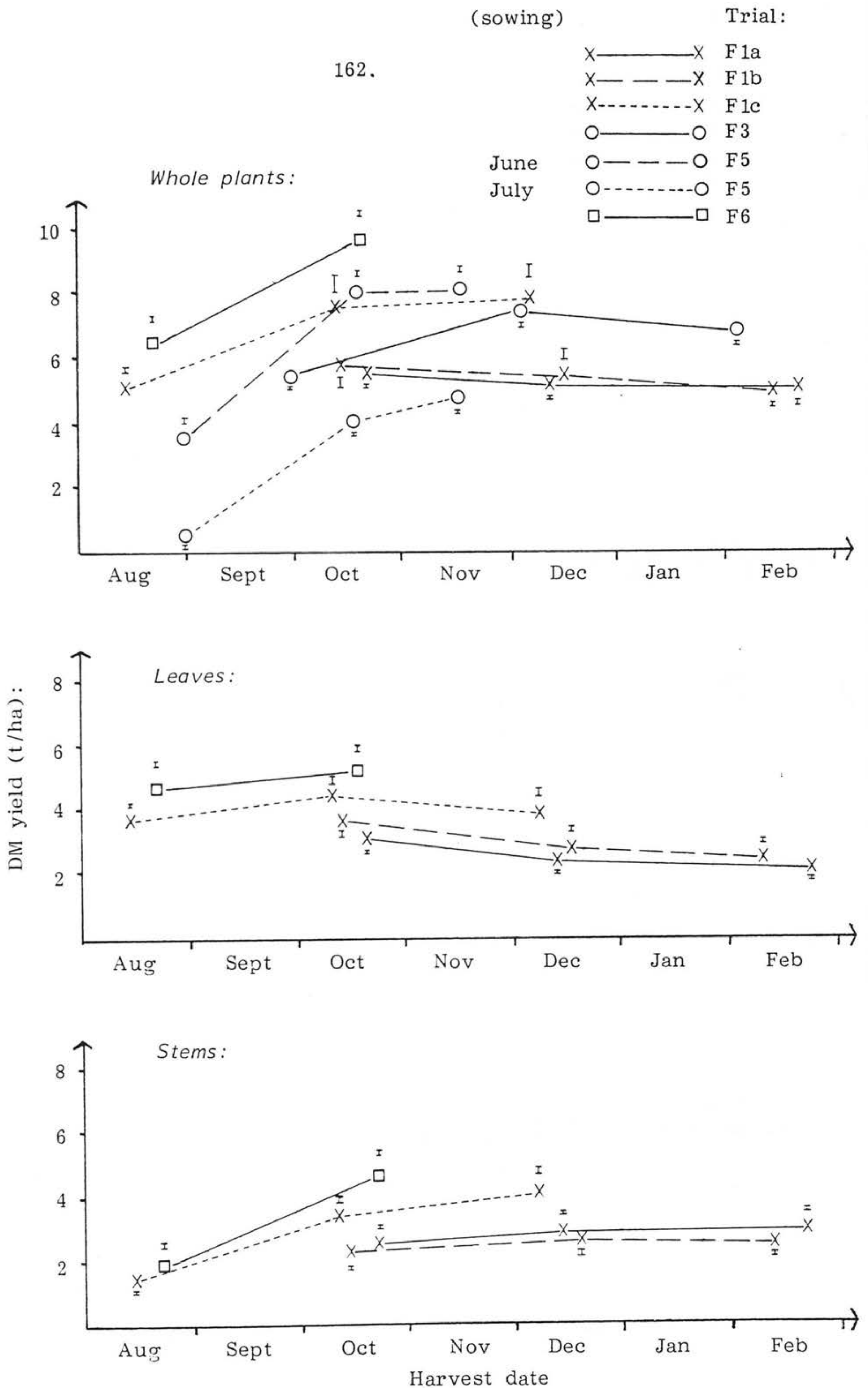


FIGURE 21: Effect of harvest date on DM yield in field trials (t/ha).

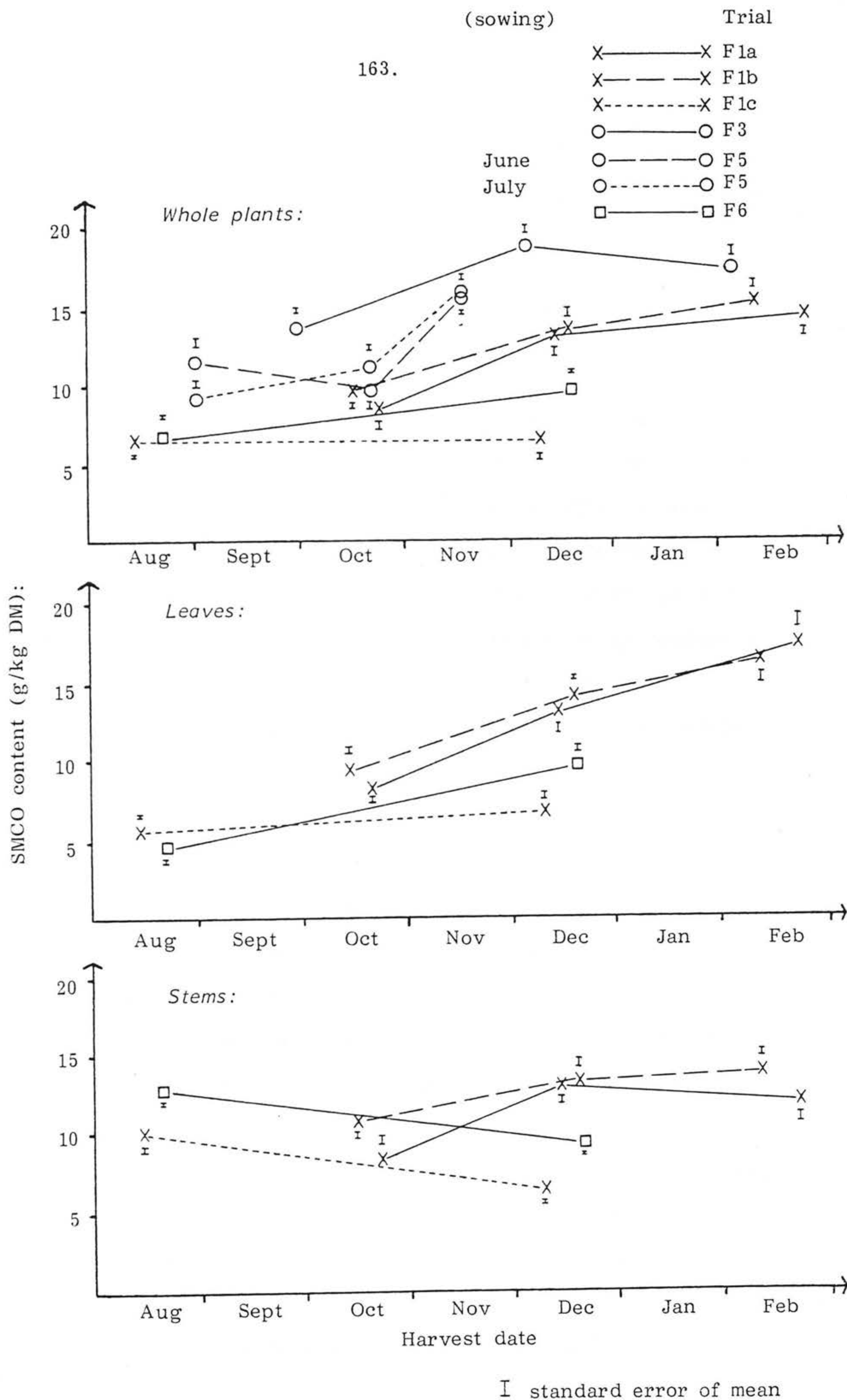


FIGURE 22: Effect of harvest date on SMCO content in field trials (g/kg DM).

trials where SMCO was measured between October and February, greater increases occurred between October and December than between December and February. Between the later harvests, SMCO increased ($P < 0.05$) only in F1b, while in F3 SMCO was significantly ($P < 0.05$) reduced. The interaction between harvest date and sowing date in F5 will be discussed later.

Significant increases in leaf SMCO levels between harvests in each trial were mainly responsible for whole plant SMCO increases. SMCO levels in stems were more consistent than in leaves, although levels decreased between August and December in the two 1981 trials (F1c and F6) and increased between October and February in F1a and F1b. SMCO was higher in stems than leaves at harvests up to approximately December, by which time levels in leaves and stems were similar. After December, SMCO became progressively higher in leaves than in stems.

The increases in whole plant SMCO with maturity confirm previous reports for a range of brassicas including kale (Smith, 1974, 1976; Whittle *et al.*, 1976; Bradshaw and Borzuki, 1980).

Although most previous reports suggest that kale is potentially more toxic to ruminant animals in terms of SMCO content as it matures, data in this thesis show that there is little difference in SMCO content between December and February harvested Maris Kestrel kale. February kale would be more toxic, however, if animals were allowed to graze leaves only for more than a few days, hence small break sizes, and thus increased grazing pressure, become more important as winter progresses in order to ensure a better total plant utilisation.

Using mean SMCO values for each plot, the correlations between leaf SMCO and stem SMCO were 0.26 (n=24), 0.44* (n=24), 0.66** (n=24),

0.34 (n=12), 0.12 (n=36), -0.52** (n=36) and 0.09 (n=48) for F1a, F1b, F1c, F2, F3, F5 and F6 respectively. Only in two of the seven trials were there significant positive correlations and these were low. The negative correlation in F5 was due to plant population and especially sowing date treatments having opposite effects on SMCO in the two components (Table 39). This inconsistent correlation between leaf and stem SMCO in kale is similar to the report by Gosden (1977) that there was no correlation between SMCO levels in 'bulbs' and leaves of individual swede plants. Correlations in these trials were affected by fertiliser, population, harvesting and sowing date treatments hence varying environmental conditions often does not affect both leaf and stem SMCO levels in a similar manner.

The main reason for whole plant, and especially leaf SMCO, increasing with maturity is not known. Possible causes include cooler temperatures, especially frosting (Evans, 1951; Gregor, 1952; Dunbar *et al.*, 1963) and the development of secondary growth and flowers as winter progresses. The effects of temperature and floral initiation were studied in separate trials and their results are presented in later sections.

HD/nitrogen content:

The effects of harvest date on N contents in each tissue type are shown in Figure 23. Whole plant N decreased in all trials between August/September and November/December. The reduction in F5 with July sown kale was very marked, showing that young kale plants are much higher in N than older plants. Changes in whole plant N between October and February were small, with N increasing ($P < 0.05$) slightly in two of the three trials. When leaf N was measured more than once, there were small reductions between August and December, but large

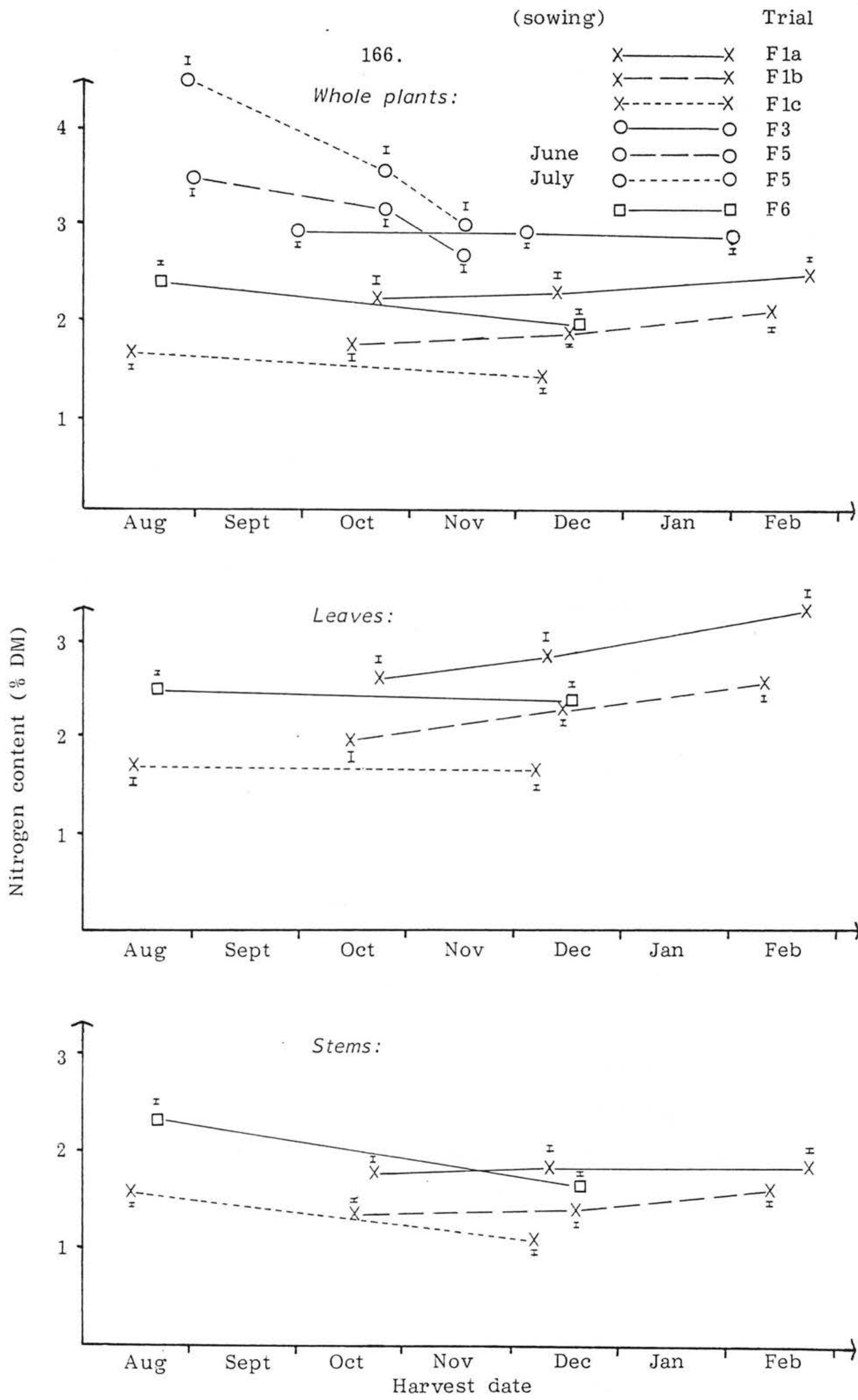


FIGURE 23: Effect of harvest date on nitrogen content in field trials (% DM).

increases between October and December and between December and February. Stem N decreased in two trials between August and December and generally remained constant after October.

The N levels of most crops are reduced as they mature and this happened here with kale harvested up to between October and December. The slight increase in N at later harvests in some trials was probably due to the average leaf N level increasing as a result of older, low N leaves falling off during colder winter conditions.

HD/SMCO and N production:

Figure 24 shows the effect of harvest date on total SMCO and N produced (kg/ha) in whole plants and leaves. Maximum SMCO production was measured in December in all trials, whereas maximum N production was measured between October and December. After December, whole plant SMCO and N productions remained constant in F1a and F1b, but were significantly ($P < 0.01$) reduced in F3. Leaf SMCO production showed small significant increases throughout the growing season in three of the four trials, while leaf N production remained constant in three of the trials and was reduced in the other.

Because SMCO production in leaves tended to increase with maturity whereas N production was relatively constant, SMCO N (% total N) also increased as was shown in Table 43. This increase was probably associated with a reduction in either protein N (% total N) or nitrate N (% total N) although these were not measured in leaves. As older leaves senesced and leaf DM yields decreased, some of the protein N in the older leaves may have broken down to amino acids (Bonner, 1950) and transferred to younger leaves where SMCO instead of protein may have been formed. This theory does not explain,

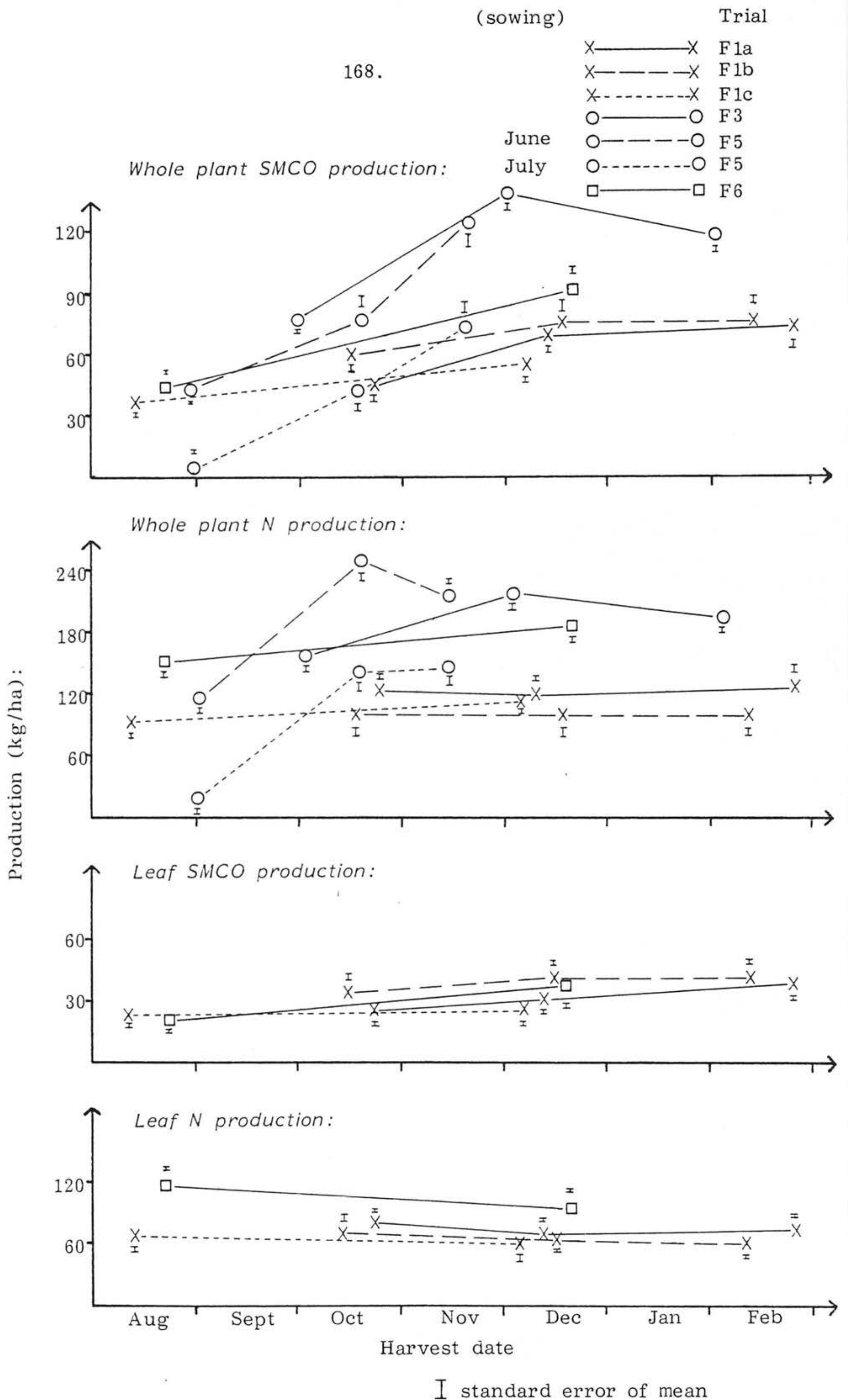


FIGURE 24: Effect of harvest date on SMCO and N productions in field trials (kg/ha).

however, why SMCO content (g/kg DM) and SMCO production (kg/ha) in F1c and F6 increased between August and December, when leaf DM yields were not reduced and there was probably little senescence of older leaves. During this autumn period, SMCO must have been formed in leaves either from other leaf N compounds or from N translocated from stems.

3.2.5 Effects of sowing date (SD)

SD/dry matter yield:

In F5, kale sown on 26 June 1980 had a higher total DM yield ($P < 0.01$) at each of the three harvest dates than kale sown on 24 July 1980 (Figure 21). Stem DM yields were also higher in the June sown kale at each harvest. With leaf yields, the differences between sowings decreased with each later harvest (Table 38). This effect of early sowings producing higher DM yields than later sowings is a common feature with kale (Stephen, 1976).

SD/SMCO content:

June sown kale in F5 had a significantly ($P < 0.05$) higher whole plant SMCO content than July sown kale at the September harvest (Figure 22). At the October and November harvests, the main effect of sowing date on whole plant SMCO was not significant, although in November there was a significant ($P < 0.05$) interaction between plant population and sowing date (Table 39). July sown kale had a higher ($P < 0.05$) SMCO content than June sown kale at the highest population density (80 plants/m^2), whereas at the lowest population density (13 plants/m^2), June sown kale had the higher level.

In November, leaf SMCO was highest ($P < 0.01$) from the earlier sowing and stem SMCO highest ($P < 0.01$) from the later sowing. In the

earlier sown kale, leaf and stem SMCO levels were similar (mean = 15.6 g/kg DM), whereas in the later sown crop SMCO was higher in stems (20.2 g/kg DM) than in leaves (13.7 g/kg DM).

Previously reported findings with kale, rape, radicole, fodder radish and stubble-turnips showed that SMCO levels were higher in July than June sown crops from an October harvest (Anon., 1978b). Findings presented in this thesis do not contradict this, apart from at an early harvest in September when SMCO was highest in the earlier sown kale. At the later November harvest, whole plant SMCO at the highest population and stem SMCO at all populations were highest in the July sown crop.

On the reasonably young plants in September, stem SMCO would have been much higher than leaf SMCO at both sowings (Figure 22). At this harvest, the July sown kale contained only 7% stem compared with 20% in the June sowing (Table 38) and the more mature leaves (June sowing) would have had higher SMCO levels than the younger leaves in July sown kale (Table 19), hence it is not surprising that the June sowing had the higher SMCO level.

The effect of sowing date on leaf SMCO in November was similar to its effect on whole plants in September, probably due to the principle that more mature leaves have higher SMCO levels as shown in Figure 22. The opposite trend tended to occur with stems in Figure 22, with SMCO decreasing as kales matured during the autumn. This is probably the main reason why the more mature stems of plants from the June sowing had lower SMCO levels than those from the July sowing. Another explanation for differences in stem SMCO in November could be that the N level in stems was higher in kale sown in July (2.37% DM) than in June (2.09% DM) (Table 42). The higher N stems probably had higher

levels of available nitrate, hence would have had more opportunity for forming SMCO, as previous trials showed that SMCO can be increased by increasing soil nitrate levels. The effect of later sown kale having a higher total N level than earlier sown kale was also reported by Boelcke (1972) who demonstrated that the nitrate content was also higher.

With stem SMCO levels being higher in later sown kale, it would be expected that as the crop matured and the percentage of total yield as stem increased, there would be an increasing tendency for whole plant SMCO to become higher in later sown kale; this occurred in Trial F5. These trials have therefore both confirmed previous work (Anon., 1978b) and showed that the effects of sowing date on SMCO are related to the proportion of leaf and stem yield and to the effects of harvest date on SMCO levels in these two components.

The interaction between sowing date and population in whole plants at the November harvest was caused by higher populations reducing SMCO in the more mature June sown kale, but having no effect on the lower yielding July sown crop (Table 39).

3.2.6 Effects of site and year

I. Variation of SMCO between trials:

The average SMCO content at harvests in individual trials ranged from 6.6 g/kg DM in F1c (December harvest) to 20.6 g/kg DM in F4 (December harvest). This inter-trial variation was larger than any variation within trials, hence possible causes were examined.

The mean SMCO content of mature kale in each of the eight field trials was calculated, based on kale harvested more than 120 days after sowing. These mean SMCO values were correlated with mean soil

sulphate, plant N, DM yield and growth rate values for each trial. Mean soil sulphate values of soil samples taken in October or early November (F2) were used. Plant N and DM yield values were based on the same samples as were used for SMCO measurements. Growth rate in each trial was calculated as the mean DM yield divided by the number of days between sowing and the mean harvest date.

Correlation coefficients (r) between SMCO content and soil sulphate, plant N, DM yield and growth rate were: -0.02, 0.95, -0.31 and 0.18 respectively (n=8 for all). Only the correlation between SMCO (Y) and plant N (x) levels was significant ($P < 0.01$), with a regression equation of $Y = 8.00x - 4.66$. Neither soil sulphate levels, DM yield or growth rate appeared to be important in explaining differences in SMCO content between trials.

The relationship between both SMCO and plant N and SMCO and soil sulphate were examined further using harvest date and fertiliser treatment differences within trials, as well as inter-trial differences. Figure 25 shows the relationship between whole plant SMCO and N contents, averaged for each N treatment, at individual harvests within trials. Data were from harvests more than 120 days from sowing. There was a close relationship ($P < 0.01$) between SMCO and N ($r = 0.92$) using the 26 data pairs available. The linear regression equation relating the two variables was similar to that formed using the mean trial values. If measurements on the more immature kale had been included, the relationship between SMCO and N would have been much less, as younger kale tended to be very high in N and low in SMCO.

The relationship between whole plant SMCO measured in December on low and high S treatments and soil sulphate levels measured in October or early November on the same plots, is shown in Figure 26.

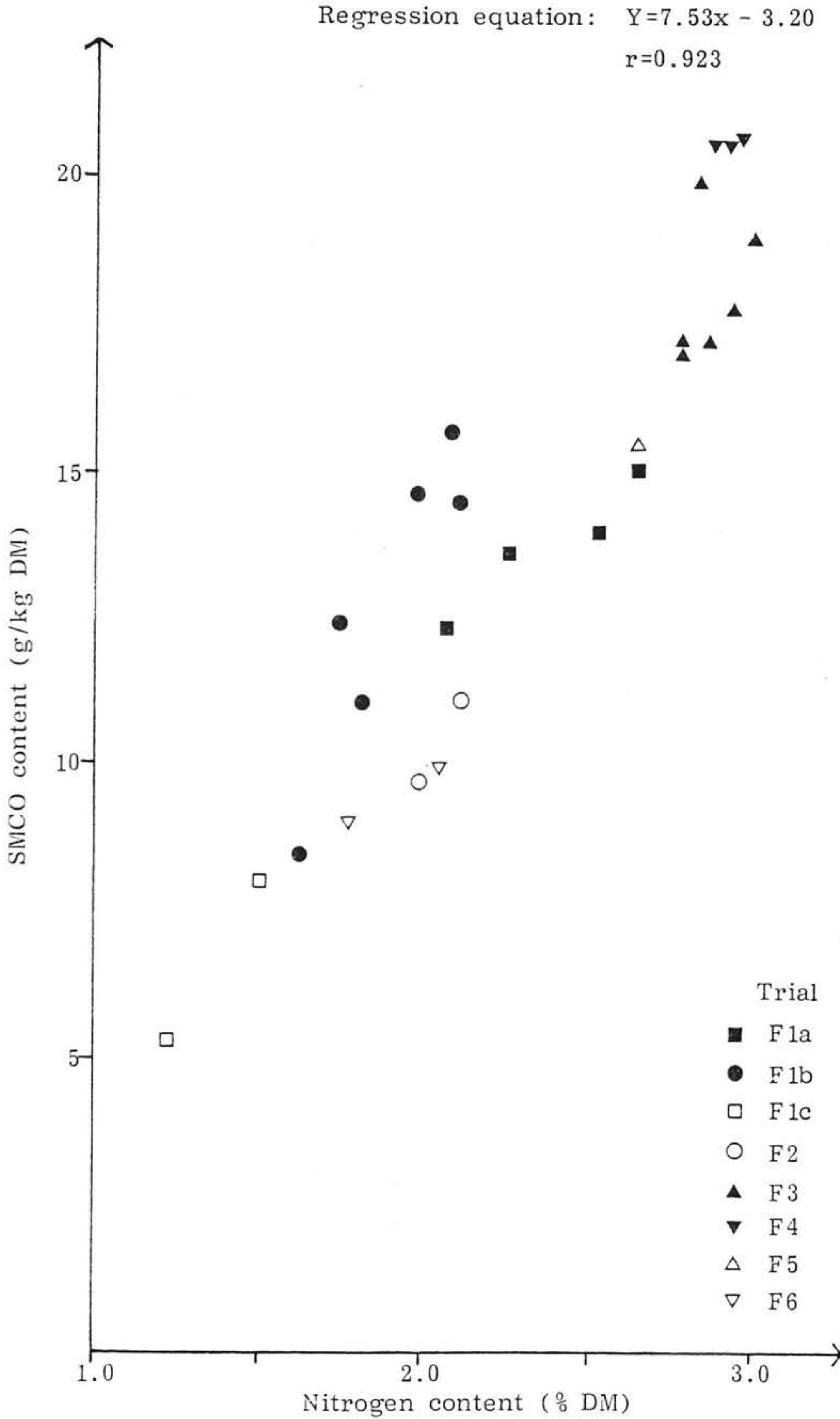


FIGURE 25: Relationship between whole plant SMCO and N contents in field trials.

(From all harvests after 120 days from sowing and on all N treatments.)

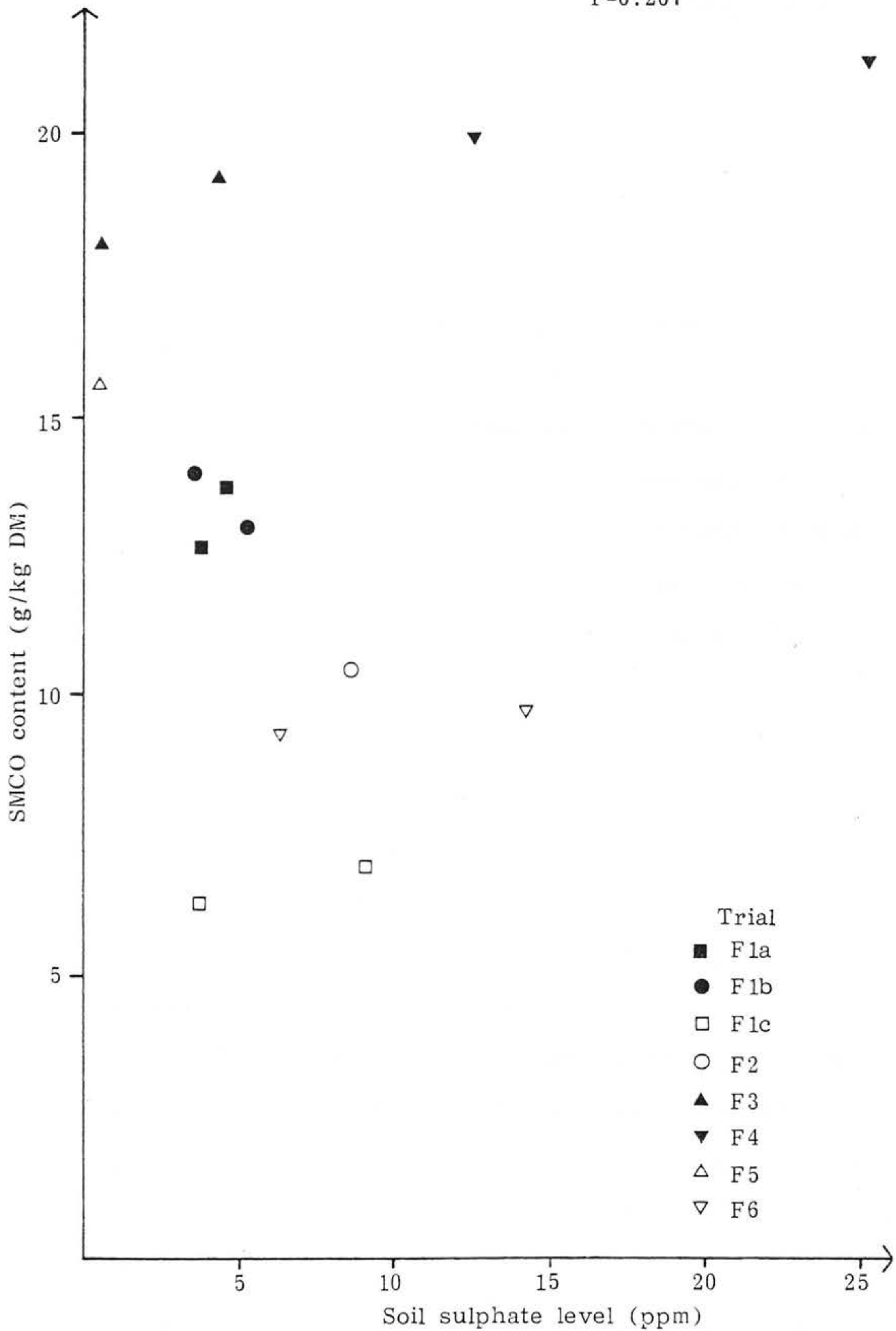


FIGURE 26: Relationship between whole plant SMCO and soil sulphate levels in field trials.

(SMCO measured in December on S treatments and soil sulphate in October or November.)

The correlation coefficient ($r = 0.21$, $n=14$) was not significant with the relationship being much poorer than that between SMCO and N.

These figures show that inter-trial variation in SMCO levels was mainly the result of variation in soil fertility and especially in N levels. The high correlation between SMCO and plant N means that possibly a reasonable estimate of the SMCO level of a Maris Kestrel kale crop could be obtained by measuring its total N content and correcting it by the linear regression equation formed in Figure 25.

Total N in plants can be readily measured by the Kjeldahl method in most agricultural soil and plant analysis laboratories, hence farmers could obtain an approximation of the SMCO level of a mature kale crop prior to it being grazed. This would allow the percentage of kale included in an animal's diet to be adjusted depending on the SMCO concentration. It is more difficult to obtain SMCO than N measurements, as in the United Kingdom there are only a few Research Institute laboratories where SMCO is analysed and its measurement is a more time consuming process than the Kjeldahl method.

Correlations between whole plant SMCO and N in individual trials, plus the ranges of SMCO and N values, are shown in Table 45. The correlations were significant in all trials except F2, where there was only 12 plots, and F5 when kale was less mature and there was again only 12 plots per correlation. The correlations within trials were lower than that obtained when values from all trials were compared. This was partly due to the smaller range of both SMCO and N values at individual trial harvests. Also reasonably high coefficients of variation with SMCO measurements in most trials (Table 46), plus the effects of other treatments on the two factors may have affected within trial correlations. With the inter-trial comparison, data were more accurate being based on

TABLE 45: Correlations between whole plant SMCO and N in field trials.

Trial	Harvest date (Sowing date)	Days from sowing	Correlation coefficient (r)	Range of N (% DM)	Range of SMCO (g/kg DM)
F1 b	Oct	112	0.44*	1.63 - 3.00	4.0 - 12.4
	Dec	161	0.72**	1.76 - 3.08	9.1 - 16.5
	Feb	235	0.64**	1.78 - 2.99	10.9 - 18.2
F1 b	Oct	140	0.64**	1.25 - 2.46	6.6 - 14.6
	Dec	200	0.50*	1.57 - 2.45	9.6 - 19.3
	Feb	258	0.45*	1.62 - 2.59	11.9 - 19.8
F1 c	Aug	107	0.87**	1.04 - 2.48	3.4 - 12.6
	Dec	224	0.69**	1.07 - 1.66	3.9 - 11.4
F2	Dec	182	0.17	1.40 - 2.59	6.4 - 13.2
F3	Oct	100	0.48**	2.51 - 3.27	9.4 - 18.8
	Dec	164	0.64**	2.23 - 3.46	14.5 - 26.8
	Feb	225	0.58**	2.42 - 3.50	12.1 - 26.2
F4	Dec	160	0.50**	2.52 - 3.30	15.6 - 28.1
F5	Sep (Jun)	67	0.37	3.04 - 4.02	9.2 - 14.9
	(Jul)	39	-0.37	4.21 - 4.79	6.8 - 11.4
	Oct (Jun)	118	0.03	2.75 - 3.51	6.7 - 14.7
	(Jul)	90	-0.02	3.18 - 3.78	7.8 - 17.8
	Nov (Jun)	144	0.55*	2.39 - 2.99	12.9 - 17.7
	(Jul)	116	0.31	2.65 - 3.29	12.9 - 17.8
F6	Aug	100	0.64**	1.51 - 3.01	3.9 - 11.9
	Dec	220	0.68**	1.49 - 2.98	6.3 - 15.5

* and ** Correlations significant at 5% and 1% levels of significance respectively.

TABLE 46: Coefficients of variation of SMCO measurements in field trials (%). (In split plot trials, sub-plot coefficients of variation are presented.)

Trial (No. plots)	Harvest date	Leaves	Stems	Whole plants
F1 a	Oct	29.7	30.3	27.4⁺
(24)	Dec	22.2	15.9	14.5⁺
	Feb	21.2	20.1	14.8⁺
F1 b	Oct	8.3*	31.8	13.4⁺
(24)	Dec	10.1*	33.8	16.8⁺
	Feb	17.1*	27.7	12.3⁺
F1 c	Aug	16.0	21.0	16.4⁺
(24)	Dec	36.6	Pith: 17.0	19.5⁺
			Cortex: 16.0	
F2 (12)	Dec	27.8	12.4	16.9⁺
F3	Oct			12.0
(36)	Dec			11.1
	Feb	14.2*	29.1	18.4⁺
F4 (36)	Dec			12.3
F5	Sep			13.7
(24)	Oct			15.1
	Nov	9.6*	15.3	8.0⁺
F6	Aug	30.9	17.4	16.9⁺
(48)	Dec	20.4	18.9	16.1⁺

* SMCO analysed by NIR analysis, whereas all other data analysed by autoanalysis method.

⁺ SMCO values calculated from measurements on leaves and stems.

many plots. The relation between SMCO and N was very poor on the younger kale at most harvests in F5.

Because within trial correlations were generally lower, using total N measurements to obtain an estimate of SMCO would probably only be reasonably accurate when comparisons were made on mature kale crops which had reasonably wide ranging levels of SMCO and N.

Based on the high correlation between SMCO and plant N, there would almost certainly be a significant correlation between SMCO and soil N. Further work is required to study the relationship between SMCO and DM yield at different levels of soil N, in order to find if soils with a particular N level would be more suitable than others for kale growth. There may be a particular soil N level above which SMCO would increase but not DM yield.

The large variation in DM yield between trials at a particular harvest date (Table 29) was mainly related to different sowing dates. The two trials, F1c and F6, which had the highest DM yields were sown earlier than the other trials. The effect of sowing date on DM yield was shown especially in trial F5 (Table 38).

II. Variation in SMCO levels within trials:

Coefficients of variation are presented in Table 46 for each tissue type at each harvest date. Sub-plot coefficients of variation are presented for the split plot trials F1 and F2.

Coefficients of variation with many of the whole plant analyses were lower than those with the leaf and stem analyses at the same harvests. This was due to whole plant values being calculated from the leaf and stem measurements. The average coefficient of variation of 15.3% with whole plants was acceptable for SMCO measurements.

Leaves analysed by autoanalysis and NIR had coefficients of variation of 25.6 and 11.9% respectively. The variations obtained using the autoanalysis method with leaves and stems (21.9%) were very high. From previous autoanalysis experiments (Table 4), normally between 6 and 10 percentage units of the variation is due to SMCO estimation errors. This analytical error was probably higher in the 1980 stem samples in F1b, F3 and F5, when samples at each harvest were analysed over several weeks. Previous work showed that analytical errors increased, as the time during which a batch of samples was analysed was increased.

Plot variation was often higher in F1 than in the other trials, hence errors in DM yields and all chemical analysis results were generally higher in this trial. Trial F1 was situated on a soil which had many small stony patches and DM yield was quite variable within plots as well as between plots.

Some samples were stored in freezers longer than the maximum recommended storage time of 9 months (Table 24). This was due to the shortage of analytical facilities which had to be shared. The October harvested leaf and stem samples in F1a and stem samples in F1b were stored for approximately 10 months. Fortunately their SMCO levels were generally quite low (<10 g/kg DM), so it was unlikely that significant amounts of SMCO were lost from these samples (see Section 2.3). The whole plant samples harvested in September in F5 may have lost some SMCO prior to being analysed, although if any was lost in this or other trials, the loss probably occurred in all samples, hence the effects of treatments would not have been affected. Only if there had been a very wide range of SMCO in the samples at a particular harvest, would the high SMCO samples have lost more than the low SMCO samples.

III. Effects of three consecutive kale crops:

In the three consecutive years of trial F1, soil sulphate levels in October, averaged over all plots, were 4.3, 4.5 and 6.8 ppm. In the plots where no S was applied, soil sulphate levels were 4.5, 3.6 and 4.1 ppm in the three consecutive years. Available S levels were reasonably low in each year and were not reduced by consecutive kale crops, hence any differences in SMCO between years were not related to changes in soil sulphate. Valid comparisons of SMCO levels in first, second and third year kale crops were not applicable in this trial (F1), as the crops were grown in different years with different sowing dates.

IV. Variety effects:

Trials F3 and F4 contained both Maris Kestrel and Vulcan kale. There were no interactions between variety and other treatments in these trials, hence Vulcan was affected by S and N treatments in a similar manner to Maris Kestrel.

There were differences, however, in DM yield and SMCO content between the two varieties. In F3, Vulcan was higher ($P < 0.01$) yielding than Maris Kestrel, having an average yield over the three harvests of 6.93 t/ha compared with 6.27 t/ha for Maris Kestrel (SE = 0.588). Maris Kestrel had higher SMCO and N levels than Vulcan. Mean whole plant SMCO levels (g/kg DM) were 17.8 and 15.3 for Maris Kestrel and Vulcan respectively in F3 (SE = 1.46), and 22.3 and 19.0 respectively in F4 (SE = 2.53). Mean N levels for Maris Kestrel and Vulcan were 3.00 and 2.79 respectively in F3 (SE = 0.153) and 3.04 and 2.82 in F4 (SE = 0.179). Differences in SMCO occurred in stem tissue only, as at the February harvest in F3, SMCO (g/kg DM) levels were

similar in the leaves of the two varieties (mean = 21.4, SE = 3.04), but significantly ($P < 0.01$) higher in Maris Kestrel stems (17.6) than in Vulcan stems (12.8, SE = 4.42).

Vulcan is generally accepted as being slightly higher yielding than Maris Kestrel (Bradshaw, 1981), but there have been no previous reports showing that Maris Kestrel had the higher SMCO content. At the October, December and February harvests in F3, the differences in SMCO content between varieties were 8, 15 and 24% respectively. The difference increased therefore with each later harvest and was not significant at the first harvest.

3.2.7 Significance of changes in SMCO on animal performance

Smith and Greenhalgh (1977) reported that sub-acute anaemia occurs in cattle on a daily diet of 10 g SMCO/100 kg LW and acute anaemia on a diet of 15-20 g SMCO/100 kg LW.

In order to keep SMCO intake at the sub-acute level, 200 kg animals grazing Maris Kestrel (20 g SMCO/kg DM) and Vulcan kales (17.1 g SMCO/kg DM), could consume only 1.0 and 1.2 kg kale DM/day respectively, or 8.3 and 9.8 kg kale fresh weight respectively (at 12% DM). A 200 kg steer requiring 6 kg DM per day (NRC, 1976) could only be fed one-sixth of its total diet as Maris Kestrel or one-fifth of its diet as Vulcan, to keep its SMCO intake at the sub-acute level.

The same animals grazing December harvested kale grown at the lowest (11.0 g SMCO/kg DM) and highest populations (8.1 g SMCO/kg DM) in Trial F6 (Table 39), would take in 11 and 8 g SMCO/100 kg LW respectively, if a third of their diets were kale. Sub-acute anaemia would therefore be likely on the sparsely sown kale, but not on the kale at the highest plant density.

These examples show that small changes in kale SMCO content, by varying agronomic treatments or kale varieties, could have significant effects on the severity of anaemia in animals grazing the crops.

Conclusions

1. Sulphur:

Results showed that both the drainage characteristics and the sulphate status of soils are important factors in assessing whether S fertiliser will affect SMCO levels in kale. On free draining soils low in available sulphate, most S fertiliser applied at sowing was leached from the top-soil within a few months. In five trials on free draining soils, S had no effect on SMCO content apart from at early harvests in August and October in two of the trials. At these early harvests, significant increases in whole plant SMCO of approximately 18% were obtained, although in one trial the increase was found only when low levels of N were applied.

On a more poorly drained soil, S increased ($P < 0.10$) kale SMCO in December by 8%. The effect was small due to a reasonably high soil sulphate level (13 ppm) on plots which had received no S. One might expect that S fertiliser would increase SMCO most on low S poorly drained soils, when it would not be advisable to apply any S in fertilisers.

Dry matter yield was not affected by S fertiliser applications in any trial. Kale nitrogen content was reduced by S in one out of six trials.

2. Nitrogen:

The effects of N fertiliser were dependent on soil fertility. In six field trials, all sown after at least three previous crops since pasture, N increased DM yield, SMCO and nitrogen contents. In another trial which was sown immediately after pasture, none of these factors were affected by N fertiliser. In the trial harvested at the same site for three years (F1), N increased whole plant SMCO levels (g/kg DM) by 8, 18 and 64% in three consecutive years. At the other three sites which had been cropped previously, the average increase in SMCO with additional N was 15%.

When varying levels of N were added in one trial, small amounts of N fertiliser which substantially increased DM yield, also caused large increases in SMCO content. It would appear, therefore, that whenever N is used to increase DM yield, it will always result in a crop more toxic to animals.

Unlike previously reported results, N application in these trials did not have the beneficial effect of reducing SMCO when S was limiting. In most of the presently reported trials, N increased SMCO even though S was limiting.

3. Plant population:

Increasing plant population generally reduced SMCO and increased DM yield in kale stems and whole plants. The effect of population on SMCO was greater in mature plants harvested after October and sown before early July. At harvests in November or December, there was approximately a 16% reduction in whole plant SMCO as plant populations were increased from 10-13 to 40-52 plants/m^2 and a further 9% reduction, as the population was increased to 80 plants/m^2. The effects of

population on SMCO occurred at high and low N and S levels and on both freely and imperfectly drained soils.

The reduction in SMCO with higher populations was not due to changes in the proportion of stem or cortex, but was at least partly due to a reduction in the proportion of total N forming SMCO. There was a corresponding increase in the proportion of total N forming protein N and nitrate N, when available N was low and high respectively. At high N levels, there was a large increase in DM yield with higher populations, and as all soils were reasonably low in sulphate, competition for available S probably also helped reduce SMCO at higher populations.

Higher populations reduced kale N levels, although reductions were small and unimportant compared with the larger beneficial effects of reduced SMCO levels and increased DM yields.

4. Harvest date:

Plant SMCO content increased during the growing season in most trials. Whole plant SMCO increased more between October and December than between December and February when the increase was significant in only one out of three trials. Leaf SMCO was lower than stem SMCO in young plants harvested in August, but increased constantly until February, when it had a much higher level than stems, which tended to decrease during autumn and slightly increase during winter. It was concluded that, in terms of SMCO content, there is probably no more danger in grazing Maris Kestrel kale in February than in December, although animals should not be allowed to graze leaf alone for a long period later in the winter.

Total DM yields increased up to between October and December depending on the site and year and remained reasonably constant through to February. Leaf yields reached a maximum in October and then tended to decrease. Whole plant N content decreased in all trials up to November or December. Between December and February, N increased slightly in two out of three trials, mainly due to an increase in leaf N.

5. Sowing date:

In September, SMCO was higher in June sown that July sown kale. As harvests were delayed, there was a tendency for the later sown crop to have the higher SMCO level, especially at high populations. At all harvests, leaf SMCO was higher in earlier sown kale due to leaf SMCO increasing as kale matured. Stem SMCO was always higher in later sown kale, due both to stem SMCO slightly decreasing with maturity and a higher N level in the July sown kale. As harvests were delayed and the proportion of stem increased, there was an increasing tendency, therefore, for the later sown crop to have the higher SMCO level.

June sown kale had a higher DM yield at all harvests, although the difference in leaf yield between sowings decreased with later harvests.

6. Site and year effects:

Variation between trials in SMCO content was more than three-fold, ranging from 6.6 to 20.6 g/kg DM. This variation in SMCO (Y) was highly correlated ($r = 0.92$, $n = 26$) with plant N levels (x) in mature kale having the relationship, $Y = 7.53x - 3.20$. Correlations between SMCO and N within trials were generally lower. It was concluded that the use of N to estimate SMCO, would be most beneficial when kale samples

covering a wide range of SMCO and N were being compared. The relationship between soil sulphate and SMCO levels was much poorer ($r = 0.21$, $n = 14$).

Variation in SMCO levels within trials was high, with coefficients of variation for leaves, stems and whole plants averaging 20.3, 21.9 and 15.3% respectively. The high errors in leaves and stems were due to errors in the autoanalysis technique, sampling errors and inter-plot variations.

In the trial where kale was grown for three successive years, available soil sulphate was low in each year and was not reduced in the third year. Any changes in kale SMCO content between years were not due, therefore, to changes in available soil sulphate.

Vulcan was affected by S and N treatments in a similar manner to Maris Kestrel. Vulcan, however, was higher yielding and contained approximately 17% less SMCO than Maris Kestrel.

3.3 Floral Initiation Effects

Introduction

One of the reasons for SMCO increasing in kale at later harvests may be related to floral initiation, as it is known that kale flowers have higher levels of SMCO than other parts of the plant (Whittle *et al* ., 1976). Any difference in the accumulation of SMCO between vernalised (flowering) and devernalised (non-flowering) plants was therefore studied in two glasshouse (G) trials.

Experimental

In trials G1 and G2 carried out in different years, Maris Kestrel seed was sown in 12.5 cm pots filled with compost and grown in a glasshouse at 15°C. In G1, a constant amount of NPK fertiliser was applied to all pots at sowing and after 98 days. In G2, fertiliser was applied after 105 days at a rate similar to that in one of the applications in G1. After the juvenile stage when plants had approximately 9 leaves, plants in each trial were separated into two groups and either vernalised at 6°C or vernalised at 6°C for 20 hours per day and subsequently treated at 22°C for 4 hours per day. The latter treatment was termed devernalisation. Vernalisation and devernalisation treatments were carried out in growth cabinets for 31 and 37 days respectively, which allowed all plants to have the same number of degree days. After temperature treatments, plants were grown in a glasshouse until the vernalised plants had flowered. During this period, plants in G2 were grown under lights at a higher temperature than those in G1. Plants in both trials were harvested prior to the temperature treatments and at flowering.

At harvests in G1, whole plants were chopped and stored at -20°C. In G2, plants were divided into leaves and stems and weighed prior to

chopping. Subsamples of each component were frozen and others oven dried (90°C) for dry matter determinations. Stem lengths in G2 were measured at the flowering harvest. The frozen samples were freeze dried and analysed for SMCO by the autoanalysis technique nine months (G1) or one month (G2) after the final harvests. N was predicted in all G2 samples using NIR analysis, which was also used in both trials to measure the moisture contents of the freeze dried samples.

Table 47 shows the number of plants harvested, the number of replicates and the times of harvests in each trial. In G1 there were

TABLE 47: Harvest times and number of plants harvested in G1 and G2.

Trial			Harvest time	
			prior to temperature treatment	at flowering
G1	Days from sowing		57	154
	Plants per treatment (Number of replicates)	Vernalised	10 (2)	8 (2)
		Devernalised		10 (2)
G2	Days from sowing		55	153-160
	Plants per treatment (Number of replicates)	Vernalised	15 (3)	12 (12)
		Devernalised		12 (12)

other harvests prior to flowering, however in that year (1979) only the few samples for which data are given could be analysed for SMCO from that trial. In G2 there were 30 plants in each treatment after vernalisation and devernalisation, but only 12 of those in the vernalisation

treatment flowered. In order to compare treatments accurately at the final harvest in G2, a devernalised plant was harvested in a replicate together with a flowering plant. Plants within replicates were similar in size and from similar positions in the glasshouse. Inflorescences at the final harvest were between 2 and 60 cm long in G1, but were all approximately 2.5 cm long in G2 when vernalised plants were harvested at this stage. In G2, inflorescences were included with the stems.

Treatment comparisons were made using an analysis of variance. Standard errors presented in this and other glasshouse trials (G1 - G8), are standard errors of means. Each was calculated by dividing the square root of the error mean square by the square root of the number of plots in the trial or treatment mean.

Results and discussion

The effects of vernalisation and devernalisation on SMCO levels in the two trials, are shown in Table 48. In G1 no accurate statistical comparison of treatments was possible with only two replicates and reduced degrees of freedom. There was a tendency in that trial, however, for whole plant SMCO levels to be slightly higher at flowering than at the earlier harvest, although flowering plants were no higher in SMCO than non-flowering plants.

In G2, stems from vernalised plants had a significant ($P < 0.05$) 28% higher SMCO level than those from devernalised plants. Leaf and whole plant SMCO were not significantly increased by vernalisation, although whole plant SMCO tended to be increased (16%) due to higher stem SMCO. Between the two harvests, both leaf and whole plant SMCO increased ($P < 0.05$), while stem SMCO decreased ($P < 0.05$).

The higher SMCO levels in vernalised stems suggests that floral initiation probably has some influence on kale SMCO increasing with

TABLE 48: Effects of vernalisation and devernalisation on SMCO levels (g/kg DM).

Trial		Harvest time					
		Prior to temperature treatment			At flowering		
		Leaves	Stems	Whole plants	Leaves	Stems	Whole plants
G1	Vernalised						10.9
	Devernalised						12.8
	Mean			9.6			11.9
	SE			1.35			2.51
G2	Vernalised				4.5	8.6a	6.4
	Devernalised				4.9	6.7b	5.5
	Mean	2.9	10.6	3.8	4.7	7.6	5.9
	SE	0.37	0.35	0.30	0.40	0.46*	0.42

* Treatment effect significant at the 5% level of significance.

plant maturity. Usually the increase with maturity occurs in leaves, although they were not affected by vernalisation in this trial. These flowering plants were further developed than most previously measured plants and inflorescences were included with the stem tissue, hence it is not surprising that if flowering was going to affect plants, differences would occur in the stems. It is not known whether leaves would have been affected by vernalisation, if they had been analysed for SMCO prior to flowering. As leaf and whole plant SMCO in non-flowering plants increased ($P < 0.05$) between harvests in G2, there are probably other factors which are also important in SMCO levels increasing with later harvests.

The increase in leaf SMCO and the decrease in stem SMCO between the two harvests are similar to previous reported findings in this thesis (Figure 22). The rate of change between harvests and the

actual SMCO levels at harvests, are dependent on available nutrients. Whole plant SMCO was higher in G1 than G2, probably because more fertiliser was applied in G1 and plants in G2 appeared at the final harvest to be deficient in minerals.

In G1 all plants in the vernalisation treatment flowered, whereas in G2 only 12 of the 30 plants flowered. None of the devernalised plants flowered in either trial. Possible explanations for some of the plants in the vernalisation treatment in G2 not flowering, are that they were slightly less mature at the beginning of the temperature treatment due to receiving less fertiliser, hence some may have still been in the juvenile stage, or the higher temperatures after vernalisation in G2 may have devernalised some plants.

The effects of vernalisation and devernalisation on plant dry weights, N levels and stem lengths in G2 are shown in Table 49. In all tissue types, N was not significantly ($P < 0.05$) affected by flowering, although stem N in particular tended to be affected in a similar manner to stem SMCO. Dry weights were similar in the two treatments, but length of stems varied due to stem extension in the flowering plants.

Plant numbers in both trials were lower than was desired for accurate treatment comparisons, as it is known that there is a large inter-plant variation in SMCO (see Section 3.1). Despite this, treatment differences were still obtained in G2, an experiment where there was sufficient replication.

Conclusion

Flowering plants in one trial had higher ($P < 0.05$) SMCO levels in stems, than non-flowering, devernalised plants grown with the same average temperature. Small inflorescences (2.5 cm long) were analysed

TABLE 49: Effects of vernalisation and devernalisation on dry weights, N and stem lengths at final harvest in G2.

Tissue type	Treatment	Dry weight (g/plant)	N content (% DM)	Stem length (cm)
Leaves	Vernalised	24.4	1.63	
	Devernalised	27.0	1.49	
	Mean	25.7	1.56	
	SE	1.90	0.170	
Stems	Vernalised	15.9	2.05	33.8a
	Devernalised	14.6	1.66	24.5b
	Mean	15.2	1.86	29.2
	SE	1.49	0.156	2.73*
Whole plants	Vernalised	40.4	1.78	
	Devernalised	41.5	1.54	
	Mean	41.0	1.66	
	SE	2.25	0.155	

* Treatment effect significant at 5% level of significance.

with the flowering stems. In the same trial, leaf and whole plant SMCO levels were not significantly ($P < 0.05$) affected by vernalisation and devernalisation, although there was a tendency for whole plant SMCO to be higher in flowering plants. In a preliminary trial where few samples were analysed, vernalisation had no effect on whole plant SMCO.

Floral initiation appears therefore to have some importance in increasing kale SMCO levels with later harvests. These trials did not show, however, that flowering is responsible for the large increase in leaf SMCO as plants mature. This would require further trials examining SMCO in the various components of vernalised and devernalised plants, at varying growth stages up to flowering. There are almost certainly

other factors as well as floral initiation, which affect SMCO increases with later harvests.

3.4 Temperature and Water Deficiency Effects

Introduction

Whittle *et al.* (1976) reported a large variation in kale SMCO levels, caused by both site and harvest date effects. SMCO tended to increase with later harvests and it has been suggested that this may be due to frosting. SMCO generally increases throughout the winter at the same time as temperatures become lower and frosts more frequent. There have never been any reports of increases in SMCO in brassicas with frosting however. The only evidence for this theory is based on animals appearing to be more anaemic after feeding on kale which has been frosted for one or several days, compared with the same animals grazing non frosted kale at different times (Evans, 1951; Gregor, 1952; Clegg and Evans, 1962; Dunbar *et al.*, 1963). Two trials examined the effects of temperature on SMCO levels in kale.

It was expected that if frosting increased SMCO, it could be due to the breaking down of proteins into amino acids within cells and some of these forming SMCO (Street and Opik, 1970). In one of the trials, therefore, a treatment was included which allowed a period of thawing and respiratory activity after frosting and prior to harvesting. It was thought that possibly this period of respiratory activity may be necessary if the plant was to produce higher levels of SMCO, although there have been no reports on thawed kale being toxic to animals.

The other major climatic factor likely to affect SMCO levels in kale, is moisture availability, hence this was also examined in a small trial.

3.4.1 Frosting

Experimental:

In two trials, G3 and G4, Maris Kestrel kale yielding approximately 5.0 and 9.0 t DM/ha respectively, were transplanted from field plots into 12.5 cm pots approximately seven days prior to the commencement of temperature treatments. In both trials, field plots were grown at a density of approximately 40 plants/m^2.

In G3 in 1979, 192 plants were arranged into 24 plots each of eight plants. The high number of plants per plot was required because of the large variability in SMCO content between individual plants. Temperature treatments were frosting (-2 to -6°C) for one or six days, 12°C for one or six days, and frosting for one or six days with each treatment being followed by one day at 12°C. All treatments were replicated twice at different times and twice within growth cabinets, which were used in order to keep temperatures constant.

In trial G4 in 1981, 144 plants were separated into 18 plots of eight plants. Plants were kept for six days at each of the three temperatures, -6°C, 2°C and 15°C. Each treatment was replicated twice at different times and three times within each time replicate.

Plants in the 15°C treatment in G4 were grown in a glasshouse due to there being only two operational growth cabinets, which were used for the two lower temperature treatments. In G4, there was difficulty in maintaining a low temperature in the frosting treatment. The temperature rose above 0°C for one 8-hour period in the first time replicate and for approximately three 6 to 8-hour periods during the second time replicate. Temperatures in the other treatments were kept reasonably constant.

In both trials, plots were daily given 8 hours light and 16 hours darkness and were watered regularly during the temperature treatments. All transplanted plants were grown in a glasshouse at 15°C for seven days prior to beginning their temperature treatments.

At the end of the temperature treatments, plants in G3 were divided into young leaves (leaves within 2.5 cm of growing point), older leaves and stems, while those in G4 were divided into leaves and stems. In both trials, sub-samples from components in each plot were chopped and stored at -20°C prior to being analysed. Total leaves and stems in G4 were weighed and also sub-sampled for oven drying at 90°C. This enabled whole plant estimates of each constituent analysed to be calculated from dry matter proportions of leaves and stems.

In G3, only the 24 young leaf samples were analysed for SMCO, due to there being a limited number of samples that could be analysed in 1979. In G4, leaf and stem samples were analysed for SMCO and total N content. The frozen samples were freeze dried prior to SMCO being measured by the autoanalysis technique. The time between harvesting and SMCO analysis was approximately nine months in G3 and four months in G4. N levels in G4 were estimated by NIR on the Infra-Alyzer 300, using the following leaf and stem calibration equations.

Predicted N (leaf)

$$= 1.97 - 28.12 \log(F2) + 122.94 \log(F5) - 84.56 \log(F7)$$

Predicted N (stem)

$$= 0.85 + 83.47 \log(F5) - 58.36 \log(F7) - 29.18 \log(F10)$$

Predicted leaf N values (x) were corrected using the correction equation for 1981 samples: Y (True N) = 0.73x + 0.54.

The moisture contents of the freeze dried young leaf samples in 1979 were measured by drying approximately 1 g of material at 90°C for

24 hours. In 1981, moisture contents were estimated on the Infra-Alyzer 300 at the same time as N was predicted, using the best leaf and stem moisture calibration equations outlined previously (see Section 2.2.3).

In both trials, SMCO levels in each component were analysed in a randomised block design, with the three temperature and two time treatments factorially arranged in G3. Interactions between each of the two replication factors and the other treatments were included in the residual error term in both trials. Standard errors of means presented were calculated as in G1 and G2.

Results and discussion:

The mean SMCO contents (g/kg DM) of the kale components are shown in Table 50. Neither temperature, length of temperature treatment or thawing frosted kale significantly affected SMCO in young leaves in G3. Temperature also had no effect on any of the components in G4.

It was unfortunate that only the young leaves could be analysed in G3, although it was expected that if frosting increased leaf SMCO, some of the additional SMCO would probably be in young leaves which contain the highest levels of N and protein.

It is unlikely that the results in G4 were affected by the variation in temperature in the frosting treatment, as for most of the six days plants remained frozen. Possibly a longer period of frosting than six days would increase SMCO levels, although if this were true, some trend after six days would be expected.

The lack of effect of frosting on SMCO, suggests that the previously observed anaemic symptoms in animals grazing frosted kale were probably not caused by increases in kale SMCO levels, but more

TABLE 50: Effect of temperature on SMCO in kale (g/kg DM).

Trial G3 - young leaves

Treatment	Length of treatment 1 day	6 days	Mean
Frosting	21.8	22.7	22.2
12°C	26.2	19.1	22.7
Frosting/thawing	25.2	24.0	24.6
Mean	24.4	21.9	23.2

SE = 1.91

Trial G4

Treatment	Plant component Leaves	Stems	Whole plants
Frosting	9.4	6.9	7.9
2°C	7.8	8.1	7.9
15°C	8.2	7.6	7.8
Mean	8.4	7.5	7.9
SE	0.67	0.68	0.62

likely by effect of colder temperatures on the animals as suggested by Clegg (1966). He suggested that frosting may affect blood circulation through the liver capillaries. Alternatively it is possible that the lower temperatures put further strains on already debilitated animals.

Another possible effect of frosting or cooler temperatures, could be to increase the amount of the crop eaten by the animals. This would increase the likelihood of anaemia, as it is related to the amount of SMCO ingested per unit liveweight (Smith and Greenhalgh, 1977). MacDonald and Bell (1958) and Webster *et al*. (1970) reported higher

DM intakes with lower temperatures by dairy cows and by beef cattle respectively. Often, however, lower temperatures only reduce the performance (liveweight gain) of animals and the efficiency of feed conversion without actually affecting intake (Milligan and Christison, 1974).

Nitrogen levels (% DM) in leaves, stems and whole plants in G4 were not affected by temperature and averaged 1.38% (SE = 0.063), 0.96% (SE = 0.035) and 1.13% (SE = 0.041) respectively. These N levels were fairly low, probably due to the plants being grown on a reasonably infertile soil.

Conclusion:

These trials showed that neither frosting of kale nor growing plants at 2°C for up to six days affected SMCO or N levels compared with kale grown at 12 to 15°C. Thawing kale at 12°C for one day after being frozen also had no effect on SMCO.

Previous reports related frosting to increased anaemia in animals, but probably the anaemia was not caused by increased SMCO levels. The anaemia was more likely due to either animals ingesting more kale and SMCO during cooler conditions, or due to the cooler temperatures affecting the health and resistance of animals to kale toxins.

These trials have shown that the effect of SMCO increasing in brassicas as they mature is probably not related to frosting or other temperature changes.

3.4.2 Moisture availability

Experimental:

Maris Kestrel kale plants growing in a field at a density of 40 plants/m^2 and yielding approximately 9.0 t DM/ha, were transplanted into 12.5 cm pots and kept at 15°C for two weeks prior to the trial. In the trial (G5), which was carried out in a glasshouse at approximately 15°C with no artificial lighting, 40 plants were watered daily and 40 plants were given no water for 11 days, which caused severe wilting.

After the 11 days, plants in each treatment were harvested in five replicates of eight plants each. The number of dead leaves (leaves fallen off the plants) in each plot of eight plants was counted and the total fresh weight of plants recorded. Plants were chopped and a representative sample of approximately 300 g taken from each plot and stored at -20°C. The frozen material was freeze dried and after approximately two months analysed for SMCO by the autoanalysis method and scanned on the Technicon InfraAlyzer 300 for moisture and N predictions.

Results and discussion:

The mean SMCO levels of the wilted and non-wilted plants in G5 were 9.6 and 8.5 g/kg DM (SE = 0.96) respectively. This non significant difference between treatments suggests that severe wilting of plants, as with frosting, has no effect on kale SMCO levels. Wilting or moisture deficiency is probably therefore not a major reason for variation in SMCO between sites.

The high coefficient of variation in this trial (24%) was probably mainly caused by errors in the autoanalysis technique and variation

in SMCO between individual plants (see Section 3.1). The high errors plus the reasonably low number of replicates which reduced degrees of freedom, reduced the likelihood of significant differences between treatments in this trial. Although the results showed no reduction of SMCO in the wilted plants, further larger trials would be necessary before it could be concluded that water deficiency has no effect on SMCO.

The N content in the wilted plants (2.24% DM) was significantly ($P < 0.05$) higher than that in the non-wilted plants (1.73% DM, SE = 0.103). This was probably at least partly because more older low N leaves were shed by the wilted plants (mean = 9.4/plot), than from the non-wilted plants (mean = 7.0/plot, SE = 0.62). With the greater loss of leaves, together with the loss of moisture and reduced photosynthesis through wilting, the average fresh weight of wilted plants was 87 g compared with 144 g in non-wilted plants.

Conclusion:

Moisture deficiency did not significantly affect SMCO levels in Maris Kestrel kale. Results, however, were affected by a high coefficient of variation.

It would appear from these frosting and wilting trials that climatic factors probably have much less effect on SMCO variation in kale between sites than soil fertility factors.

Wilting increased dry base N levels, probably mainly due to more old low N leaves being shed by the wilted plants, leaving them with a higher average N content.

3.5 Effects of Chopping and Wilting

Introduction

There have been reports that anaemia in animals eating brassica material is reduced if the crop is chopped or wilted prior to feeding, although neither process has become a major practice by farmers (Gregor, 1952; Schubert, 1954; NZDA, 1955). The objective of these trials was therefore to check these reports by measuring any changes in SMCO levels in Maris Kestrel kale after it was chopped and wilted. A further objective was to study how quickly SMCO was lost from samples after harvesting, especially if the material was chopped. This is especially important for plant breeders and researchers, who find it necessary to chop up several plants from trial plots in order to obtain representative samples for dry matter and chemical determinations. It was important therefore to know whether SMCO was affected by such a technique.

Experimental

The effects of chopping and wilting on SMCO content in Maris Kestrel kale were studied in three trials (G6, G7 and G8). In October 1979, a small experiment (G6) was carried out prior to any field trials being harvested, in order to study the effect of chopping kale into small pieces on SMCO content. From Maris Kestrel kale yielding approximately 6.0 t DM/ha, six plants of similar size were chosen for each of four replicates. Each group of six plants was divided into stems and leaves. Within each replicate, two stems were kept whole, two were each cut into three pieces and sliced down the centre and the remaining two were chopped into pieces 1.5-2.0 cm long. The leaves of the six plants were divided into two similar portions which were either kept whole or chopped into pieces of lamina approximately 5-8 cm square

and pieces of petiole 2-4 cm long. All plant material was frozen (-20°C) approximately two hours after plants were cut from the field. The time between chopping and freezing was approximately 15 minutes. The 20 samples were freeze dried and analysed for SMCO and N within one month of being harvested. SMCO was measured by the autoanalysis technique, nitrogen by the Kjeldahl method and the moistures of freeze dried samples estimated by drying them in an oven at 90°C for 24 hours.

In trial G7, whole plants and chopped plants were both subjected to four different wilting periods (0, 6, 24 and 48 hours) at two temperatures (2° and 15°C). All treatments were replicated four times. Within each replicate, 64 plants were cut in February 1980 from a crop of very wet kale yielding approximately 5.0 t DM/ha. Half the plants were kept whole and half chopped by machine, producing pieces of stem 1-3 cm long and bits of leaf 5-10 cm square, in a manner similar to that achieved by a forage harvester. Plants were sorted randomly into treatments of approximately four plants, which were weighed before and after wilting in order to measure any loss or gain of dry matter. After wilting, whole plants were partly chopped and sub-samples taken from each treatment for both freezing (-20°C) and oven drying (90°C). The frozen samples were freeze dried and stored for approximately six months before being analysed for SMCO by the autoanalysis technique and scanned by the InfraAlyzer 300 to predict nitrogen and moisture levels.

In trial G8, whole plants and chopped plants were wilted for 0, 24, 48 and 96 hours. The major differences in this trial from G7, were the longer wilting period and the wilting of all plants at 15°C for 18 hours per day and at approximately 30°C under lights for six hours per day. Treatments were replicated four times as in G7. In October 1981,

80 plants were harvested per replicate from kale yielding approximately 8.0 t DM/ha and half the plants were chopped by machine as previously. Ten plants were randomly chosen for each treatment and processed in the same manner as in G7, including weighings to measure changes in dry matter during wilting and samples being kept after wilting for both chemical and dry matter percentage determinations. Samples were stored approximately two months prior to being analysed for SMCO, N and moisture as in G7.

All trials were in a factorial randomised block design. Standard errors of means were calculated as previously.

Results and discussion

SMCO content:

SMCO content (g/kg DM) did not differ significantly ($P < 0.05$) between treatments in either G6 (mean = 7.4, SE = 0.09), G7 (mean = 14.5, SE = 0.46) or G8 (mean = 11.1, SE = 0.36). In G8, the main effect difference in SMCO levels (g/kg DM) between chopped (10.4) and whole kale (11.8, SE = 0.52) was significant at the 10% level of significance, however this trend did not occur in the other trials. As a consequence of SMCO levels not being significantly ($P < 0.05$) affected by chopping prior to freezing, it was decided that chopping would be used in all future trials in order to obtain representative samples from plots.

Trial G6 had fewer plants than were desirable for treatment comparisons, due to the large variation in SMCO between individual plants (see Section 3.1). In G7 and G8 plant numbers were adequate, especially for main effect comparisons.

The lack of effect of wilting on SMCO levels was in contrast to the conclusions of previous workers. In G7, 48 hours wilting of chopped

plants at a reasonably high temperature did not reduce SMCO, although possibly the very wet plants had some effect on the results. In G8, there were more plants per treatment and the wilting period was extended to 96 hours, but there was still no loss of SMCO. The only previous work that reported an actual reduction in SMCO with wilting, was with kale flowers (Allison and Borzuki, 1978). All other reports on effects of wilting were based on whether animals appeared to be anaemic on wilting or non-wilted kale (Gregor, 1952; NZDA, 1955). It is probable from these results that wilting kale for up to four days will not directly reduce the chances of an animal becoming anaemic in terms of available SMCO. Wilting may, however, affect other factors by reducing palatability, in which case an animal would probably eat less kale (Willey, 1964) and hence reduce its chance of becoming anaemic.

Dry matter content:

Dry matter contents were 13.0, 13.6, 14.7 and 14.7% after 0, 6, 24 and 48 hours storage respectively in G7. In G8, dry matter contents were 14.0, 14.8, 16.2 and 17.9% after 0, 24, 48 and 96 hours storage respectively. There was therefore some wilting after 48 hours in both trials, although the degree of wilting at this time was slightly higher in G8. Kale after 96 hours storage in G8 was much more wilted than kale stored for 48 hours.

Dry matter changes:

The actual loss of dry matter from plants was measured in terms of dry matter at the time of oven drying as a percentage of fresh matter harvested. This was not affected by any treatments in G7 (mean = 13.3%, SE = 0.09), although in G8 the main effect of wilting was significant ($P < 0.05$). Values in G8 were 14.0, 13.4, 13.0 and 13.0% respectively

after 0, 24, 48 and 96 hours wilting (SE = 0.22). In this trial, 24 hours wilting caused a significant amount of dry matter to be lost from the plants by respiration, although there was no significant loss between 24 and 96 hours wilting.

No dry matter was lost from plants after 48 hours wilting in G7, whereas in G8 dry matter was lost after a similar wilting period, probably due to a slightly higher rate of wilting in G8 as a result of a higher temperature. The higher temperature would have increased the rate of respiration and hence the dry matter loss.

These results show that harvested kale plants stored for up to 24 hours at a temperature greater than 15°C are likely to lose dry matter, although if stored at a lower temperature, there is unlikely to be any dry matter loss.

Nitrogen content:

Nitrogen levels were not affected by chopping either stems or leaves in G6, although the level in each of the 2 leaf treatments (mean = 2.99% DM) was significantly ($P < 0.05$) higher than that in any of the 3 stem treatments (mean = 2.28% DM, SE = 0.113). Chopping also had no effect on N in G7 (mean = 2.67% DM, SE = 0.043) and G8 (mean = 2.28% DM, SE = 0.058).

Wilting, however, increased N levels in both G7 and G8 as shown in Table 51. Wilting for 48 hours compared with no wilting, increased N by 14% and 26% respectively in G7 and G8. The greater increase in G8 was at least partly due to loss of dry matter in that trial, which would have helped increase nitrogen as a percentage of dry matter. Why 24 hours wilting significantly increased N levels in G7 but not in G8 is not known, although even in G8 the N content tended to increase after 24 hours. With N levels, there were no interactions between wilting and chopping or wilting and temperature in either trial.

TABLE 51: Main effects of wilting on nitrogen levels in kale in trials G7 and G8 (% DM).

Trial G7		Trial G8	
Time wilted (hr)	Nitrogen (% DM)	Time wilted (hr)	Nitrogen (% DM)
0	2.52 b	0	2.00 b
6	2.45 b	24	2.14 b
24	2.81 a	48	2.53 a
48	2.88 a	96	2.47 a
SE	0.086**	SE	0.116*

* and ** Main effects of wilting significant at 5% and 1% levels of significance respectively.

Conclusion:

SMCO levels in Maris Kestrel kale plants were not significantly ($P < 0.05$) affected by either chopping or wilting for up to 96 hours. Only in one out of three trials did chopping tend to reduce SMCO levels when the difference between treatments was significant at the 10% level of significance.

It was concluded that, in order to obtain representative kale samples from trial plots, plants could be chopped and mixed together before sub-sampling without risking a significant loss of SMCO. It would appear that probably neither chopping nor wilting would be practical methods farmers could use to reduce SMCO levels in kale. Previously reported beneficial effects of wilting appearing to reduce incidences of anaemia in animals grazing kale probably resulted not from SMCO levels being reduced, but may have been caused by wilting reducing crop palatability and hence reducing the intake of kale and SMCO.

Significant losses of plant dry matter were noted in one trial when kale was stored for 24 hours or longer at a temperature exceeding 15°C. Kale was stored for 24 hours at a lower temperature (2°C), however, without any loss of dry matter. Wilting also tended to increase N levels in kale. This was partly due to loss of dry matter with wilting.

3.6 Cultivar and Plant Component Effects

Introduction

Two field trials were carried out to examine SMCO levels in several kale cultivars and in different components within each cultivar. A specific aim of the trials was to study the relationship between SMCO in whole plants and SMCO in young leaves.

Experimental

- *a) Treatments and experimental design:*

In 1979, a field trial (F9) was laid down containing the four kale cultivars, Canson, Maris Kestrel, Merlin and Vulcan. Canson is a short thousand-head type kale, while the other three cultivars are short, medium and tall marrow-stem varieties respectively.

At harvest, plants within each plot were divided into five components. Young leaves, approximately one-third expanded, were first separated and the remainder of the leaves were divided into laminae and petioles, while stems were divided as evenly as possible into upper and lower portions. The trial was originally sown with two sowing dates and two plant spacing treatments as well as the four cultivars, all randomised in a factorial design within each of four blocks. Only a limited number of samples could be analysed for SMCO, however, due to shortage of facilities, hence the only results presented are from the four varieties in two of the blocks, together with the five components within each of the eight field plots.

A second trial (F10) in 1980, contained six kale cultivars and three blocks. The same four cultivars were used as in the previous year, plus Chrysol and Bittern. Chrysol is a French bred kale which has large erect leaves and Bittern is a new triple-cross variety developed from brussel sprout and marrow-stem kale lines.

Plants in each of the 18 plots were divided into the five components as in trial F9. This trial was sown with four blocks and harvested on two occasions, however again due to only a limited number of samples being able to be analysed for SMCO, results are presented for only three blocks at one harvest date.

- *b) Cultural details:*

Sowing was carried out with a Stanhay precision seeder (5 x 50 cm rows) on 30.5.79 (F9) and 9.5.80 (F10). Plot lengths were 10.0 m and intra-row seed spacing was 2.5 cm in both trials. Basal NPK fertiliser was applied to all plots prior to drilling at 157.78.78 kg/ha (F9) and 179.90.90 kg/ha (F10). With both trials, Treflan (48% trifluralin) was sprayed and incorporated into the soil prior to sowing. A steerage hoe was used to remove weeds four to six weeks after sowing. Seed was treated with Gamma Betoxin (a lindane-thiram mixture) to control flea beetle and Dursban granules (chlorpyrifos) were applied at sowing to control cabbage root fly.

Bulk soil samples (15-20 cm depth), six from trial F9 on 31.10.79 and four from trial F10 on 14.10.80, were analysed for soil sulphate by the method of Sinclair (1973). Mean soil sulphate levels were 10.9 and <0.5 ppm respectively for F9 and F10.

- *c) Harvesting details:*

In F9, approximately 15 plants were cut to 5 cm above ground level from each plot on 31.10.79. These plants were divided into the five components which were weighed, chopped and further sub-sampled for freezing (-20°C) and oven drying (90°C) for chemical and dry matter determinations respectively. From fresh weights and dry matter contents, the proportion of total dry matter yield for each component was calculated.

In early November 1979, an 8 m x 1.5 m (3 rows) area from each plot was harvested by a modified Maisprinz forage harvester. The chopped material was weighed and a sub-sample taken for dry matter determination in order to calculate the total dry matter yield.

In F10 on 11.12.80, plants within a sample area of 1.5 m x 1.5 m (3 rows) were cut to approximately 5 cm above ground, weighed and a sub-sample of approximately 12 plants taken for division into the five components. After the components were separated, they were weighed, chopped and sub-sampled for freezing and oven drying, as in F9.

- *d) Chemical determinations:*

The 40 samples in F9 and the 90 samples in F10 were analysed for SMCO by the autoanalysis technique approximately 11 and 7 months respectively after being harvested. The samples were analysed for nitrogen (N) and moisture contents on the NIR InfraAlyzer 300 at approximately the same time as being analysed for SMCO.

In both years, N in young leaves and laminae was predicted using the 1979 entire leaf calibration equation, while N in petioles, upper and lower stems was predicted using the 1979 entire stem calibration equation. The predicted N values (x) for young leaves, laminae, upper and lower stems were corrected by one of the following linear regression equations, derived from Figures 4 and 5.

Component	Regression equation
Young leaves	$Y = 0.70x + 1.59$
Laminae	$Y = 0.79x + 0.60$
Upper stems	$Y = 1.10x + 0.09$
Lower stems	$Y = 1.29x + 0.08$

The moisture contents of the freeze dried young leaf and lamina samples were predicted using the entire leaf moisture calibration equation, while the petiole, upper and lower stem samples were predicted using the entire stem moisture calibration equation. These calibration equations should have given reasonably accurate moisture contents according to Table 17, although young leaves and to a lesser extent laminae would, in retrospect, have both been better predicted using the whole plant moisture calibration equation.

- *e) Statistical methods:*

Each variable in the two trials was analysed by an analysis of variance, with cultivars in main plots (MP) and components in sub-plots (SP). Within whole plants, entire leaves and entire stems, cultivars were compared using a simple randomised block design incorporating only cultivars and blocks. Standard errors of main plots and sub-plots are presented with each split plot analysis. A main plot standard error, SE(MP), was formed by dividing the square root of the main plot error mean square by the square root of the number of sub-plots within a main plot. The sub-plot standard error, SE(SP), was formed by taking the square root of the sub-plot error mean square. When whole plants or entire leaves or stems were analysed in simple randomised block designs, the standard errors presented are the square roots of the error mean squares. From these standard errors, standard errors of differences and least significant differences can be calculated to compare means of any factor.

Results and discussion

- *a) Dry matter yield:*

The dry matter (DM) yields of cultivars and components for each trial are shown in Table 52. In F9, Vulcan was significantly ($P < 0.05$) higher yielding than Merlin or Maris Kestrel, these two cultivars were both significantly higher yielding than Canson. In F10, Merlin was significantly higher yielding than Maris Kestrel, but similar to Vulcan and Bittern. Canson and Chrysol were lower yielding than the other four cultivars. These trends confirmed the findings of Bradshaw (1981), who also reported Canson to be lower yielding than the marrow-stem cultivars, of which Maris Kestrel tended to be lower yielding than most of the other taller cultivars.

The proportion of total yield contributed by each component varied in different cultivars. The three highest yielding kales, Bittern, Merlin and Vulcan, consisted predominantly of stem, having only between 25 and 36% of their total DM yield as leaf (young leaves, laminae and petioles). In comparison, the proportion of total yield as leaf in Canson, Chrysol and Maris Kestrel averaged 60, 63 and 54% respectively. Within each cultivar, there was usually a similar yield of laminae and petioles and a lower yield of young leaves (Table 52).

- *b) SMCO:*

SMCO concentrations in whole plants, entire leaves and entire stems, are shown for each cultivar in Table 53. These concentrations were calculated by multiplying the SMCO concentration of each component by its proportion of total yield and adding the products. Whole plant SMCO levels were not significantly different between cultivars in either trial, although in F10, Chrysol tended to have a higher SMCO level than the other cultivars. SMCO in entire leaves did not differ

TABLE 52: Dry matter yields of kale cultivars and their components (t/ha).

Trial F9

Cultivar (MP)	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean	Total
	Component (SP)						
Canson	0.67	1.78	2.26	1.21	1.57	1.50c	7.49c
Maris Kestrel	0.70	1.89	2.30	1.69	2.83	1.88b	9.41b
Merlin	0.82	1.27	1.23	2.58	3.85	1.95b	9.75b
Vulcan	0.80	1.36	0.96	2.91	4.60	2.13a	10.63a
	d	c	c	b	a		
Mean	0.75	1.58	1.69	2.10	3.21	1.86	9.32

Significant effects: Cultivar**, Component**, Cult. x Comp.**

SE(MP) = 0.041; SE(SP) = 0.264; n = 40.

Trial F10

Cultivar (MP)	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean	Total
	Component (SP)						
Bittern	0.91	1.52	1.24	3.21	3.80	2.14ab	10.69ab
Canson	0.82	1.75	2.07	1.52	1.93	1.62c	8.09c
Chrysol	0.81	1.75	2.89	1.28	1.95	1.74c	8.68c
Maris Kestrel	0.84	1.59	2.03	2.14	3.31	1.98b	9.91b
Merlin	0.77	1.65	1.49	3.35	3.72	2.20a	10.98a
Vulcan	0.82	1.11	0.72	3.76	4.30	2.14ab	10.71ab
	d	c	c	b	a		
Mean	0.83	1.56	1.74	2.54	3.17	1.97	9.84

Significant effects: Cultivar**, Component**, Cult. x Comp.**

SE(MP) = 0.118; SE(SP) = 0.329; n = 90.

significantly between cultivars in F9, although in F10 the two leafiest varieties, Chrysol and Canson, had higher SMCO levels ($P < 0.05$) than Bittern, Maris Kestrel or Merlin. Within stems, Canson tended to have a lower SMCO level than the other cultivars, although differences were only significant in F9. Leaf and stem SMCO levels were similar in all cultivars except Canson, which had higher levels in leaf than in stem.

TABLE 53: Effect of cultivar on SMCO in entire leaves, stems and whole plants (g/kg DM).

Trial F9

Cultivar	Entire leaves	Entire stems	Whole plants
Canson	11.3	4.8 c	8.8
Maris Kestrel	8.2	6.8 b	7.5
Merlin	8.9	9.1 a	9.0
Vulcan	11.3	8.0 ab	8.9
Mean	9.9	7.2	8.6
n	8	8	8
SE	2.14	0.43**	1.21

Trial F10

Cultivar	Entire leaves	Entire stems	Whole plants
Bittern	7.7 c	7.9	7.8
Canson	13.4 ab	6.5	10.4
Chrysol	14.3 a	11.0	13.1
Maris Kestrel	8.1 c	10.3	9.3
Merlin	8.2 c	9.4	8.6
Vulcan	10.6 bc	9.8	10.1
Mean	10.4	9.1	9.9
n	18	18	18
SE	1.99**	2.64	1.89

TABLE 54: Effect of cultivar and plant component on SMCO concentration (g/kg DM).

Trial F9

	Component (SP)					
Cultivar (MP)	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean
Canson	24.3	12.0	6.6	7.9	2.4	10.6
Maris Kestrel	16.8	9.2	4.6	10.2	4.8	9.1
Merlin	13.1	8.2	6.6	13.7	5.9	9.5
Vulcan	16.6	10.7	7.5	15.4	3.3	10.7
	a	b	c	b	c	
Mean	17.7	10.0	6.3	11.8	4.1	10.0

Significant effects: Component**, Cult. x Comp.**

SE(MP) = 1.14; SE(SP) = 1.77; n = 40.

Trial F10

	Component (SP)					
Cultivar (MP)	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean
Bittern	9.6	8.2	5.5	9.7	6.4	7.9 c
Canson	27.5	12.7	8.3	7.1	6.0	12.3 ab
Chrysol	21.4	14.3	11.9	14.8	8.3	14.2 a
Maris Kestrel	13.4	8.0	5.9	10.3	10.3	9.6 bc
Merlin	16.7	7.2	4.1	13.0	6.1	9.4 bc
Vulcan	15.0	9.0	7.8	12.3	6.5	10.1 bc
	a	b	c	b	c	
Mean	17.3	9.9	7.3	11.2	7.3	10.6

Significant effects: Cultivar*, Component**, Cult. x Comp.**

SE(MP) = 1.72; SE(SP) = 3.06; n = 90.

SMCO concentrations in the five components in each cultivar are shown in Table 54. The higher entire leaf SMCO levels in Canson and Chrysol compared with the other cultivars were mainly due to higher SMCO levels in young leaves and laminae. There were large differences ($P < 0.01$) between components within plants and in both trials the relationship between components was similar. The main effect comparisons showed young leaves to be highest in SMCO, followed by laminae and upper stems with similar levels, while petioles and lower stems had lowest concentrations. This trend between components varied in some cultivars, although in most cultivars young leaves had highest SMCO levels and petioles and lower stems lowest levels. Canson had a lower SMCO concentration in its upper stems than in its laminae, whereas the tall marrow-stem cultivars, Vulcan and Merlin, had higher SMCO levels in their upper stems than in their laminae.

The total amount of SMCO in each component (kg/ha) is shown in Table 55. Total SMCO production did not vary significantly between cultivars, although there were significant differences between components and between cultivars within components. Chrysol and Canson contained between 69 and 80% of their SMCO in the leaf components, whereas Bittern, Vulcan and Merlin contained only 27 to 39% of their SMCO in leaves. Maris Kestrel was intermediate, having 56% and 39% of its SMCO in leaf tissue in F9 and F10 respectively.

These SMCO results show that the higher yielding cultivars, Bittern, Vulcan, Merlin and to a lesser extent Maris Kestrel, have no disadvantage in terms of high SMCO levels. Chrysol appeared to have a higher SMCO level than these cultivars and previous reports have suggested that thousand-head kale varieties, such as Canson, usually also have higher SMCO levels (Whittle *et al.*, 1976, Bradshaw and

TABLE 55: Effect of cultivar and plant component on SMCO production (kg/ha).

Trial F9

Cultivar (MP)	Component (SP)					Total
	Young leaves	Laminae	Petioles	Upper stems	Lower stems	
Canson	16.2	21.9	14.9	9.6	3.7	66.3
Maris Kestrel	12.1	17.3	10.6	17.2	13.7	70.9
Merlin	11.1	10.4	8.2	35.4	22.5	87.6
Vulcan	13.3	14.6	7.2	44.6	15.1	94.8
	bc	b	c	a	bc	
Mean	13.2	16.0	10.2	26.7	13.7	80.0

Significant effects: Component**, Cult. x Comp.**

SE(MP) = 1.80; SE(SP) = 4.42; n = 40.

Trial F10

Cultivar (MP)	Component (SP)					Total
	Young leaves	Laminae	Petioles	Upper stems	Lower stems	
Bittern	9.5	12.9	7.1	30.9	24.3	84.7
Canson	22.7	22.4	17.1	10.8	11.6	84.6
Chrysol	17.7	25.4	35.0	19.2	16.1	113.4
Maris Kestrel	11.5	12.4	12.2	22.3	33.8	92.2
Merlin	12.9	12.6	5.8	42.0	20.9	94.2
Vulcan	12.6	10.2	5.3	46.5	28.3	102.9
	c	c	c	a	b	
Mean	14.5	16.0	13.7	28.6	22.5	95.3

Significant effects: Component**, Cult. x Comp.**

SE(MP) = 4.15; SE(SP) = 6.73; n = 90.

Borzuki, 1981). The highest yielding cultivars had the majority of their SMCO in stems, which are often only partly eaten by grazing animals. In comparison, Canson and Chrysol had most of their SMCO in the leaf components, hence they are probably more likely to induce anaemia in animals grazing the crops. The greatest effects of SMCO on grazing animals occur during the first three to six weeks on kale (Barry *et al.*, 1981a). During this time, reduced liveweight gains and possibly deaths would be even greater on the leafy kale cultivars, Canson and Chrysol, especially if grazed in large breaks when predominantly leaf would be eaten. Grazing in smaller breaks and ensuring animals eat some stem would be the safest way to utilise these two leafy kales, during animals' first few weeks on the brassicas.

These results show that when kale varieties are being compared in terms of dry matter yield and SMCO content, there is an advantage in dividing plants into leaves and stems rather than making all comparisons on whole plants only. This is especially important when comparing reasonably leafy kale cultivars such as Canson and Maris Kestrel, which have similar total SMCO levels. Canson, however, has higher leaf SMCO levels than Kestrel but lower stem SMCO levels, hence animals grazing on Canson would probably be more likely to become anaemic than animals on Maris Kestrel.

These trials did not substantiate the results of field trials F3 and F4, which found that Maris Kestrel had a higher SMCO content than Vulcan. This was probably because there was not enough replication in trials F9 and F10 for accurate cultivar comparisons. This lack of replication was also a major reason for the sub-plot coefficients of variation for SMCO production being 27.6% (F9) and 35.3% (F10). These were high mainly due to the 17.7% (F9) and 28.9% (F10) coefficients

of variation in SMCO concentration. As well as the lack of replication resulting from there being only a limited number of samples that could be analysed for SMCO, these large errors were also partly due to reasonably high errors in sampling and analysing for SMCO (see Section 3.1). In F9, the 11-month delay between harvesting and analysing the samples for SMCO may have resulted in loss of SMCO from some samples (see Section 2.3). Any effects of SMCO loss, probably affected differences between components more than differences between cultivars. This would occur as a result of varying rates of SMCO loss in different components, due to different levels of SMCO. The relationship between components, however, was similar in both F9 and F10, hence any losses of SMCO probably had little or no effect on the results.

Correlation coefficients are presented in Table 56A for comparisons between SMCO levels in whole plants and SMCO levels in each component. The correlation coefficients between whole plant SMCO and young leaf SMCO were poor, being only 0.11 and 0.56* in F9 and F10 respectively. There was a shortage of plants on which to base such a comparison, especially in F9 (n=8), but the results suggest that an accurate comparison of kale cultivars can not be obtained by measuring SMCO in young leaves instead of in whole plants. This confirms findings published recently by Bradshaw and Borzuki (1981). The component most highly correlated with whole plants over both trials was petioles, however it is not practical to harvest petioles without destroying whole plants. In F10, where there was the greater number of plants (n=18), all three leaf components were significantly ($P < 0.05$) correlated with each other, but none of them were correlated with upper or lower stems. All five components in this trial were significantly ($P < 0.05$) correlated

with whole plant SMCO, although none of the correlation coefficients were very high.

TABLE 56: SMCO and nitrogen correlations between kale components.

A. Correlations between SMCO in components and SMCO in whole plants (r)

	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Whole plants
Trial F9 (n=8):						
Young leaves		0.45	0.13	-0.72*	-0.60	0.11
Laminae			0.62	-0.27	-0.20	0.53
Petioles				0.34	0.37	0.76**
Upper stems					0.37	0.20
Lower stems						0.09
Trial F10 (n=18):						
Young leaves		0.56*	0.51*	-0.10	0	0.56*
Laminae			0.49*	0.13	0.16	0.73**
Petioles				0.13	0.13	0.70**
Upper stems					0.30	0.56*
Lower stems						0.49*

B. Correlation coefficients relating SMCO and and nitrogen in components (r)

	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Whole plants
Trial F9	0.14	0.33	0.82*	0.23	0.29	0.80*
Trial F10	0.43	0.28	0.24	0.77**	0.38	0.79**

- *c) Nitrogen:*

Nitrogen levels for each cultivar and component are shown in Table 57. Whole plant N levels, calculated by multiplying the N level of each component by its proportion of total DM yield and adding the products, showed no significant differences between cultivars. There

TABLE 57: Effect of cultivar and plant component on nitrogen content (% DM).

Trial F9

Cultivar (MP)	Component (SP) Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean	Whole plants
Canson	4.23	3.40	1.41	2.28	1.70	2.61	2.34
Maris Kestrel	3.89	3.37	1.20	1.80	1.62	2.38	2.06
Merlin	3.95	3.69	1.93	2.34	2.04	2.79	2.47
Vulcan	4.48	3.96	2.21	2.42	1.69	2.95	2.43
Mean	a 4.14	b 3.60	d 1.69	c 2.21	d 1.76	2.68	2.32

Significant effects: Component**
SE(MP) = 0.117; SE(SP) = 0.314; n=40. SE= 0.131

Trial F10

Cultivar (MP)	Component (SP) Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean	Whole plants
Bittern	3.44	3.39	1.70	1.94	1.87	2.47	2.22
Canson	3.89	3.22	1.52	1.78	1.55	2.39	2.18
Chrysol	3.96	3.77	1.79	2.39	2.02	2.78	2.53
Maris Kestrel	3.83	3.24	1.66	2.16	1.94	2.57	2.30
Merlin	4.07	3.59	1.76	2.28	1.85	2.71	2.36
Vulcan	4.12	3.70	2.27	2.32	2.16	2.92	2.53
Mean	a 3.88	b 3.49	d 1.78	c 2.14	d 1.90	2.64	2.35

Significant effects: Component**
SE(MP) = 0.229; SE(SP) = 0.202; n=90. SE= 0.257

were large differences between components however. Within all cultivars, N was higher in young leaves and laminae than in the other three components. Measured over all cultivars, N was higher in young leaves than laminae, and lower in petioles and lower stems than in upper stems. The differences between components with N were similar to differences between components with SMCO.

The correlation coefficients relating SMCO and N levels in each component, over all cultivars, are shown in Table 56B. In whole plants in both trials, there was a significant correlation between SMCO and N, although in individual components, the relationship was only significant in petioles in F9 and in upper stems in F10. The higher correlation in whole plants than in most of the components was at least partly due to the greater accuracy of whole plant values which were each based on five component samples. This high correlation between SMCO and N in whole plants was found in several other trials in this thesis and its importance has been discussed previously. It is important to note that this relationship is not only true for comparisons of plots within a cultivar as found previously, but it also occurs when comparing cultivars. It appears less likely to occur in plant components, however, therefore it is mainly applicable when using whole plant levels.

- *d) Dry matter content:*

There were significant differences in whole plant dry matter content between cultivars, as shown in Table 58. Bittern and Canson had very high dry matter contents whereas Chrysol had quite a low content. Chrysol's low dry matter content was the major reason why its total dry matter yield was lower than that for the marrow-stem cultivars and Bittern, as its total fresh weight was similar to that in the other cultivars.

TABLE 58: Dry matter content of kale cultivars and their components (%).

Trial F9

Cultivar (MP)	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean	Whole plants
	Component (SP)						
Canson	12.4	16.6	11.3	15.1	18.2	14.7a	14.7a
Maris Kestrel	13.2	16.6	13.4	12.4	15.3	14.2a	14.4a
Merlin	12.0	15.6	10.3	11.3	12.0	12.2b	12.1b
Vulcan	12.4	14.5	9.6	10.4	13.8	12.2b	12.5b
Mean	b 12.5	a 15.8	c 11.2	b 12.3	a 14.8	13.3	13.4

Significant effects: Cultivar*, Component**, Cult. x Comp.* SE= 0.56*
SE(MP) = 0.52; SE(SP) = 1.04; n=40. n=8

Trial F10

Cultivar (MP)	Young leaves	Laminae	Petioles	Upper stems	Lower stems	Mean	Whole plants
	Component (SP)						
Bittern	15.7	15.6	14.0	17.7	18.8	16.4a	17.2a
Canson	13.7	14.9	13.2	16.6	19.5	15.6a	15.9b
Chrysol	9.6	13.7	8.8	11.4	13.9	11.5d	11.4e
Maris Kestrel	14.0	14.8	13.6	14.4	15.5	14.4b	14.6c
Merlin	13.8	15.1	12.2	13.0	13.4	13.5c	13.4d
Vulcan	13.6	14.8	11.6	12.4	12.9	13.1c	12.9d
Mean	d 13.4	b 14.8	e 12.2	c 14.2	a 15.7	14.1	14.2

Significant effects: Cultivar**, Component**, Cult. x Comp.** SE= 0.61**
SE(MP) = 0.49; SE(SP) = 0.65; n=90. n=18

There were significant differences in dry matter content between components and these differences varied depending on the cultivar. Highest contents generally occurred in lower stems or leaf laminae and lowest contents in petioles.

The importance of dry matter content in brassicas on the liveweight gain of animals grazing them is not clear, although probably animal liveweight gains would not be limited by the dry matter content of any of these six kale cultivars. Levels of SMCO and glucosinolates in kales are probably more important than any differences in dry matter content (Barry *et al.*, 1981a).

Conclusion

Canson and Chrysol kales had lower total dry matter yields than Bittern, Vulcan, Merlin and Maris Kestrel. The two lowest yielding cultivars, however, had a higher percentage of their dry matter yield as leaf components, than the other cultivars.

SMCO concentrations in whole plants did not differ significantly ($P < 0.05$) between cultivars, although in one trial Chrysol (13.1 g/kg DM) tended to have a higher SMCO content than the other cultivars (mean = 9.2 g/kg DM). Canson and Chrysol had higher levels of SMCO in their young leaves and laminae than the other cultivars, which resulted in approximately 73% of the SMCO they produced being in their leaf components. This compared with an average of 33% in Bittern, Vulcan and Merlin and 48% in Maris Kestrel leaves. It was considered that animals grazing large breaks of kale were more likely to become anaemic on Canson and Chrysol than on the other varieties. These results showed the desirability of dividing plants into leaves and stems when comparing kale cultivars in terms of dry matter yields and SMCO contents.

In all cultivars, SMCO concentration was highest in young leaves and lowest in petioles and lower stems, with levels in laminae and upper stems being intermediate.

There was a poor correlation between whole plant and young leaf SMCO concentrations in both trials F9 ($r = 0.11$, $n=8$) and F10 ($r = 0.56$, $n=18$), hence it was concluded that for comparing SMCO in kale cultivars, young leaves should not be used instead of whole plants.

Nitrogen levels were similar in all cultivars, although N was higher in young leaves and laminae than in upper stems, which generally had higher levels than petioles or lower stems. The trials showed that the significant correlation between SMCO and N, which was previously found to occur between plots of Maris Kestrel kale, also occurs when comparing kale cultivars at the same site.

The whole plant dry matter contents of the cultivars ranged from 17.2% in Bittern to 11.4% in Chrysol. Amongst the components, lower stems and leaf laminae had the highest dry matter contents and petioles the lowest.

CHAPTER IV

General Conclusions

1. SMCO in kale can be minimised by growing high density crops on soils low in available sulphate and N, and restricting applications of S and N fertilisers.

2. Applied S has little effect on SMCO in kale on free draining soils, with the exception of early autumn harvests. S is most likely to increase SMCO on soils which have impeded drainage and are low in available sulphate.

3. The effects of N fertiliser on SMCO are greatest when levels of available soil N are low. Generally N increases both SMCO and DM yield, therefore when N is restricted crop yield is usually also reduced.

4. SMCO content is reduced and dry matter yield increased by increasing the plant density within the range 10 to 80 plants/m^2. This effect on SMCO is greatest at later harvests and occurs on soils of varying fertility and drainage characteristics.

5. As plants age, leaf and whole plant SMCO levels increase, especially during the autumn. Stem levels are more consistent, although they tend to decrease during the autumn. The SMCO increase in whole plants with maturity is probably more related to floral initiation than frosting, as stems with small inflorescences contained more SMCO than non-flowering stems.

6. Kale sown early (before July) usually has a lower SMCO content at late autumn and winter harvests than a crop sown later (July). This is due to stems of later sown kale being higher in SMCO as a result of both the small reduction in stem SMCO with maturity and a higher nitrate level in later sown stems. The sowing date

effect does not affect earlier harvests when stems have less influence on whole plants.

7. Variation in SMCO between sites is mainly due to differences in plant N levels, presumably as a result of varying available soil N. Climatic factors, such as temperature and soil moisture availability, do not appear to affect SMCO. The relationship in mature kale between SMCO and plant N content over all trials and N treatments, showed a very high correlation. Plant N could therefore be measured in a kale crop prior to grazing and using this relationship, an estimate of the SMCO level could be calculated and the amount of crop fed to an animal adjusted accordingly.

8. SMCO levels in kale are not affected by either wilting for up to 96 hours, or finely chopping plants.

9. In cultivar trials, leafy kales such as Canson and Chrysol are usually lower yielding than marrow-stem varieties, and Chrysol in particular tends to have a slightly higher SMCO content. These leafy kales contain most of their SMCO in leaf tissue and animals fed with these cultivars are more likely to become anaemic than animals fed with marrow-stem varieties, especially if grazed in large breaks. SMCO content varies in different plant components, with young leaves having higher SMCO levels than laminae of older leaves and upper stems, while lower stems and petioles have the lowest levels.

   When comparing kale cultivars for SMCO content, comparisons should be based on whole plant rather than young leaf values. There is the added advantage, however, in measuring DM yield

and SMCO content in both entire leaves and stems, as this enables varieties with most of their SMCO in leaf tissue to be identified.

10. NIR analysis accurately measured N and moisture in several different kale tissue types and could be used routinely for these measurements in brassica breeding programmes. NIR estimations of SMCO in kale leaves and young leaves were acceptable for plant breeding purposes, however in stems and whole plants, only low and high SMCO samples could be separated. It was found that different calibration equations are required for most tissue types and that yearly corrections to the equations are required for all SMCO and some N measurements. These NIR studies were mainly based on Maris Kestrel kale. Further work is required using other kale cultivars. The effect of particle size of freeze dried and milled material on SMCO predictions also needs to be examined.

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APPENDICES

APPENDIX A: Sequence of events used in NIR analysis on Neotec 6350

1. SCANNING	
2. DATA TRANSFORMATION	(Spectral smoothing to reduce interference from electrical noise.)
3. REGRESSION ANALYSIS	(Determines regression wavelength combinations which best correlate with constituent of interest.)
4. PREDICTION OF UNKNOWN SAMPLES	(Comparison of reference with predicted values.)

APPENDIX B: Procedure for finding wavelength combinations which correlate highest with SMCO (whole kale calibration population.

Stage 1: Two wavelength combinations (correlation coefficients)

Target forward regression pair combination: r = 0.62

Wavelengths	Individual correlation	1120	1220	1260	1416	1620	1884	1914	2016	2114	2174	2268	2318	2432
1120	0.52	-	-	-	-	-	-	-	-	-	-	-	-	-
1220	0.52		-	-	-	-	-	-	-	-	-	-	-	-
1260	0.52			-	-	-	-	-	-	-	-	-	-	-
1416	0.05				-	0.62	-	0.64[1]	-	0.61	-	0.62	0.60	-
1620	0.50					-	0.64[2]	0.64[3]	0.63	-	-	-	-	0.63
1884	0.05						-	0.64[4]	-	0.62	0.60	0.63	0.62	0.60
1914	-0.18							-	0.66[5]	0.64[6]	0.62	0.64[7]	0.64[8]	0.64[9]
2016	-0.01								-	0.62	0.61	0.63	0.63	0.61
2114	0.51									-	0.60	-	-	0.63
2174	0.43										-	0.69[10]	-	-
2268	0.50											-	0.64[11]	0.61
2318	0.44												-	0.63
2432	0.27													-

All correlations below 0.60 have been eliminated for clarity.

From the above, 11 combinations were tuned as follows (overleaf).

APPENDIX B (cont):

Selection number	Initial combinations	Correlation coefficient (r)	Tuned combinations	Correlation coefficient (r)
1	1416 + 1914	0.64	1426 + 1914	0.65
2	1620 + 1884	0.64	1620 + 1836	0.66*
3	1620 + 1914	0.64	1616 + 1918	0.64
4	1884 + 1914	0.64	1886 + 1912	0.64
5	1914 + 2016	0.66	1924 + 1986	0.70*
6	1914 + 2114	0.64	1924 + 2092	0.64
7	1914 + 2268	0.64	1924 + 2242	0.64
8	1914 + 2318	0.64	1924 + 2320	0.64
9	1914 + 2432	0.64	1924 + 2404	0.65
10	2174 + 2268	0.69	2184 + 2212	0.77*
11	2268 + 2318	0.64	2266 + 2294	0.67*

* The best four pair combinations were taken to the next stage.

Stage 2: Three wavelength combinations

Target forward regression three wavelength combination: $r = 0.63$

Selection number	Initial combinations	Correlation coefficient (r)	Tuned combinations	Correlation coefficient (r)
2	1620 + 1836 + 1810*	0.71	1618 + 1828 + 1810	0.72
5	1924 + 1986 + 1426*	0.71	1924 + 1980 + 1426	0.72
10a	2184 + 2212 + 1844*	0.79	2184 + 2212 + 1844	0.79
10b	2184 + 2212 + 2096*	0.79	2184 + 2208 + 2086	0.80
11	2266 + 2294 + 2156*	0.72	2260 + 2290 + 2150	0.75

* Wavelengths chosen by forward regression. With number 10, two different wavelengths were chosen after forward regression, for forming three-way combinations.

All five three wavelength combinations were taken to the next stage (overleaf).

Stage 3: Four wavelength combinations

Target forward regression four wavelength combination: $r = 0.65$.

Selection number	Initial combinations	Correlation coefficient (r)	Tuned combinations	Correlation coefficient (r)
2	1618 + 1828 + 1810 + 1784*	0.75	1618 + 1820 + 1810 + 1792	0.75
5	1924 + 1980 + 1426 + 1702*	0.78	1918 + 1986 + 1430 + 1702	0.79
10a	2184 + 2212 + 1844 + 2070*	0.83	2184 + 2208 + 1844 + 2070	0.83
10b	2184 + 2208 + 2086 + 1808*	0.82	2184 + 2208 + 2074 + 1808	0.83
11	2260 + 2290 + 2150 + 1702*	0.76	2260 + 2290 + 2150 + 1702	0.76

* Wavelengths chosen by forward regression.