Onobrychis viciifolia Scop.
(syn. *O. sativa* Lam.)

Sainfoin

A STUDY OF GROWTH AND MANAGEMENT OF SAINFOIN (*Onobrychis viciifolia* Scop.)

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ABSTRACT

This thesis reports on studies designed to examine the production patterns of sainfoin (Onobrychis viciifolia Scop.) subject to defoliation. Dry matter yield estimates, seasonal distribution of production, plant disease and long term survival were considered for three sainfoin cultivars in a field trial run over a three year period. Assimilate partitioning and closer examination of plant morphology was studied using two of the cultivars grown under controlled environment conditions.

Sainfoin was successfully established in the spring period and early growth rates and dry matter production were at least as good as those of lucerne. All cultivars grown showed a tendency towards relatively early flowering when compared to lucerne. Only Fakir, the earliest flowering of the three cultivars, regrew sufficiently to be harvested a second time under the conservative harvesting regime imposed to maximise uninterupted growth during the establishment year. The results of the two different cutting heights provided little evidence to support the higher of the two cuts (12-15cm). The main outcome was to leave about 25% of the dry matter behind as stem with little gain in residual leaf area to provide a photosynthetic surface for regrowth.

Subsequent growth and regrowth cycles in the field gave yields of up to 12 t/ha of herbage dry matter in a single season. All cultivar and management combinations tended to show poor autumn growth, with the plant adopting a rosette habit when lucerne was still actively growing. Also while the cultivar and management combinations gave a variable number of harvests in a given season, there was no evidence to support any one

management approach as being superior in production. The later maturing cultivar Melrose did provide some indication that higher yields may be possible from a later maturing plant, but also provided evidence of the potential for marked leaf loss when the plant was maturing under drying conditions. Leaf loss aggravated the situation of low leaf area indices that was shown for all three sainfoin cultivars.

Plant losses in the field trial resulted in uneven stands and contributed to the sampling variability which reduced the sensitivity of the experiment. While some of the losses were possibly related to the plants consistently harvested at a vegetative stage, all sainfoin plants were susceptible to a crown-root rot complex. This was indicated by necrosis of the crown tissue and vascular tissue of the tap-root which was often extensive and extending well below ground level.

Controlled environment studies provided further evidence of the poor regrowth ability displayed in the field. This would appear to be a result of a combination of the poor development of any new shoots to provide a start point for regrowth, little leaf area remaining at the base of the plant after harvest to provide a photosynthetic surface, and losses of root and nodule tissue from the plant after the stress of harvest. This tissue was subsequently replaced, possibly at the expense of top growth. Movement of assimilates to the root system did not tend to support any hypothesis of a build-up phase which may have interacted with management.

Indication of the within-cultivar variation for sainfoin was clearly shown under the controlled environment conditions. Multivariate analysis of the data provided a preliminary estimate of the gains that might be possible if certain groups of attributes formed the base of a selection programme. Future prospects for sainfoin in grassland farming were proposed in the light of this information, and that gained from the field trial. These focused on the need to more fully evaluate sainfoin as a species, or group of species, and establish its demands rather than assume it will conform to the management model provided by other summer active forage crops.

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

Sainfoin (*Onobrychis viciifolia* Scop.), is a summer active forage legume that has attracted particular attention in New Zealand due to its quality to ruminant animals. The major area of attraction is that sainfoin does not cause bloat,a widespread and expensive problem with dairy cattle grazing summer pastures containing other actively growing legumes such as white clover, red clover and lucerne. Estimates for the New Zealand dairy industry of an annual cost of about $17 million (Towers, 1984), arising from deaths, production losses and control costs, provide an indication as to the basis for producer enthusiasm for a plant like sainfoin.

The non-bloating character has been in part attributed to the presence of condensed tannins in the plant tissue (Jones and Lyttleton, 1971; Reid and Clarke, 1974), and also to the lack of cell wall disruption in the rumen (Howarth *et al.*, 1979). An associated effect of the tannins is the protection of the protein from rumen degradation, and most studies indicate that this results in improved nitrogen utilisation by the animal (Thomson *et al.*, 1971; Ulyatt *et al.*, 1977; Ulyatt and Egan, 1979). Another key consideration for any forage species is the acceptability by the animal, and under various feeding conditions, it has been observed that voluntary intake for sainfoin is invariably very high (Ulyatt *et al.*, 1977; Parker and Moss, 1981; Wilman and Asiedu, 1983).

To this array of feed quality features, which would seem enough in themselves to encourage further consideration of sainfoin, it is also important to examine other attributes. In North America, where lucerne is the major legume, pest problems and difficulties with dryland sowings have led to an examination of alternatives. The result of this has been the release of several cultivars (Eslick et al., 1967; Hanna et al., 1970; Melton, 1977), and an improvement in the understanding of crop management (Ditterline and Cooper, 1975). More recently, New Zealand and Australia have displayed some interest in sainfoin due to its comparative resistance to aphids which have devastated large areas of lucerne (Lance, 1980). Some work has also continued in Great Britain where a few farmers with thin calcareous soils have persisted with sainfoin cultivation, and also a little ongoing research has been inspired (Doyle et al., 1984). It is of interest that the starting point for studies on sainfoin has in many cases been due to a recognisable inadequacy in lucerne, as distinct from the need for a greater understanding of the general biology and agronomy of a forage legume that has been utilised variably over a similar period of recorded history.

This is not, however, just the result of a lack of interest but rather that sainfoin has displayed a number of problems under experimental and production situations. Of particular importance is the relatively poor yield, regrowth and persistance that is recorded in many comparative studies, mainly with lucerne. Associated with these features, and in some cases perhaps causal, are reports of root disease, nutritional disorders, poor and irregular nodulation, and poor nitrogen fixing ability. Unfortunately, the unifying factor with many of the problems is their unpredictability between the various experiments and locations which tends to make any single approach to problem solving difficult. This is best illustrated by using seed production as an example. In North America it has been shown that seed yields of up to 1400 kg/ha are possible for sainfoin (Ditterline and Cooper, 1975), and yet poor seed production is given as one of the reasons for the reduction in breeding effort in France (Chesnaux and Demarly, 1970). Just why this apparent contrast exists is not clear, but the end result tends to be moderately expensive seed which can have a major influence on the cost structure of establishing the crop (Scott, 1979; Doyle et al., 1984).

While the list of problems associated with sainfoin seem to rapidly balance and even outweigh the benefits, it should be clearly stated that many of the problems are not unique to sainfoin. Indeed, if the history of lucerne is even briefly examined (Hanson, 1972), it can been seen that breeding and selection has been continuously aimed at producing solutions to a similar group of inadequacies. There is no reason to suspect that plant performance for sainfoin cannot be eventually improved further by a clearer understanding of the problem areas.

Therefore the objectives of this work on sainfoin were

a) To consider factors contributing to establishment and early growth of sainfoin under local field conditions.

b) To examine the morphological characteristics of three reasonably diverse cultivars of sainfoin and to evaluate their response patterns to various harvest regimes. The reponse patterns were measured in terms of dry matter production, seasonal distribution of production, and seasonal indicators of perennial performance such as plant disease incidence and plant survival.

c) To determine patterns of assimilate partitioning between plant components in defoliated sainfoin.

d) To identify plant components that determine the more productive sainfoin plants and to consider the relationships that may exist between components.

These objectives required the establishment and maintenance of a field trial which yielded data over three consecutive spring and summer periods. The trial used Melrose, Remont and Fakir sainfoin under different harvesting regimes, and also plots of Saranac lucerne were grown to provide some comparative data from this better documented species. The second stage of the work involved a pot experiment conducted under controlled environment conditions. Only Fakir and Melrose sainfoin were used for the examination of growth and regrowth patterns, and the distribution of assimilates as indicated by ^{14}C techniques. While the field trial gave an indication of the variability that existed both within, and between sainfoin cultivars, the data from the plants grown under the constant conditions of the controlled environment facility provided a clear picture of the extent of specific component variation.

CHAPTER 2: LITERATURE REVIEW

2.1 BACKGROUND

From its origins in the near Eastern Centre e.g. Turkey, Iran, Transcaucasica (Vavilov,1951) sainfoin has spread eastwards to mediterranean and central Europe and as a result has probably been cultivated in most European countries. As a component of native pastures, Onobrychis spp. date back several millenia (Hely and Offer,1972) but European cultivation has only been recorded for about 400 years (Chorley, 1981).

The actual area of sainfoin currently cultivated is difficult to ascertain but interest is most active in Italy (Orsi,1978), Romania (Varga,1968), the Soviet Union (Andreev,1963), Canada (Hanna et al.,1972) and the United States (Ditterline and Cooper,1975). Surveys of abstracts (C.A.B.,1982) would indicate that only eastern Europe and the Soviet Union regard sainfoin as a significant component of their grassland based agriculture.

Reasons for the apparent lack of interest in sainfoin are not immediately clear. Possibilities such as lack of opportunity for accidental introduction (Hely and Offer,1972), poor adaption to the changing needs of agriculture (Hutchinson,1965), inadequate evaluation of the basic requirements for plant growth, and misleading visual estimates of the plants worth due to its stemmy nature (Eslick,1968) have all been suggested.

In addition, the success of lucerne as a summer growing forage legume has probably led to some neglect of possible alternatives. Because of major similarities between lucerne and sainfoin, reference to lucerne will often be made in this review. Direct comparison, however will tend to favour lucerne in many cases, as it rightly should, considering the vast research effort that has gone into the plant over the past 100 years (Hanson,1972).

A confounding factor when examining any legume is alluded to by Hutchinson (1965), is that for many areas, particularly Europe, the total area of all legumes has declined markedly during the twentieth century (Rogers,1975). This is due to the overall intensification of the agricultural system, the ready access to cheap artificial nitrogen and the higher levels of management skills required to maintain legume productivity. As Rogers (1975) points out, the energy crisis of the 1970's has made it fashionable to extol the virtues of legumes in terms of value to ruminant animals and contribution to the nitrogen economy of grassland and subsequent crops. Obviously with a declining area of legumes sown there needs to be some caution in separating the fashion and economic realities.

In some agricultural systems, economics force closer examination of alternatives. The development of sainfoin research programmes in the United States and Canada provides an example. The areas involved have traditionally relied on lucerne as the main summer forage legume and have hot dry summers and extremely cold winters. Interest in sainfoin arose because of the need for dryland legumes and also a possible substitute for lucerne on irrigated areas where weevil (Hypera postica) infestation was a problem (Hanna et al.,1972; Ditterline and Cooper,1975). Over the past 20 years these programmes have contributed information on the biology of the plant, management problems and cultivar development. While it is quite clear that sainfoin does have problems, one has only to examine lucerne's recent history to see a large number of improvements from plant introduction and breeding programmes (Hanson,1972). It is not unreasonable to expect that further gains can be made with sainfoin.

2.2 Nomenclature

Any study of sainfoin literature encounters the problem of nomenclature. This arises for several reasons

i) There is a large number of Onobrychis species, and varieties within species, frequently with poor descriptions (e.g. Chapman and Yuan (1968), Darlington and Wylie (1955)).

ii) There are both perennial and annual Onobrychis species (e.g. Simmonds (1976)).

iii) There is a range of common names that appear to encompass a number of species (e.g. Kernick (1978)).

iv) Within cultivated Onobrychis viciifolia, early and late or common and giant types are sometimes assigned separate varietal status (e.g. Stebler and Schroter (1889)). Giant and common sainfoin are taxonomically indistinguishable, and the recognition of a plant as belonging to one or the other variety depends on growth behaviour (Thomson,1938). Giant types generally have the ability to flower at least twice in a growing season, while the common types flower once and then adopt a rosette habit. Comparison is sometimes made with the single and double-cut varieties of red clover (Trifolium pratense) which are well established groupings (Spedding and Diekmahns,1972).

v) Sinskaya (1955) has described an approach where new species are established on the basis of biological and ecological differences rather than distinctive morphological characters. While these differences may be apparent in a given locality there is no reason to expect them to remain so in markedly different environments, resulting in questions as to the validity of separate species status.

vi) At times there is question as to whether the genus referred to in some literature is even correct (e.g. Chorley,1981).

It may well be that the genus Onobrychis, particularly cultivated forms, covers a range of freely crossing species that results in a complex array of hybrids as documented for cultivated lucerne (Medicago-sativa-falcata-glutinosa complex) (Lessins and Gillies,1972). Only more detailed taxonomic studies would verify this and also perhaps indicate interspecific crosses that may be of value.

In this study the name sainfoin will refer to Onobrychis viciifolia unless stated otherwise.

2.3 Morphology

Sainfoin is an upright perennial legume. It develops hollow stems with a concentration of nodes at the soil surface which form the crown of the plant. The axillary buds that develop at these nodes give rise to subsequent generations of new stems. During early development and in winter the stem remains compact and the plants appear as rosettes. Branching of sainfoin stems appears to be quite uncommon.

The leaves, borne on long petiole are imparipinnate with 5-14 pairs of leaflets and a terminal leaflet. Size of leaves may vary markedly from small and compact in the rosette stage, to large and open leaves on the upper nodes of an elogated stem. With some varieties of sainfoin, a range of leaf sizes is often present concurrently, resulting in a relatively open appearance of the canopy.

The inflorescence is an erect raceme with 5-80 papilionaceous florets, and is borne on a long axillary stalk. Flowers are usually a deep pink colour, but white flowers have been observed and used as genetic markers in pollination studies (Knipe and Carleton,1972). Pollination occurs through intermediary insects, mainly honeybees. The crop is so attractive to bees that is held in high regard for honey production (Dubbs,1968). Following pollination, pods form which are single seeded, indehiscent and have marked reticulate ridges. The seed itself tends to be kidney shaped and olive brown in colour and is somewhat larger than those of other forage legumes. Seed weights range from 13.2 - 16.8g per 1000 seeds for milled seeds to 18.2 - 23.6g per 1000 seeds for unmilled seed.

The root system of sainfoin develops from a single seedling taproot which may branch in older plants. Numerous fine lateral roots normally develop from the taproot and these provide sites for nodule development. The nodules range considerably in size from about 1mm spheroid structures to large (3x6mm) and wedge shaped, and orange-white in colour. Reference to the depth and extent of the root system is variable. Comparison is often made with lucerne (*Medicago sativa*) which is well known for having a deep taproot and a root system capable of exploring a large volume of soil *eg*. Spedding and Diekmahns (1972).

2.4 Physiology

2.4.1 Seedling growth and development

Viable sainfoin seed germinates rapidly and produces vigorous seedlings (Hanna *et al*., 1977). Over a range of temparatures from 5 to 25° C, Townsend and McGinnies (1972) found sainfoin to be relatively temperature insensitive for total germination. Other studies have shown that while low temperatures may reduce the rate of germination (McElgunn, 1973), sainfoin still had the ability to germinate at near freezing temperatures (Young *et al*.,1970).

The earliest emergence of sainfoin seedlings and most rapid development is associated with lines of larger seeds (Fransen and Cooper, 1976). When the cotyledons emerge in these lines, the seedlings can derive energy from photosynthesis while still utilising stored reserves (Cooper, 1977). During this phase the total weight of the seedling decreases due to respiratory losses. Cooper and Fransen (1974) have shown that this can be about a 38% loss over the first nine days for sainfoin. However any estimates of weights or remaining reserves after a given time are primarily affected by sowing depth (Black,1959).

Fransen and Cooper (1976) have also shown correlations between leaf area of first leaves and seed weight, which they suggest is a reflection of the rapid development of seedlings from heavier seed. Another factor that is evident with the first leaf produced is a variable number of leaflets. While it is usually simple, two, three or four leaflets are not uncommon (Thompson,1938; Cooper,1974). Despite variation in leaf area of these different types of leaves, Cooper (1974) has found no evidence to suggest improved plant performance in selecting a given type.

2.4.2 Vegetative growth

Sainfoin seedlings usually produce about six leaves before any stem development becomes noticeable. At this stage lateral buds develop, each producing numerous leaves from the internodes. With the giant, or double-cut types the upper internodes of some of the buds elongate at this stage resulting in a plant with relatively sparse upper foliage and a tuft of leaves at the base (Thompson,1955). The common or single-cut types also produce an array of lateral buds but elongation is delayed. This results in the prostrate appearance that characterises the early season growth of these types (Thompson,1955). In some cases, individual plants from northern latitudes may not elongate in the first year (Sheely,1977).

In a comparative study of a single and a double-cut type, Cooper (1972) has shown that the single-cut type maintains a greater proportion of the plant mass as leaf and also achieves a higher leaf area index. Cooper (1972), suggests that this enables the single-cut types to efficiently intercept the increasing amounts of energy available with increasing daylengths which results in high yields from a single harvest. Despite this difference that occurs in leaf area development and total leaf area between sainfoin cultivars, it is pertinant to note that when compared with lucerne, sainfoin has a much lower leaf area index (Sheehy and Popple, 1981). This is more associated with the very much higher specific leaf area (cm^2/g) for lucerne, rather than sainfoin being a poor producer of leaf. Indeed, Sheehy and Popple (1981) have shown that the yield advantage of lucerne, at a given harvest is almost entirely due to a greater stem mass. For this reason they suggest selecting sainfoin with a greater specific leaf area. Attempts at modifying specific leaf area for improved photosynthetic efficiency have been made for lucerne (Delaney and Dobrenz, 1974), without particular success. This may be in part due to strong environmental interactions which effect specific leaf area (Cooper and Qualls,1967; Christian,1977), and so the modification approach may have merit if interactions can be minimised in experiments.

In line with the theory of Monteith (1965), Sheehy and Popple (1981) have shown that for the low leaf area of sainfoin, the prostrate crop utilises light efficiently for photosynthesis. This adds support to Cooper's (1972) suggestion that the more prostrate single-cut types may be more efficient at intercepting light than the more upright double-cut types. These ideas may need to be considered if selections were made on the basis of erectness (e.g. Rumball,1982).

2.4.3 Flower and Seed Development

Many authors have made general comments about the flowering patterns of sainfoin, particularly the fact that the common types may not flower in the seeding year (e.g. Thomson, 1938). The only detailed study on floral initiation and development was conducted by Sheely (1977).

Sheely (1977) noted four distinct reproductive stages for the range of sainfoin cultivars examined. These stages included a) an increase in angle to the horizontal of leaves coupled with bud swelling, b) stem elongation, c) inflorescence initiation, and d) development of the inflorescence to maturity. There is some question (Lang, 1965) as to whether any stages prior to initiation are reproductive stages. This is partially true for sainfoin where the cultivar Common displays an increase in leaf angle but progresses no further, and the cultivars Melrose and Krasnodar had some plants that showed stem elongation but no inflorescence initiation (Sheely, 1977). However, as there were strong correlations between measurements of the two earlier stages and flowering, Sheely (1977) maintained them as reproductive stages.

From further data of Sheely (1977), it would appear that when the plants are subject to short days and low temperatures, floral initiation precedes the altering leaf angle and stem elongation. This might be expected to occur in the first year if sowing is in autumn or particularly early in spring, and may explain some reports of common types flowering in the first season (Fagan and Rees, 1930). Otherwise the normal pattern of reproductive development for sainfoin would seem to require some low temperature pretreatment, especially for common types and any originating at extreme northern latitudes, followed by daylengths of about 14 hours or longer.

Floret maturation occurs from the base of each flower spike and is evident when the floret becomes perpendicular to the rachis. As well as good nectar production, the explosive tripping of the staminal tube that occurs in lucerne is not a feature of sainfoin, and so the plant is very attractive to honeybees. Once pollination has occurred, seed development is rapid. The actual flowering period is relatively limited (St John-Sweeting, 1980) and this may reflect a finite number of initials developed under cool conditions (Sheely,1977). It would appear for sainfoin that seed maturity is an important physiological state as new basal shoots do not start to develop in uncut plants until this stage (Cooper and Watson, 1968).

2.5 Cultural Aspects

2.5.1 Adaptation

The initial problem encountered in examining the adaptation of sainfoin is due to the range of ecotypes and species (Section 2.2) that have been described in most situations except the tropics. A further complication is that some of the more detailed studies have used either a limited range of cultivars (e.g. Koch *et al.*,1972) or a limited range of environments (e.g. Evans,1961).

As sainfoin occurs in localities ranging from Mediteranean regions with hot, dry summers to northern latitudes with severe winters, it is reasonable to assume that climatic requirements will vary considerably depending on the origin of the type or cultivar. This variation can be well illustrated by flowering time (Sheely,1977) and winter survival (Hanna and Smoliak,1968; Eslick _et al._,1967), both of which are dependant on latitude and altitude of origin.

However, a common factor with sainfoin is that it appears to be well adapted to drought in terms of morphological features and time of maximum growth (Koch _et al._,1972). The adaption to short growing seasons is also demonstrated by the ability of sainfoin to germinate and grow at low temperatures and thus withstand an early change to a hot, dry climate (Young _et al._,1972). Because of these characteristics, United States workers (Ditterline and Cooper,1975) recommend sainfoin for areas with a minimum rainfall of about 330mm. The upper limit would be based on relative merits of other legumes as the climate becomes more temperate and also the likelihood of waterlogging which has been shown to adversely effect sainfoin quite rapidly (Heinrichs,1970).

For best growth sainfoin is reputed to require free-draining soils that have an alkaline pH and are high in lime (Spedding and Diekmahns, 1972). Stebler and Schroter (1889) suggest that the reason that calcareous soils are most suitable for sainfoin is simply that they satisfy physical conditions for growth better than other soils. This idea is supported in studies where sainfoin has been successfully established in acid sand (St John-Sweeting, pers.comm.) and was also grown in vermiculite using a nutrient solution of pH 6.2, without any apparent adverse effects (Smoliak _et al._,1972). However as nutrient toxicity may occur at lower pH levels, this factor cannot be ignored. Rorison (1965) notes that sainfoin is excluded from certain acid grasslands, in the United Kingdom, mainly because of the toxic effect of aluminium in the soil solution.

2.5.2 Establishment

As there is a recent extensive review on the establishment and development of legume seedlings, including frequent reference to sainfoin (Cooper,1977), only certain specific aspects will be discussed here.

The high seeding rates recommended for sainfoin may make establishment costs very dependant on seed price (Scott,1979; Doyle et al., 1984). Rates of 55-60 kg/ha for milled seed and 90-100kg/ha for unmilled seed have been suggested by Spedding and Diekmahns (1972). However, with 1000 seed weights of 13.2 - 16.8g for true seed and 18.2 - 23.6g for milled seed (Spedding and Diekmahns,1972), the seeds are somewhat larger than those of many other commonly used pasture or forage legumes e.g. lucerne 2.15g/1000 seeds (Gunn,1972). The recommended lucerne seeding rates of about 10kg/ha (Langer,1973), are therefore similar to sainfoin rates in terms of numbers of seeds sown. Unless it can be shown that the larger seeds of sainfoin enhance the chance of seedling survival, or that improved cultural practices can reduce the seeding rates, the problem will only be overcome by minimising costs of seed production. This will be a function of management and seed yielding ability of a given cultivar.

Sowing depth is of importance as it, and seed size determine emergence rate (Cooper,1977). Despite its large seed size, sainfoin does not emerge well from deep sowings and so planting depth should be approximately 1-2cm (Hanna et al.,1972). In instances where sowing depth has accidentally been increased e.g. loose, sandy soils, emergence was noticeably poorer and delayed, resulting in severe weed competition (Fortune, unpublished data).

The next factor is whether the indehiscent pod should be removed prior to sowing as it has been shown to contain germination inhibitors in laboratory germination tests (Carleton et al.,1968; Smith,1979). However these have not shown any effects on field establishment (Carleton et al., 1968). Due to its persistent nature, the seed pod can also damage the developing root and thus provide a site for disease infection (Sears,1974). Perhaps the best argument for removing the pod is that of Thomson (1952), who showed that the friction of milling reduces the number of hard seeds. Also milling would permit mechanical sorting of seed to remove shrivelled seed and result in more even seed lines as well as reducing the weight of material for transport. Even given these reasons for removing the pod, most seedings in the United States are made with the pod intact (Ditterline and Cooper,1975), and so while it may be desirable to remove the pod it cannot be regarded as essential.

An important consideration at seeding for any legume is the use of an inoculum. As sainfoin Rhizobium appears specific (Burton and Curley, 1968), new areas of cultivation require seed inoculation with viable bacteria prior to sowing. Even when this criterion is met, there are still problems with both lack of nodulation, and ineffective nitrogen fixation by apparently well nodulated plants (Ditterline and Cooper,1975; Hume,1981). However the situation is complicated as deficiency symptoms may occur at any stage of growth, but do not always occur under cultivated conditions (Hanna et al.,1972). Observations of nodule presence and function in native habitats indicate a reasonable symbiotic relationship (Hely and Offer,1972), which suggests factors other than strain of Rhizobium may be causal. A possibility is mineral nutrition, which for other legumes has known effects on the symbiotic relationship resulting in an array of growth defects and foliar symptoms (Epstein,1973).

Finally there is the question of sowing time which will depend on the local circumstances. In cooler climates, spring sowing will probably be most successful and also prevent some of the problems of early weed control as the plant will be able to grow rapidly as the temperatures increase (Ditterline and Cooper,1975). However if summer conditions are particularly harsh as in Mediterranean climates, autumn sowing will have an advantage (St John-Sweeting, pers. comm.).

2.5.3 Weed Control

Despite the vigorous growth of young sainfoin plants they are often regarded as poor competitors with weeds (Bland,1971; Ditterline and Cooper, 1975). The reason for this is not clear as sainfoin has a similar early growth rate to lucerne (Smoliak et al.,1972) which does not seem to suffer such a severe problem with early weed ingress. One possibility is that with sowing rates generally being about 35kg/ha (Hanna et al.,1972; Ditterline and Cooper,1975), the plant populations are not sufficient to effectively smother the weeds. However, some of the taller types currently available may produce insufficient leaf area to shade weed seedlings even when high plant populations are present (Fortune and Withers,1980).

Some care should be exercised with herbicides as, being a relatively minor crop, only a limited number have been extensively tested. Currently such chemicals as trifluralin, 2,4-DB, MCPB and cyanazine appear reasonably safe (James and Atkinson,1978), and no doubt others will become available with further testing. It is possible that judicious grazing will also offer some degree of weed control as responses to grazing become better understood. An example of this is provided by Kilcher (1982) where heavy grazing reduced the strong competition from the grass component of the sward and enhanced both yield and persistence of sainfoin.

2.5.4 Fertiliser

Studies on fertiliser application suggest that sainfoin has a slightly lower demand for major elements such as potassium (Spedding and Diekmahns, 1972) and phosphate (Roath and Graham,1968) than lucerne. The low phosphate requirement may be related to the high cation exchange capacity of sainfoin roots and their ability to extract phosphorus from the soil (Fox and Kacar,1964). The effect of sainfoin on the soil phosphate availability is further reflected by the lack of phosphate response in subsequent cereal crops sown on the same area (Fox and Kacar,1964; Kernick, 1978).

Owing to the previously mentioned problems of poor nitrogen fixation ability (Section 2.5.2), small amounts of nitrogen have often been applied but the responses have been varied (Meyer,1975; Smoliak and Hanna,1975). These variable responses to applied nitrogen may be in part due to the considerable variation reported in the nitrogen fixation rates of sainfoin as measured by acetylene reduction techniques (Major et al.,1979; Hume, 1981; Krall and Delaney,1982). It is unfortunate that this problem has not been resolved as it places limitations on the evaluation of sainfoin yield, unless nitrogen was to be totally supplied as fertiliser.

Further complications arise when micronutrient requirements are considered, particularly when heavy applications of lime are used for pH modification. For example other legumes such as lucerne (Sherrell and Toxopeus,1978) and trigonella (Trigonella foenum-graecum) (Molgaard and Hardman,1980) can suffer moderate to severe boron deficiency under these circumstances. Many of the symptoms associated with boron deficiency (Epstein,1973) may have been observed in sainfoin and attributed to other factors e.g. altered nutrient uptake effecting root and shoot growth (Ross and Delaney,1977).

Associated with nutrient requirements of the plant is the effect of nutrients on herbage quality. As the promotion of sainfoin is centred on quality aspects it is important to consider recent work with Lotus pedunculatus, another legume with high levels of condensed tannins in the herbage (Barry and Forss, 1983). Barry and others (Barry and Duncan, 1984; Barry and Manley,1984) have shown that soil fertility can markedly effect the concentration of tannins in the herbage. In particular the herbage from low fertility sites contained higher concentrations of condensed tannins than that from fertile areas, and therefore the pattern of digestion in the ruminant animal tended to differ. While the structure of tannins in sainfoin is different from those in L. pedunculatus, it would seem possible that their concentration in the plant tissue could be effected in a similar manner, resulting in quality variation.

2.5.5 Defoliation and Herbage Dry Matter Production

2.5.5.1 General Concepts

Defoliation is defined by its frequency, intensity, uniformity and timing in relation to the development phases of plants or swards (Harris, 1978). While regrowth is influenced by these factors, genetic differences, and the weather conditions during the harvest and post-harvest period, the interactions between all factors creates difficulties with the description of optimal management strategies (Vickery,1981).

Therefore, examination of influences on regrowth after defoliation is necessary in understanding the plants overall performance. Success in maintaining growth will be dependant on genetic differences in growth rate, plant capacity for tillering, maintenance of leaf canopy, accumulation of carbohydrate and other reserves, and rate of recovery of root growth and nutrient and water uptake (Harris,1978; Humphreys,1981).

An understanding of these factors will help in designing a management that will be useful in ensuring long-term stand survival and production of a quanitity of high quality forage for either conservation or direct consumption by grazing animals.

2.5.5.2 Sainfoin

As sainfoin is adapted to areas that limit growth to the period from spring to mid-summer (Hanna _et al._,1972; Koch _et al._,1972), and as it has been used for haycutting with aftermath grazing (Spedding and Diekmahns, 1972), the crop has generally been cut at an advanced flowering stage. This practice has been successful in maximising yield as regrowth is often poor. Also any decline in quality as sainfoin matures is small (Baker et al.,1952; Koch et al.,1972), and even at advanced stages it does not appear prone to leaf loss (Carleton et al.,1968). For the late flowering or single-cut types, this timing also coincides reasonably well with the natural emergence and extension of any new basal shoots (Cooper and Watson, 1968).

Because of the trend towards late cutting, there has been little emphasis on other management possibilities which may be desirable in areas where rainfall can be adequate for continuous summer growth and grazing is often used as well as conservation. To improve the options available a combination of improved regrowth in the later flowering types (Varga,1968; Carleton,1968; Melton,1977), and improved longevity in the early flowering or double-cut types that do have the potential for rapid regrowth (Chesneaux and Demarly,1970) may be required. Even with these constraints it has been shown that the late flowering types can tolerate cyclical cutting (Thomson,1951; Percival and McQueen,1980), which can encourage regrowth and result in a more even distribution of yield through the season. However, despite the management used for the early flowering types which can produce yields of as much as 15t D.M./ha/year (Chesneaux and Demarly,1970), they suffer from rapid decline in yield and may only be useful for 2-3 years (Evans,1961). Just why there is this apparent linkage between regrowth ability and longevity is not clear but may be related to the way in which assimilates are internally distributed and utilised. In the only study on this aspect, Cooper and Watson (1968) have shown that for a late flowering type (cv. Eski), total available carbohydrates (T.A.C.) in the roots do not reach peak levels until seed maturity. A more recent study (Krall and Delaney, 1982) has also indicated that the levels of T.A.C. are low when compared with those found in lucerne. Despite the limited nature of these studies, the possibility is raised that regrowth will be affected by T.A.C. reserves and that frequent, severe cutting may result in a depletion of these reserves. Percival and McQueen (1980) have shown that for the relatively late flowering cultivar Melrose, a frequent cutting regime can result in both a decline in yield and plant numbers. Therefore, until this area of management is more fully understood, caution would have to be exercised with early, frequent cutting.

Until some of the plant factors involved in the problem of poor regrowth are resolved, it is likely that defoliation practices will have to be related to the growth stage of the plant that offers a balance between yield and long term survival of the stand. Flexibility may be introduced by choosing a range of early to late flowering types.

2.5.6 Mixtures

Mixtures make better use of the area by either providing a better quality material with a maximum yield, or extending the period of time over which material is available for grazing or conservation. The possibility of combining sainfoin and lucerne to reduce bloat was a novel idea, but the high palatability of sainfoin (Smoliak and Hanna, 1975) could necessitate some form of separation of the two species. Also, if the yield of sainfoin was too low, drenching was still a more viable proposition for bloat control with lucerne (Scott, 1979). Grasses such as timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and cocksfoot (*Dactylis* glomerata) have shown promise in the United Kingdom (Spedding and Diekmahns,1972). More vigorous grasses should be avoided as they are too aggressive and effect stand survival. Kilcher (1982), has shown that sainfoin did not persist well beyond two years when grown in a mixture with Russian wild ryegrass (Elymus junceus), even though some improvement was noted if seeding was in alternate rows.

One mixture that has shown potential in the United States has been sainfoin and birdsfoot trefoil (Lotus corniculatus), where the mixture produced greater yields than either species grown alone (Cooper,1973; 1979). Cooper (1973) attributes some of this gain to the growth patterns of the two species, with sainfoin being most active in the earlier part of the season and birdsfoot trefoil in mid-summer. Apart from these points of interest, mixtures do complicate management, and so at present effort may be better spent on understanding sainfoin in monoculture.

2.5.7 Seed Production

As high seeding rates seem to be important for successful establishment, seed costs for sainfoin can be a major expense. Low production resulting from poor seed set has been reported in France (Chesnaux and Demarly,1970). The situation elsewhere suggests that, with reasonable management, the seed yield can be as much as 1400kg/ha (Ditterline and Cooper,1975). This results from a combination of both prolific flowering and high seed weight (Carleton and Weisner,1968). The attraction of sainfoin to bees means that it is a very useful honey crop and that pollination presents no particular management difficulties (Dubbs, 1968).

In general seed harvesting and handling does not appear to cause any unique problems. Seed should be harvested at about 40% moisture as after this seed shedding can occur (Carleton et al.,1967). Long term viability appears to be a problem with storage (Rogers,1975). Germination tests on imported seeds would tend to support this observation (Fortune, unpubl, data), as batches of seed have been found with germination rates of considerably less than 50% and getting progressively worse with retests, even when stored under cool-room conditions.

2.5.8 Pests and Diseases

One advantage of sainfoin is that it generally seems resistant to a number of pests that can attack other species, and in particular lucerne (Lance,1980). Potential pests have been examined by Wallace (1968) and include some sitona weevils which can attack the root system and the sainfoin bruchid (*Bruchidis unicolour*) which can cause extensive damage to the developing seed head. Field observations would indicate that while many pests seem to avoid the foliage, the seed head is the target of a number of species of insects (Fortune, unpubl.).

The crown and root rot complex appears to be the main disease problem as it can cause a rapid decline in stand density, particularly under irrigation (Sears et al.,1975). However, this complex is not clearly understood as it was initially thought that *Fusarium solani* was the main organism involved (Sears et al.,1975), but more recent studies suggest the causal organisms are bacteria (Gaudet et al.,1980). Despite these uncertainties with the epidemiology, Auld et al., (1977) have shown potential for selection of disease resistant types.

2.6 Genetics and Breeding

One of the first problems encountered in considering aspects of genetics and breeding with sainfoin is that mentioned in Section 2.2, the question of taxonomic identity. This is further complicated by the occurrence of both diploid and tetraploid species with chromosome numbers of 14 and 28 respectively (Chapman and Yuan, 1968; Simmonds, 1976). A detailed study by Heyn (1962) showed additional chromosomes (16 and 32) for a pair of related *Onobrychis* species and also highlighted some of the problems in separating species on morphological grounds. Obviously if a large scale breeding programme was to be undertaken these factors would have to be taken into account as there may be incompatabilities if certain crosses are attempted.

As sainfoin is usually cross-pollinated (Thomson, 1938), a variety will be composed of a collection of genotypes. The degree of heterogeneity will depend on the number and constitution of the basic plants, isolation from foreign pollen and rogueing of off-types. While self-pollination can occur fairly readily (Knipe and Carleton, 1972), the effects of inbreeding appear immediately. Thomson (1938) reported poor seedling establishment and vigour and Rogers (1975) reported reductions in yield, fertility and disease resistance and an increase in abnormalities after only one generation of selfing. However, the ease of inbreeding may be useful in genetic studies (Rogers, 1975).

Most of the breeding efforts to date with sainfoin have been aimed at improving the dry matter yield and regrowth ability by phenotypic selection of elite plants followed by a polycross of the selected plants (Carleton, 1968; Hanna,1968; Varga,1968; Melton,1977; Rumball,1982). Superior winter hardiness has also been of particular importance at more extreme latitudes (Hanna,1968,1980). There is only limited published evidence of any heritability estimates for characters subject to selection (e.g. Varga, 1968). Despite the small scale of the breeding efforts thus far, there are a number of cultivars registered and maintained in various countries.

2.6.1 Population evaluation and data handling

In establishing any preliminary breeding or evaluation programmes, the handling and recording of data from an enormously variable array of material presents some unique problems. Some of these involve the pure logistics of growing the material and some are associated with the treatment of accumulated data. While the logistics will often be an economic question, the treatment of data is vital to understanding plant performance. To aid in data exploration and interpretation there is a range of univariate and multivariate techniques. With increasing availability of computing facilities and power, the multivariate techniques are now both feasible and particularly suited to data sets encountered with plant introduction work. The data reduction available with the techniques can be useful in clarifying taxonomic groupings as well as indicating associations between various recorded plant characters (Pearce,1969; Williams,1983). Useful examples of this approach are provided by Broschat (1979) for the use of principle components analysis and Hayward et al.,(1982) for pattern analysis. As the techniques often involve a number of possible algorithims and general approaches to achieve a solution, there is some conflict over which of the procedures is superior. However, this should not detract from the usage of multivariate methods as long as the biological or ecological basis for the groupings seems reasonable and may be tested by further measurement or experimentation.

CHAPTER 3: THE PERFORMANCE OF SAINFOIN IN THE ESTABLISHMENT YEAR

3.1 Introduction

As sainfoin was essentially a new introduction to New Zealand there was a conflict between using a large, diverse germplasm collection or a limited array of material to be examined in more detail. The former approach presents logistics problems if more than basic measurements and scores are taken or if the management involves more than one or two basic treatments. The latter approach, investigation of a more limited array, permits management flexibility and more detailed measurements but does have the danger of poor initial germplasm selection. Since the programme was initiated in response to questions concerning management and production, and D.S.I.R. did have a germplasm collection under examination (Rumball, 1982), the choice of a more limited base was made.

The next two problems confronted were of a more practical nature and related to the anticipated pattern of use by the farmer. The first was whether to use sainfoin as a monoculture and the second was the choice of cutting or grazing the trial. In both instances the choice was made to keep the trial as simple as possible. By using monocultures competitive effects were limited, and by mechanical harvesting the problems of treading, non-uniform deposition of excreta and selective grazing were avoided. While this approach places limitations on the extrapolation of findings, it does avoid the interactions that may have resulted (Watkin and Clements, 1978).

The main aim of the first season was to examine crop establishment as indicated by plant development and performance. As it was intended to be the start of a trial that was to run for several seasons, any management in this season tended to be conservative. With the relative paucity of field establishment data for New Zealand, the trial provided some guidance with issues like sowing time, sowing rates and herbicide usage that may be of use in planning future work.

3.2 Experimental

The field trial was established in October, 1979 at Massey University, Palmerston North, New Zealand (latitude 40.23S, longitude 175.37E, 34m a.s.l.).

Palmerston North has a cool temperate climate with an annual average rainfall of 1000mm that is well distributed throughout the year (Appendix 1). Temperatures range from about 14.8/6.6 °C (mean daily maximum and minimum), in early spring and late autumn, with a summer peak of 22.3/12.8 °C in February. The only noticeable deviation from 50 year averages for the establishment year was a low February rainfall, and a high rainfall for March. The latter resulted in minor overnight flooding of the trial site in early April which only caused deposition of removable debris.

The soil at the trial site was a Manawatu fine sandy loam. This was a free draining soil underlain by gravels at a depth of 0.5 to 1.0m. In the preceding spring the trial area had been ploughed and about 2000 kg/ha of lime applied and worked into the soil. By the time the area was reploughed for sowing the trial in October, 1979, soil tests showed a pH of about 6.8 and adequate levels of phosphorus and potassium.

Immediately prior to sowing trifluralin was applied to give pre-emergent weed control. Sainfoin plots of about 60m^2 (20x3m) were sown with a cone-seeder at a calculated rate of 60 kg pure live seed/ha. Similar size plots of Saranac lucerne were sown at the same time at 10 kg/ha to provide comparative information. All seed was coated with suitable Rhizobium in a peat slurry before sowing. The herbicides 2,4-DB and dinoseb acetate were used in the young stand to control a range of weeds. An application of nitrogen (20kg N/ha) as calcium ammonium nitrate was also made to help avoid the possibility of nitrogen deficiency in the seedlings which had been indicated as possible by other workers (e.g. Hanna et al., 1972).

3.2.1 Cultivar Description

Having made the decision to limit the cultivars used in this trial (Section 3.1), the cultivars were then chosen to represent a range of flowering times and regrowth potentials.

Fakir was bred in the mediterranean climate of southern France and was expected to be early flowering and have the ability to regrow rapidly (Chesnaux and Demarly, 1970).

Remont was bred in Montana, U.S.A. in an attempt to provide a more uniform yield distribution by improved regrowth than had been possible with the "single-cut" cultivar Eski (Cooper, 1972). This situation is similar to that observed with the English cultivars Cotswold Common (single-cut), and Giant (double-cut) and therefore may also result in variation in longevity (Evans, 1961; Spedding and Diekmahns, 1972). Remont was expected to be later maturing than Fakir.

Melrose was bred in Canada at about the same time as the Montana programme was working with Remont (Hanna et al., 1970). The objective was to produce winter hardy plants with the ability to regrow after cutting. Work of Percival and McQueen (1980), in New Zealand indicated that it would be a useful choice for a late-flowering type.

The lucerne cultivar, Saranac, was a highly recommended commercial cultivar for New Zealand at the time the trial was established (Janson, 1979).

All sainfoin seed was obtained from its country of origin and local supplies of lucerne seed were used.

3.2.2 Experimental Design and Procedure

The trial was set up as a randomised complete block design incorporating three cultivars, three cutting patterns and two cutting heights. Only one management treatment was used for the lucerne check-plots, and all treatments were replicated three times.

The following were the cutting pattern treatments

1) Early/Early (E/E) The plants were cut as the first floral buds developed. The reasoning for this cutting pattern was to attempt to maximise cutting frequency and maintain high quality material. However in light of other work (e.g. Percival and McQueen, 1980) it was also anticipated that this treatment might diminish stand vigour and longevity.

2) Full/Early (F/E) The plants were permitted to reach full flower on the first cycle of growth each season, and subsequent harvests were as for E/E. Full flower was when about 90% of the plants had at least one expanded floret that was perpendicular to the rachis. With this treatment there was an attempt to simulate the conditions under which a forage crop might be used for a high yielding hay or conservation crop with subsequent grazing of the regrowth.

3) Full/Full (F/F) The plants were permitted to reach full flower on successive growth cycles before any harvesting occurred. This treatment was to permit the plant's growth cycle to be as little impaired as possible, without actually going to the stage of producing seed.

Lucerne was cut to coincide with the emergence of new basal shoots, or at about 1% flower as recommended to maximise production (Janson, 1982).

The cutting treatments were two heights, about 4-6cm and about 12-15cm above ground level, with lucerne only being cut at the lower height. These were based on the expectation of leaving differing amounts of leaf tissue (i.e. leaf area), crown and stubble tissue, and numbers of nodes to provide starting "capital" for regrowth. The management for the first season was to permit the plants to grow through to full flowering to ensure uninterrupted plant development. This meant that only Fakir was cut for a second time.

Main harvests were taken from $10m^2$ sub-plots pegged within the $60m^2$ plots. Estimates of early establishment growth rates from sowing were made on individual plants harvested from the field by cutting below the cotyledonary node. Plant counts were done using a $0.1m^2$ quadrat at a number of random points within the main plots as well as within the measurement sub-plot. Excess plot area was harvested to the desired height with a flail-type machine which permitted complete removal of the cut herbage. The sampling area was then cut using a sickle-bar mower which left the herbage intact. This was then immediately picked up by hand raking and weighed in the field. Large sub-samples (about 5kg), were then taken and sealed in plastic bags for subsequent processing in the laboratory. Most sampling was attempted in the morning to avoid higher temperatures and all samples were well shaded until removed from the field. Some of the late summer growth was lost when the plot area was cleared of superficial debris after flooding.

In the laboratory the field sub-samples were divided up to determine the sainfoin and weed component, and a bulk sample dried at 80° C to determine moisture content. Similar samples of sainfoin stems were stripped of their leaves to conduct leaf area determinations using an automatic leaf area meter.

3.2.3 Data Analysis

The design of the experiment was a randomised complete block with six management treatments (3 cutting patterns at each of 2 heights) and three cultivars. For the first season only the two cutting heights were used for cultivar comparisons.

Analysis of variance was used to determine differences between treatments and cultivars, and linear regressions were used to examine growth until flowering. The nature of the data was such that natural logarithmic transformations were satisfactory for the regression analysis. T-tests were used to examine whether slopes of regression lines were similar (Steel and Torrie, 1960). All statistical analysis was conducted using major packages such as SPSS (Nie et al., 1975) and BMDP (Dixon et al., 1981).

3.3 Results

3.3.1 Primary Growth

The data for the establishment phase (Table 3.1, Fig 3.1) mainly shows the pattern of vegetative growth and stem elongation as the final individual plant samplings were made before any flower or seed development. Despite variation in seed size between lucerne and sainfoin, the pattern of growth over the first eleven week period was similar (Table 3.2) and while all regressions are highly significant, comparisons between slopes (relative growth rates) are non-significant. The plot of weight per plant (Fig 3.1) shows that the three sainfoin cultivars are similar at each sampling time, while the differences between sainfoin and lucerne are often significant.

The marked decline in relative growth rate for both sainfoin and lucerne between days 42-55, as shown in Table 3.1, was in response to the desiccating effects of the herbicide dinoseb acetate. The difference shown for the subsequent time period (55-70 days), may also have been a reflection of this, but it is also indicative of the earlier stem elongation that occurred for Remont and Fakir.

Table 3.1 Relative growth rates (g/g/week) over the first eleven weeks from sowing.

	Sample Period (days)				
Cultivar	21-32	32-42	42-45	55-70	70-76
Melrose	0.86	0.70	0.40	0.37	0.43
Remont	0.80	0.70	0.25	0.57	0.63
Fakir	0.81	0.76	0.19	0.61	0.30
[a] S.E.	0.07	0.06	0.04	0.05	0.15
[b] Sign.level	n.s	n.s	*	*	n.s
[c] LSD (5%)			0.15	0.18	
Lucerne	0.87	0.57	0.33	0.66	0.35

a - Standard error of treatment means within a harvest date.

b - Significance levels: probability of statistical differences:

*<0.05; **<0.01; ***<0.001.

c - Least Significant Difference.

Table 3.2 Regressions with yield against time during first eleven weeks of growth (yield = ln yield).

Cultivar	Constant	Slope	R^2
Melrose	-3.03	0.07	0.96
Remont	-3.18	0.08	0.98
Fakir	-2.92	0.07	0.97
Lucerne	-4.05	0.08	0.98

- all regressions significant (p<0.001)

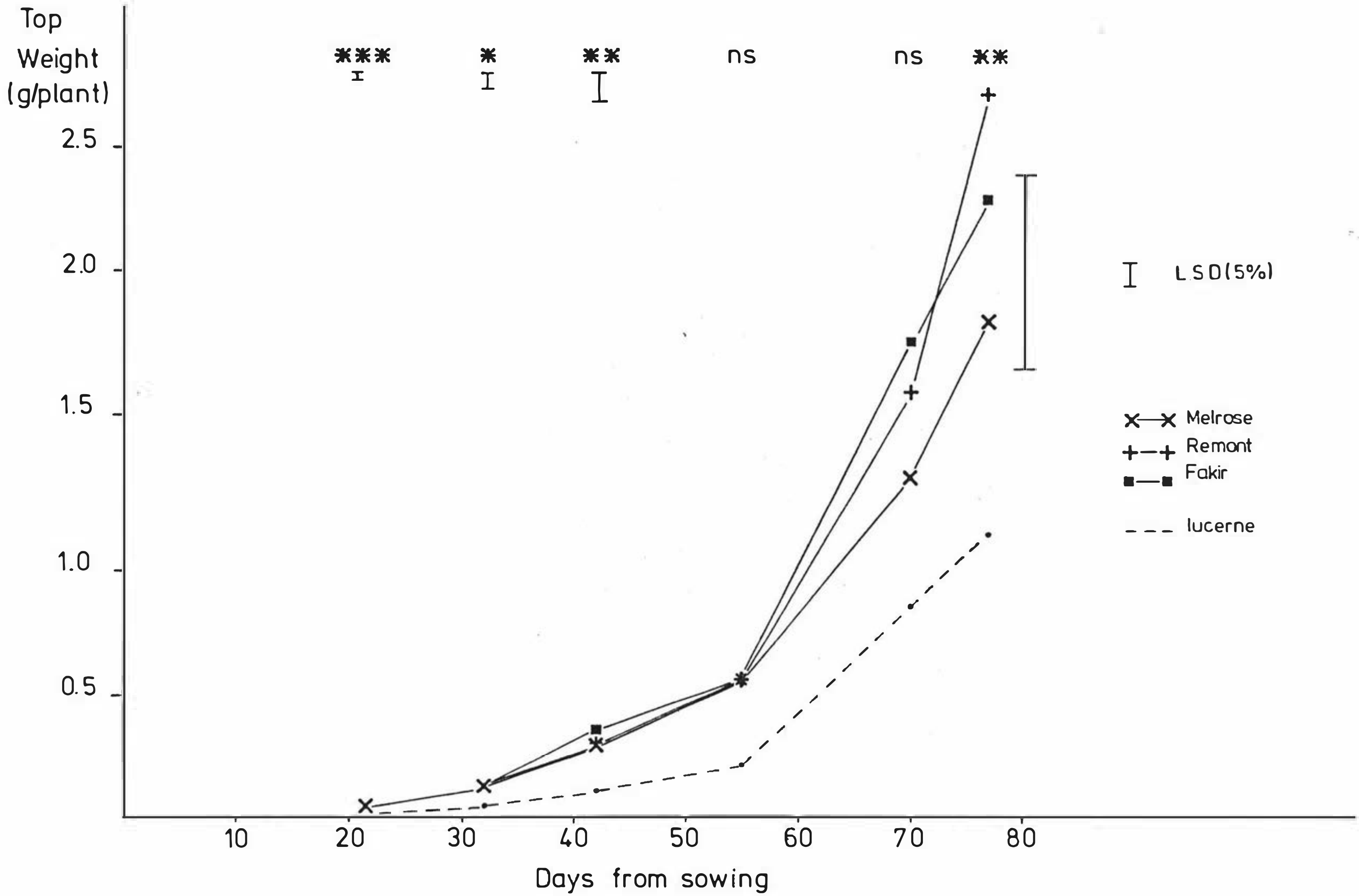


Fig.3.1 Top weights of plants sampled over first eleven weeks of growth

3.3.2 Main Harvests

Both cutting height and cultivar effects were significant for the first main harvest (Table 3.3a). In all cases the yield from the low cut was considerably higher than the 12-15cm cut, but as will be shown later, this difference was principally due to stem material. In each case, Melrose was superior to the other cultivars which performed similarly at the low cut, but with Remont being poorest under high cutting.

Table 3.3a

Main harvest yields (kg DM/ha) of sainfoin for two cutting heights.

Cultivar	High (12-15cm)	Low (4-6cm)
Melrose	3561	4206
Remont	2045	3252
Fakir	2882	3509
SE	220	218
Sign. Level	***	*
LSD (5%)	642	638
Lucerne	-	3742

Table 3.3b

Mean growth rates (kg/ha/day) for main plots for low cutting height (4-6cm) only.

	growth rate	days growth
Melrose	40.8	103
Remont	36.1	90
Fakir	29.5	119 #
SE	2.2	
Sign. Level	*	
LSD (5%)	6.4	
Lucerne	41.6	90

Note: Fakir data is the sum of two harvests with similar growth rates for each period. Therefore only the total is presented.

The growth rates for the period up to full flower (Table 3.3b), were a reflection of the total herbage accumulated and the time to reach maturity. The late maturing Melrose had the maximum mean growth rate for the period concerned. This compared favourably with lucerne which was cut at an earlier stage of reproductive development. The greater growth rate of Melrose was not just the result of stem accumulation as can be seen from the higher leaf to stem ratio shown in Table 3.4.

Table 3.4

Ratio of leaf to stem combined over both harvest heights.

Cultivar	Leaf/Stem
Melrose	1.11
Remont	0.59
Fakir	0.64
SE	0.04
Sign. Level	***
LSD (5%)	0.11
Lucerne	0.89

The leaf area index (LAI) of each of the sainfoin cultivars at full flower differed significantly and all were lower than that measured for lucerne (Table 3.5). This lower LAI was associated not so much with a particularly low leaf mass when compared with lucerne, but rather a low specific leaf area (SLA, cm^2 /g) as also shown in Table 3.5. The lower SLA for Melrose was influenced by dry conditions at harvest resulting in some wilting of samples, and from earlier preliminary samples, might have been of similar order as the other two cultivars for much of the growth period. Therefore the LAI for Melrose may have been an underestimate of that which applied for much of the growth period.

Table 3.5

Leaf Area Index (LAI) and Specific Leaf Area (cm^2/g leaf) as combined means of both cutting heights.

	L.A.I.	S.L.A.
Melrose	1.84	94
Remont	1.08	111
Fakir	0.94	117
S.E.	0.06	2
Sign. Level	***	***
LSD (5%)	0.16	6
Lucerne	3.28	189

While the higher cutting did leave significantly more residual leaf area than the low cutting (Table 3.6), the total area of leaf remaining after harvest was very small and represented less than $10g/m^2$ of leaf tissue in the best case.

Table 3.6

Residual leaf area (cm^2/m^2) remaining on stubble after harvesting.

	High (12-15cm)	Low (4-6cm)
Melrose	460	140
Remont	560	90
Fakir	1210	310
SE	120	30
Sign. Level	***	***
LSD (5%)	350	100

Only Fakir regrew sufficiently after the first harvest to permit a further cut at full flower. The remaining sainfoin plants tended to adopt a rosette habit and produce little more than compact leaves and showed no internode elongation. Lucerne was subject to aphid infestation which appeared to cost some autumn production.

3.4 Discussion

The combination of limiting cutting to when cultivars reached full flower, and the unusually dry February resulted in only Fakir being sufficiently rapid in its cycle of growth and development to reach a second harvest. This, however, did not preclude the gathering of certain basic information about the field establishment of sainfoin and achieving a desired aim of some familiarity with the species under local conditions.

From the establishment data covering the first eleven week's growth it was apparent that the early growth and development of sainfoin can be at least as fast as lucerne. This is in agreement with other work (Smoliak *et al.*, 1972) conducted under similar temperatures but in a greenhouse. One problem that was evident was the temporary slowing of growth caused by herbicide damage to leaves from dinoseb acetate, a recommended herbicide for sainfoin (Ciorlaus, 1975). While no long term effects were apparent, this did provide an example of one of the problems of handling a new species and following recommendations developed elsewhere.

The time of peak flowering for the three sainfoin cultivars was quite different but in general all were early when compared with lucerne. The higher yields of leafier herbage from Melrose may well indicate the desirability of the later flowering material under New Zealand conditions, assuming Melrose is representative of this type. Even Fakir, which managed to maintain a similar growth rate over two harvests, totalling 119 days of growth, did not outyield Melrose. This would appear to have been due to its rapid reproductive development which seemed to inhibit further vegetative growth to some degree.

Sainfoin had a much lower LAI than lucerne, which was in part due to the production of thicker, fleshier leaves by all cultivars. This resulted in a low SLA, a feature that has been observed in other studies (Cooper and Qualls, 1967; Sheehy and Popple, 1981). Therefore, while it may be possible to improve leaf area by selection of plants with a greater leaf mass, it may also prove useful to look for plants with greater SLA. However, caution may be necessary as it has been suggested that there is a link between lower SLA and improved photosynthetic efficiency in species such as lucerne (Delaney and Dobrenz, 1974). This point will be expanded in subsequent chapters with additional supporting data.

The effect of cutting height appeared to be a reduction in yield approximately in proportion to the difference in remaining stem. In the mature stands the lower nodes supported little leaf area. With an internode distance of between 5-15cm, the difference between the two cutting heights in terms of residual leaf area may have been due to either one small leaf of part of the one mature leaf. The apparently greater residual leaf area for Fakir may have been due to better survival of basal leaves in the canopy due to the comparatively low leaf area, as indicated by LAI, permitting greater light penetration to lower leaves. As there were also difficulties in achieving uniformity in plots cut at a high level, especially in removing all flower buds and thus removing potential effects of apical dominance, the examination of cutting height was eliminated from subsequent experiments.

The high stem yield of sainfoin, particularly Remont and Fakir, may not be critical as evidence suggests that stem material is of reasonable nutritive value and more readily grazed than lucerne stems (Parker and Moss, 1981). It may well be that it is a problem of visual perception as argued by Eslick (1968), as other comparative experiments have shown that the yield advantage of lucerne over sainfoin is principally due to a greater stem yield (Sheehy and Popple, 1981). While lucerne stems are finer in appearance they do have poor acceptability and nutritive value. Of greater importance may be the internal competition for nutrients and assimilates when the plant is rapidly elongating. The stems may be a strong sink and develop to the detriment of leaf and root tissue.

Due to reports of apparently poor nitrogen fixation in establishing sainfoin (Ditterline and Cooper, 1975), care was taken with inoculation and a small amount of fertilizer nitrogen was applied. Even though no measurements were taken, the sampling of plants revealed many apparently functioning nodules, when they were examined for the characteristic pink colour of leghaemoglobin. Foliage colour and final yields seemed to indicate that either the nitrogen fixation system was operating or the species is very efficient at utilising soil nitrogen.

The main conclusion from the first season was that given reasonable care with seed-bed preparation, sowing and weed control, sainfoin can be successfully established under the prevailing temperate spring conditions.

Associated with this was a modest yield of herbage which would be useful for off-setting establishment costs. With most perennials the expectation of full utility is in the second and subsequent seasons of growth. The aim of familiarisation with a new species was also met and the preliminary data collection provided the basis for further experimental work.

CHAPTER 4: DEFOLIATION MANAGEMENT AND ITS EFFECTS ON PLANT PERFORMANCE AND HERBAGE DRY MATTER PRODUCTION OF SAINFOIN (*Onobrychis viciifolia*)

4.1 Introduction

Once a perennial crop has been established it is important to devise management systems that will give high yields as well as maintain stand vigour and longevity. In some species, strong consideration may also have to be given to the effects of cutting time on herbage quality. However, this would not seem to be an issue with sainfoin as the decline in forage quality with maturity is small (Baker *et al*., 1952; Koch *et al*., 1972).

The idea of any optimum management for a particular forage or pasture system presents difficulties as the problem encompasses an entire ecosystem and all its interactions (Vickery, 1981). The approach is therefore to gain flexibility by altering defoliation frequency, intensity and timing in relation to the developmental phases of the plant in question (Harris, 1978). In many investigations the frequency has been set by a time interval. While this may make planning easier, harvests will occur at varying stages of development. This is due to genotype and environmental effects on growth rates and may result in reduced yields if cutting occurs at a critical growth stage. Therefore it is important that management recognises the importance of certain key phenological events. In sainfoin these are flower bud development, stem elongation, development of inflorescence to maturity and the development of the next generation of basal buds.

Intensity is dependent on both the quantity of the plant removed at a given time and the time this takes. The quantity removed is usually equated with a given cutting height, while the time factor is the grazing duration. Under grazing, the plant is generally exposed to the effects of defoliation over a longer period and so a new array of problems may arise. The management of lucerne provides a useful example of how new growing points may be prematurely removed if animals are permitted a long grazing duration (Janson, 1978; 1982). With machine harvesting the question of duration does not arise as the removal of herbage is relatively instantaneous. However, detailed examination of the growth pattern of the plant should give some indication of the potential for interaction with the grazing animal.

If management practices weaken the plant then the resulting reduction in stand vigour may manifest itself in various ways. Not only do plants show a decline in yield, but usually survival is also affected. This is due to increased weed competition and often an increase of insect pests, and diseases in the weakened stand. While disease control generally requires the use of resistant cultivars, weeds and insect pests can be in part controlled by the cutting regime. Therefore, it may be important to consider the costs of chemical control in designing practical management strategies. Again lucerne provides a useful example where winter weed control can be achieved either by chemical means, or by careful grazing of the comparatively inactive, winter stand (Butler, 1982).

This experiment was to examine the growth and development of three sainfoin cultivars and their interactions with cutting management. The aim of this was to determine factors contributing to yield, stand vigour and longevity.

4.2 Experimental

4.2.1 Trial Site

The trial site for this work was discussed in Section 3.2. In winter 1980, herbicide damage (accidental over-application), to part of the trial site necessitated repegging the trial on the remaining unsprayed area. In effect this reduced the mean plot size used from about 60m^2 to about 20m^2. As potential for crop damage had been displayed, hand weeding was used extensively in the last two years, 1980-81 and 1981-82, of the trial. The main problem weeds encountered were storksbill (*Erodium cicutarium*) and scrambling fumitory (*Fumaria muralis*) in winter, and white clover (*Trifolium repens*) and black nightshade (*Solanum nigrum*) in summer. White clover tended to present particular problems when its stolons completely entangled the crowns of the sainfoin plants.

All pathways between plots were mown regularly and the plots were fertilised with 400 kg/ha of potassic superphosphate each year. Lucerne required semi-regular spraying with Malathion to control aphids, which only seemed to attack developing seeds on sainfoin.

In the second season (1981-82) some attempt was made to minimise the effects of water stress by watering the plots with a garden-type sprinkler system. The site location prevented full irrigation but it was felt that the water applied was a useful buffer in the shorter dry spells, and immediately after harvests were taken each plot was watered.

4.2.2 Timing of Harvests

Timing of harvests was based on stage of development rather than a rigidly defined frequency. The reason for this was, while a set frequency would make the experimental procedure easier, there would be a range of interactions with the three cultivars, particularly flowering time and harvest date, that would tend to confuse any attempt at defining potentially useful management strategies. By timing the harvests to a growth stage it should be possible, subsequently, to give an approximate frequency as a useful guide or display clearly the weakness of this approach. The details of the cutting treatments were given in Section 3.2.1 and are briefly outlined again.

Early/Early (E/E) The plants were always cut in the early reproductive stage in an attempt to maximise cutting frequency and maintain high quality material.

Full/Early (F/E) After permitting the first cycle of spring growth to flower, any subsequent regrowth was cut at the early reproductive stage.

Full/Full (F/F) These plants were only harvested when they reached full flower.

Lucerne was managed to maximise the production of high quality forage. ie. cut to coincide with emergence of new basal shoots.

For calculations of growth rates, the first of September of each season was chosen as the starting point for spring growth. This date was chosen as it was about the time when the relatively horizontal leaves of the overwintering rosette plants began to increase in angle to be more upright. This was regarded as an indicator of the plant response to improving environmental conditions, notably light and temperature.

4.2.3 Yield Determinations

As it was decided after the establishment year to persist with only one cutting height, all yields refer to material cut from above 4-6cm for both sainfoin and lucerne.

Sub-sampling prior to the main harvests was by cutting the material within a $0.1m^2$ quadrat with hand shears from the extra area outside the main harvest plots. Three replicates per plot was the usual sampling intensity, and small plastic markers were used to indicate sampling locations, and thus avoid sampling from those areas again.

For the main harvests the bulk of the plot area was cut using sickle-bar mowers, mainly an Auto-scythe. The cut herbage was either picked up or raked from the plots. The sampling area of $1m^2$ was then harvested by hand. Ten individual plants within this area were identified with different coloured wire rings at their base. Immediately prior to harvest these plants were located and their stems disentangled from the stand and wrapped within a wire loop so that they could be kept separate. Yield estimates were therefore based on the combined total of all herbage taken from the $1m^2$ area. The individual plants were weighed complete, while the remaining material was sub-sampled and dried at 80°C to provide dry matter percentage estimates.

4.2.3.1 Plant Dissections

Plant material, mainly from the single plants, was used to count stem and node numbers, separate leaf and stem, and estimate leaf area using a Hayashi Denko automatic leaf area meter. From the dry weights of this material, percentage leaf and specific leaf area (SLA, cm^2/g) was estimated.

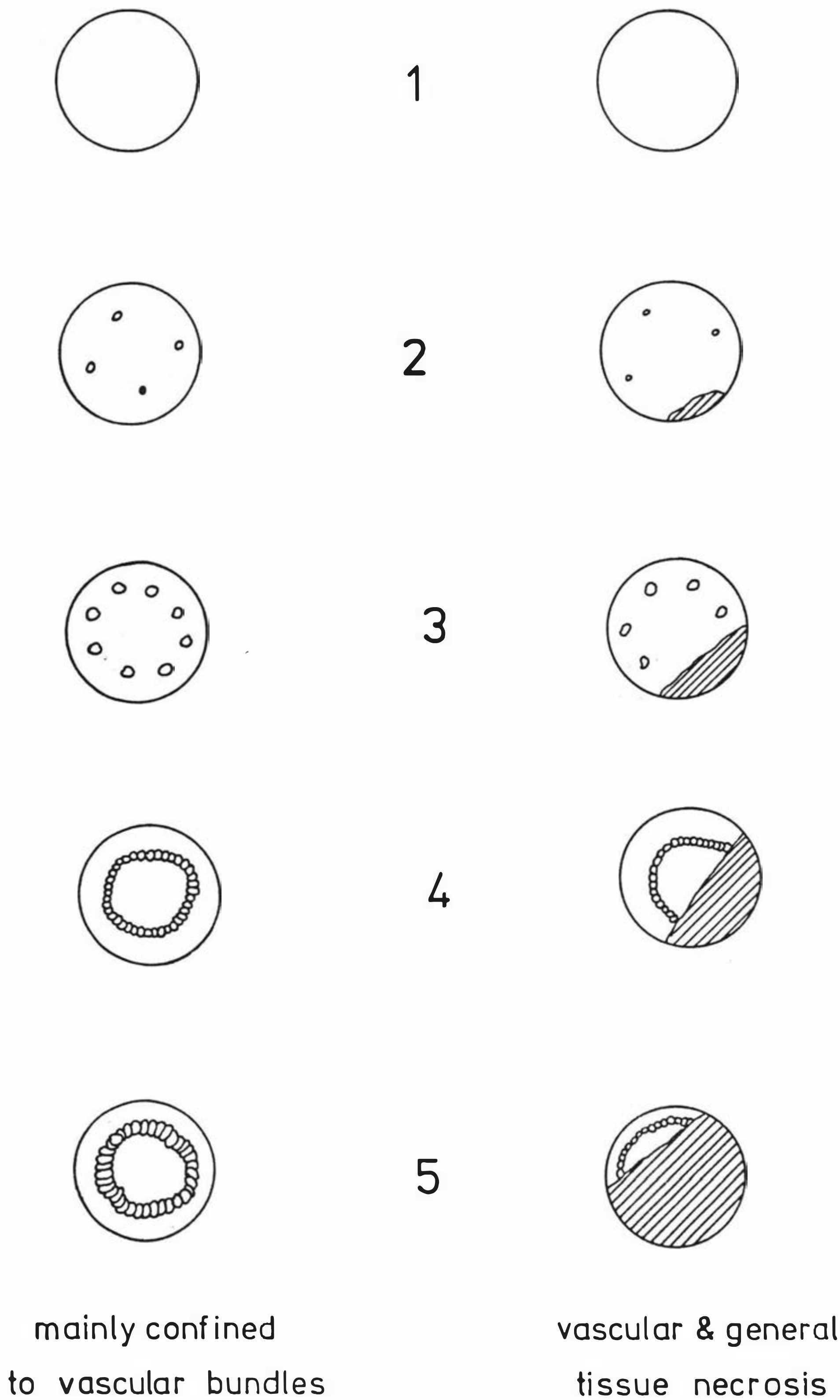
4.2.4 Excavated Quadrats

In May, 1981 each plot was sampled by digging around a $0.1m^2$ quadrat to a depth of about 20cm. The entire quadrat was then removed, placed on a large sieve and the soil gently hosed from the root system. The plants were then stored in a cool room until examination within the following few days.

In the laboratory the plants were washed again to remove any residual soil, counted, and separated into leaf material, crown (mainly stem bases) and the root mass immediately below the crown. The cut surface of the crown, tap root interface was visually assessed for disease intensity. The scoring system (Fig 4.1) used was based on a 1-5 scale, with a score of 1 indicating healthy tissue ranging through to 5 where both vascular and all other tissue were severely necrotic. The disease is referred to as "crown-root rot complex" and was thought to be similar in type to that referred to by Auld et al., (1977) and Gaudet et al., (1980). Both bacterial and fungal pathogens were implicated (Wenham, pers. comm.).

The tap root was stripped of all adhering fibrous roots and cut to a

Fig. 4.1 Crown root rot: disease intensity scores



length of 15cm to provide an indicator of comparative storage potential between the various managements, and also with lucerne.

All plant material was oven dried at 80 °C in a forced draught oven.

4.2.5 Plant Survival Quadrats

After the final harvest in 1982, all plots were assessed for plant survival and distribution. The entire plot area for each treatment was divided into 1m² areas and each of these scored for surviving plants. A scoring system of 0-5 was used, with each number representing about ten plants. A score of 5 usually indicated more than 50 plants, and a zero score indicated no surviving sainfoin plants.

4.2.6 Data Analysis

The data from each year was analysed as a randomised complete block design. A split-plot-in-time analysis was conducted, but due to the variation in the second season, it did not add to the interpretation of the data and was discarded.

Where polynomial fits have been used, the final equations have been chosen to maintain some degree of parsimony while still describing the data. The programme used for deriving these equations, BMDP5R (Dixon *et al.*, 1981), does actually test for additional information gained in fitting a higher order polynomial. This programme was particularly useful as it also fits simple linear regressions. Where natural log transformations were also fitted, growth parameters like absolute and relative growth rates could be derived directly. Derivatives of the equations were useful for defining maximum values.

Chi-square tests were used to test for deviations from distribution patterns for disease scores and also plant density and distribution.

4.3 Results

4.3.1 Timetable of Events and General Climatic Conditions

Figs 4.2a and 4.2b provide a graphic representation of the main harvest events and the interval between them for the 1980-81 and 1981-82 seasons. One feature that is clear, especially in Fig 4.2b, is that the intervals between harvests were irregular and often extended when compared with lucerne. This was due to a combination of the different flowering times between the sainfoin cultivars, and also a lack of uniformity in timing of flowering that was more exaggerated at the second and subsequent harvests.

The 1980-81 season was characterised by poor rainfall in December, January and early February. January was particularly critical as the rainfall was only 15.5mm, while the raised pan evaporation was 203mm (Appendix 1). It was during this period that many of the harvests occurred and therefore plants were subject to shortage of soil moisture as regrowth was expected to commence. The total rainfall for the first four months of 1981 was well below the long-term average (185 vs 297mm). Conditions for the 1981-82 season were much closer to the long-term average.

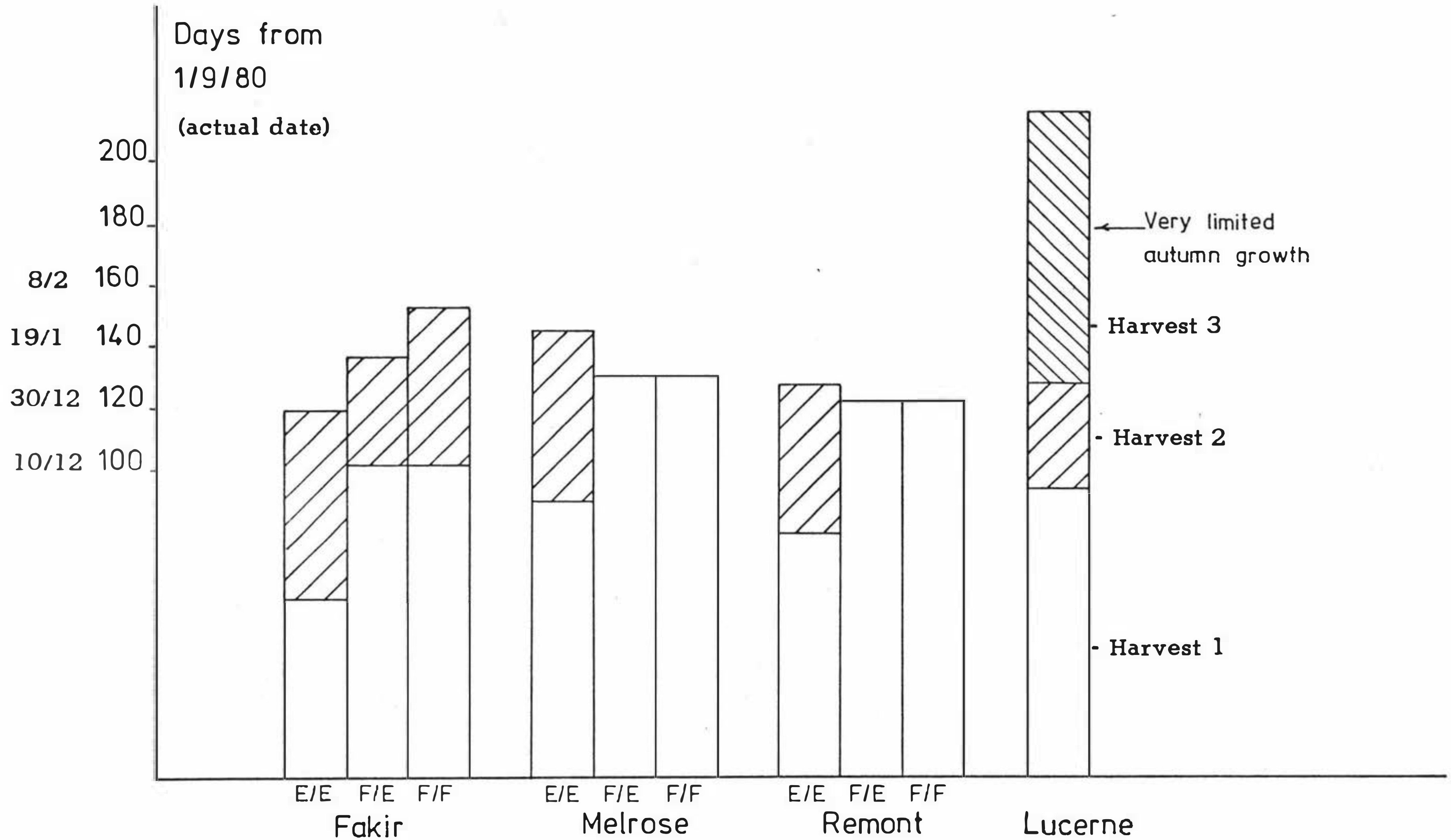


Fig.4.2a Harvest schedule 1980-81

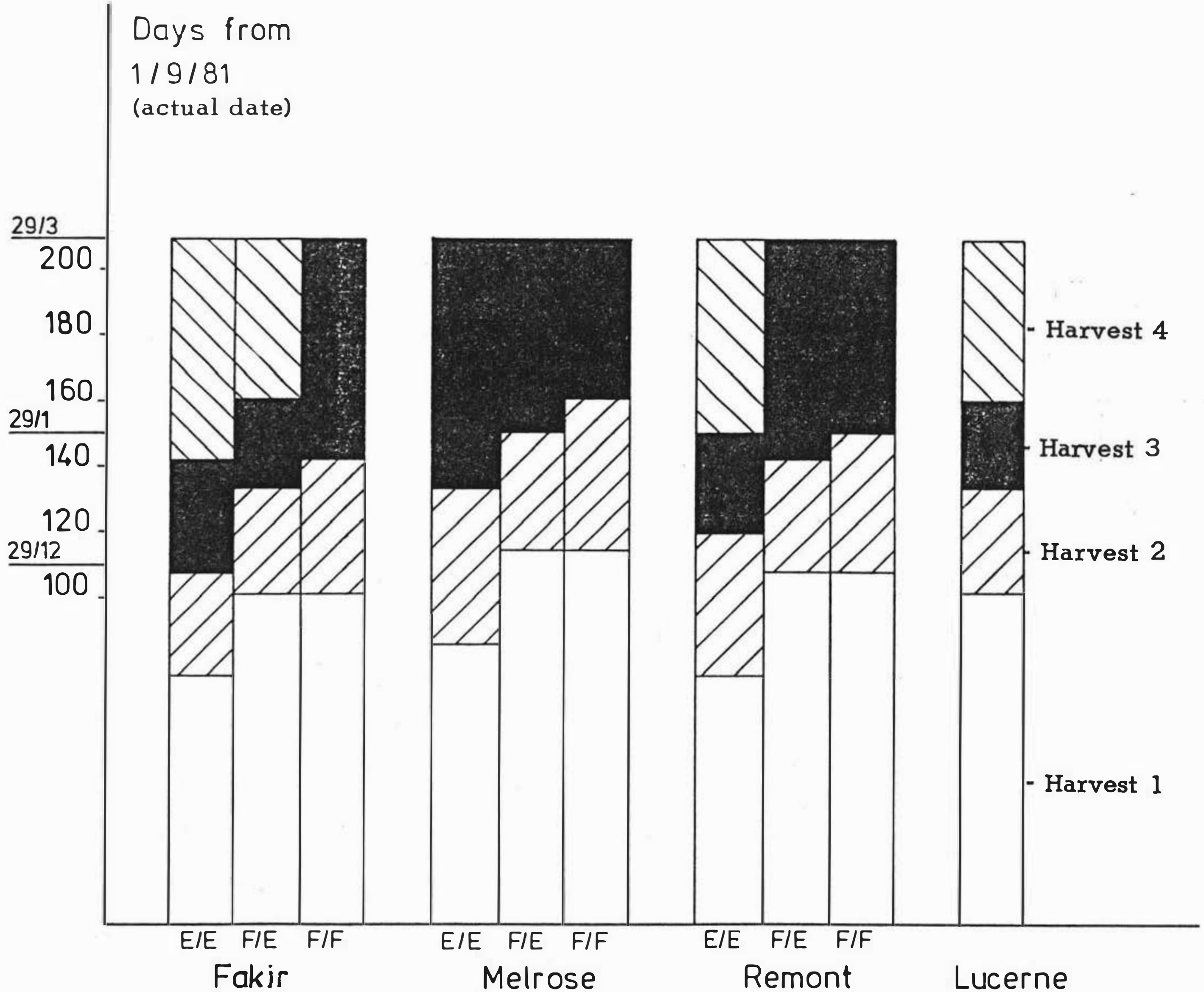


Fig.4.2b Harvest schedule 1981-82

4.3.2 Yield

4.3.2.1 Total Herbage Dry Matter Production

During the 1980-81 season there were significant differences between cultivars in their average yield over all treatments (Table 4.1). The relatively poor performance of the Remont when cut at full flower was in part due to a combination of turbulent weather conditions immediately prior to harvest causing leaf shed, and strong white clover competition in some of the plots. It should, however, be noted that as the F/E and F/F regimes for Remont and Melrose were both only harvested once, the small difference between these two treatments means for each cultivar merely reflects plot and sampling variation. Melrose, the slowest maturing of the three cultivars, gave the highest yield under all treatments and was comparable with lucerne.

The 1981-82 season provides an excellent illustration of the increasing variation with time, as reflected by the large standard error. This variation contributed to the non-significant treatment effects. Despite this it is interesting to note that Melrose still appeared with the highest cultivar mean. However, it is of some concern that six of the nine sainfoin treatments showed a decline in the second season's production, despite the better growing conditions as indicated by the increase in lucerne production.

Table 4.1
Dry matter production (kg DM/ha) for 1980-81 and 1981-82.

1980-81

Cultivar	Treatment E/E	F/E	F/F	Mean	
Melrose	11830	10830	10535	11065	
Remont	9915	6645	6310	7625	Treatment mean
Fakir	7980	9940	9155	9025	S.E. = 522, ***
Mean	9910	9140	8665	9240	(LSD 5% = 1566)
Lucerne	-	-	-	11105	

1981-82

Cultivar	Treatment E/E	F/E	F/F	Mean	
Melrose	9360	10910	8470	9580	
Remont	7955	8265	8390	8205	Treatment mean
Fakir	6250	9630	8330	8070	S.E. = 2588, n.s.
Mean	7855	9600	8400	8620	
Lucerne	-	-	-	14700	

Total of both seasons

Cultivar	Treatment E/E	F/E	F/F	Mean	
Melrose	21190	21740	19005	20645	
Remont	17870	14910	14700	15825	Treatment mean
Fakir	14230	19570	17485	17095	S.E. = 2961, n.s.
Mean	17765	18740	17065	17855	
Lucerne	-	-	-	25805	

The total yield of the two seasons displayed quite clearly the comparative advantage of Melrose over Fakir and Remont. It is also clear that at best, sainfoin yields were only 80-85% of that obtained for lucerne. The two year total gave little indication of the relative merit of the harvest treatments, but it would appear that Melrose was comparatively insensitive to the managements imposed while Remont and Fakir seemed to respond to the E/E treatment. The maximum dry matter production from Remont was with the E/E treatment, while for Fakir it was the minimum.

4.3.2.2 Leaf Yield

In the 1980-81 season the leaf yields (Table 4.2) are useful to emphasise two main points. The first is that the generally higher yields for Melrose that are apparent in Table 4.1 were only maintained for the E/E cutting, indicating a high stem production to achieve the yields shown for the F/E and F/F treatments. The second is that the E/E cutting appeared superior to the F/E and F/F cutting stages in terms of leaf production for each cultivar. It is of note that the Fakir F/E treatment, which was cut a second time, yielded more leaf than the remaining F/E and F/F treatments.

Differences in the 1981-82 season were less apparent than for 1980-81, but the superiority of the E/E treatment was not evident, and for Fakir it was in fact the poorest treatment. The potential for leaf shed was still obvious for the F/F treatments.

The total from the two seasons suggested a generally better leaf yield from the E/E cutting. Melrose also displayed a marked difference with cutting treatments that was not evident when examining total herbage yield

Table 4.2
Leaf yield (kg DM/ha) for 1980-81 and 1981-82

1980-81

Treatment

Cultivar	E/E	F/E	F/F	Mean	
Melrose	5140	1755	1545	2815	
Remont	3595	1930	1840	2455	Treatment mean
Fakir	3530	3305	2400	3080	S.E. = 162, ***
Mean	4090	2330	1930	2785	(LSD 5% = 485)
Lucerne	-	-	-	4870	

1981-82

Treatment

Cultivar	E/E	F/E	F/F	Mean	
Melrose	4715	4730	3280	4240	
Remont	3770	3405	3225	3465	Treatment mean
Fakir	3040	4270	3200	3505	S.E. = 1060, n.s.
Mean	3840	4135	3235	3735	
Lucerne	-	-	-	6085	

Total of both seasons

Treatment

Cultivar	E/E	F/E	F/F	Mean	
Melrose	9855	6485	4825	7055	
Remont	7365	5335	5065	5920	Treatment mean
Fakir	6570	7575	5600	6580	S.E. = 1098, n.s.
Mean	7930	6465	5165	6520	
Lucerne	-	-	-	10955	

(Table 4.1). For the E/E cut about 46% of total production was leaf while for the F/F cut, leaf represented only about 25% of the total. The other feature of the leaf yield from the E/E cut was the comparative stability over the two seasons which would assist any management planning.

Lucerne generally displayed a clear advantage over sainfoin in leaf yield, but individual sainfoin treatments e.g. Melrose E/E, indicate that its superiority is not as marked as for total yield. Also the lucerne cutting time was well defined and more closely related to the E/E type cuts and so the stands were not subject to the leaf loss observed with the late cut sainfoin.

4.3.2.3 <u>Growth Rates</u>

The growth rates tended to show a noticeable improvement during the period covering the second harvest for the season, particularly in the 1981-82 season (Fig 4.3a & 4.3b). An example of this was where the maximum growth rates of about 100kg/ha/day occurred for the Fakir F/E second harvest in 1980-81, and the Melrose second harvest in 1981-82 respectively.

It is evident that the yield advantage shown by Melrose in 1980-81 was not just a function of the time to harvest, but also of high growth rates (Fig 4.3a). The poor performance of Fakir F/F when compared to Fakir F/E (second harvests) was due to the extra 16 days required to reach floral maturity, during which time leaf loss was occurring. Despite improvements in growth rates through the mid-season period, none of the sainfoin approached the average growth rate of 143kg/ha/day shown by lucerne for its second harvest period. The combined mean for lucerne in Fig 4.3a for all

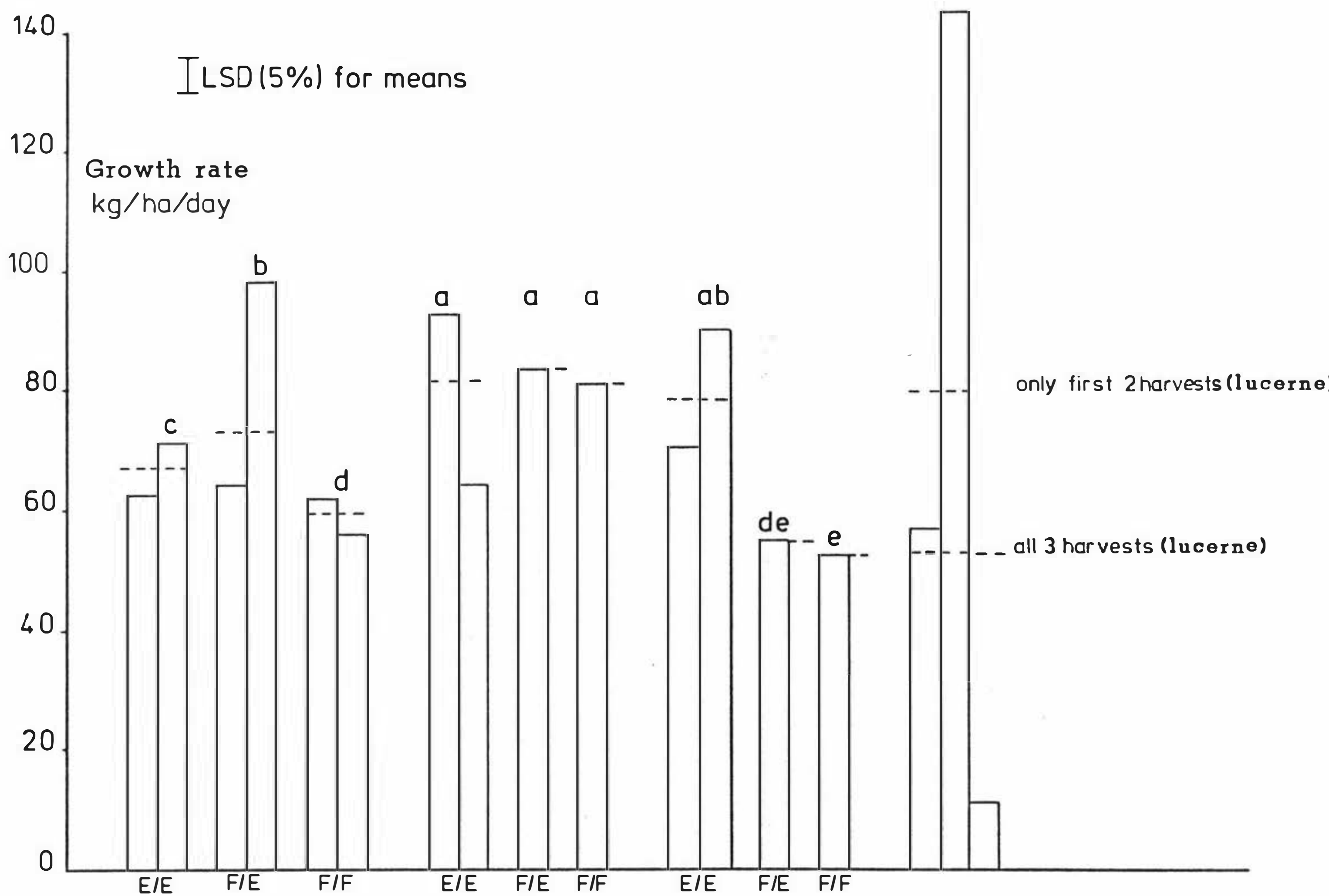


Fig. 4.3a 1980-81 growth rates for individual harvests (--- mean of combined harvests)

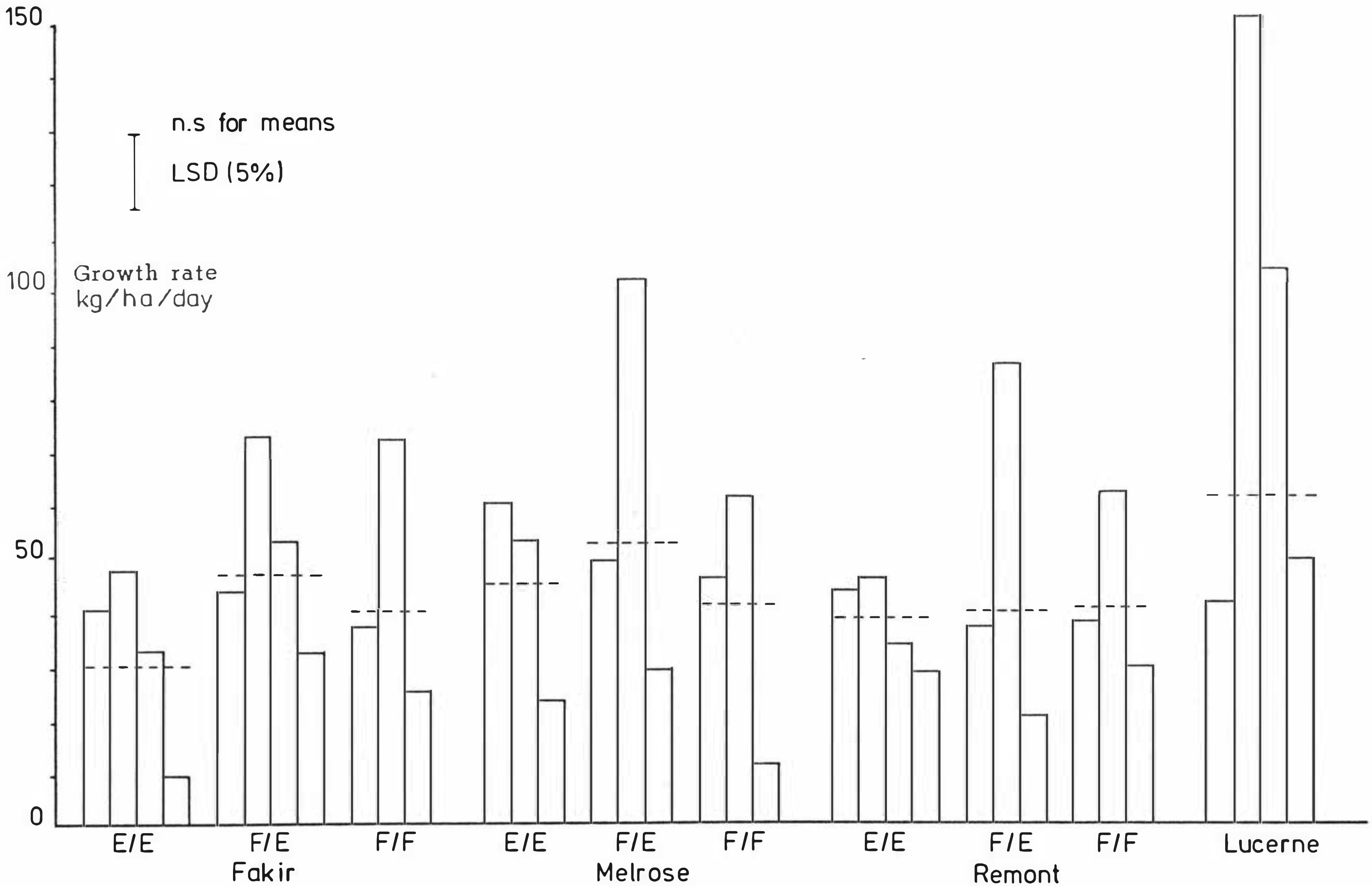


Fig.4.3b 1981-82 growth rates for individual harvests(--- mean of combined harvests)

three harvests tended to provide undue weight to the slow autumn growth and so the mean for the first two harvests was also indicated.

With all treatments terminating on the same date in the 1981-82 season (29th March 1982), the average growth rates for the whole time were directly related to yield. Therefore the non-significant treatment effects for yield also apply to growth rates. All treatments generally had lower growth rates in the 1981-82 season than in 1980-81. As with the previous season, lucerne displayed a marked mid-season advantage over sainfoin. Also with the milder summer and early autumn conditions its growth ability for that period was clearly shown by the maintenance of growth rates of about 50kg/ha/day for a fourth harvest, a figure that was often not achieved by sainfoin in the first growth cycle of the 1981-82 season.

4.3.2.4 Distribution of Production

In the 1980-81 season four of the nine sainfoin treatments were restricted to a single harvest (Fig 4.4a). Of the remaining treatments, the Fakir E/E and Remont E/E gave approximately equivalent production for the two harvests, while the later cut Fakir and Melrose E/E produced about two-thirds of their yield at the first harvest. Lucerne yielded evenly for the first two harvests and also produced a small amount of autumn growth.

As shown in Fig 4.4b, the generally good growing conditions experienced in the 1981-82 season resulted in three or four harvests for each treatment. Again there was the tendency for all sainfoin treatments to yield of the order of 50% or more of their production at the first harvest. Any remaining harvests successively diminished in yield. Only lucerne came close to a degree of uniformity in the distribution of production over its four harvests.

4.3.3 Plant Development

Data from the regular $0.1m^2$ sub-samples in the 1980-81 spring growth period provided the basis for the following results on the pattern of development in sainfoin.

4.3.3.1 Dry Matter Production and Growth Rates

Plant growth was found to be adequately described by quadratic polynomials. Appendix 2 provides a summary of the fitted equations with the dry matter yields being used to provide estimates of average growth rates, and the natural log transformations providing the basis for estimates of relative growth rates.

The curves in Fig 4.5 indicate that for at least the first 80 days the relative growth rates were similar for all cultivars. As the growth rates were similar, the final dry matter yield in the spring period tended to reflect the length of time to reach flowering for sainfoin, and early flowering for lucerne.

Fig.4.4a 1980-81 % distribution of total dry matter yield for each harvest

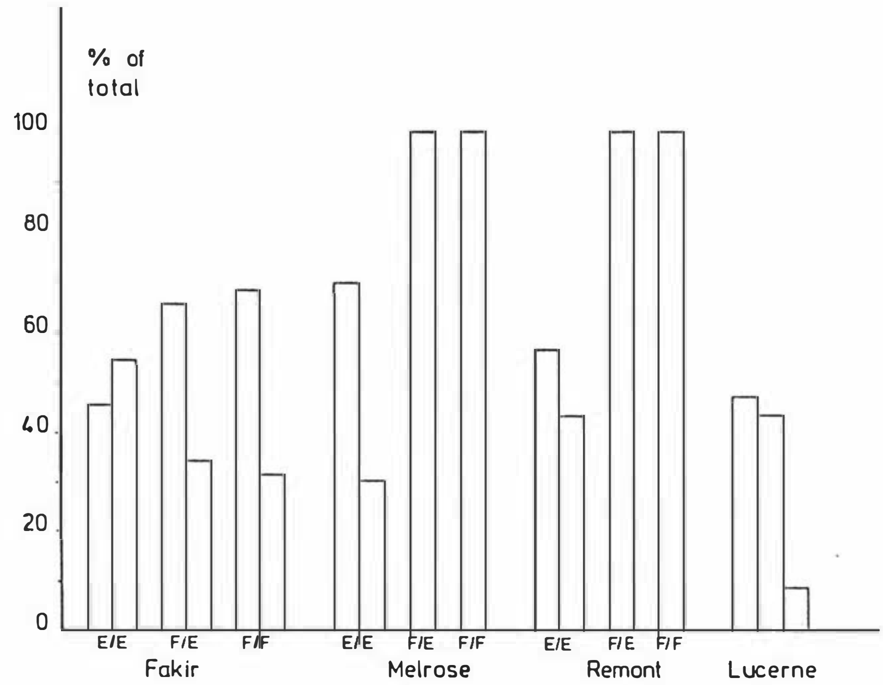
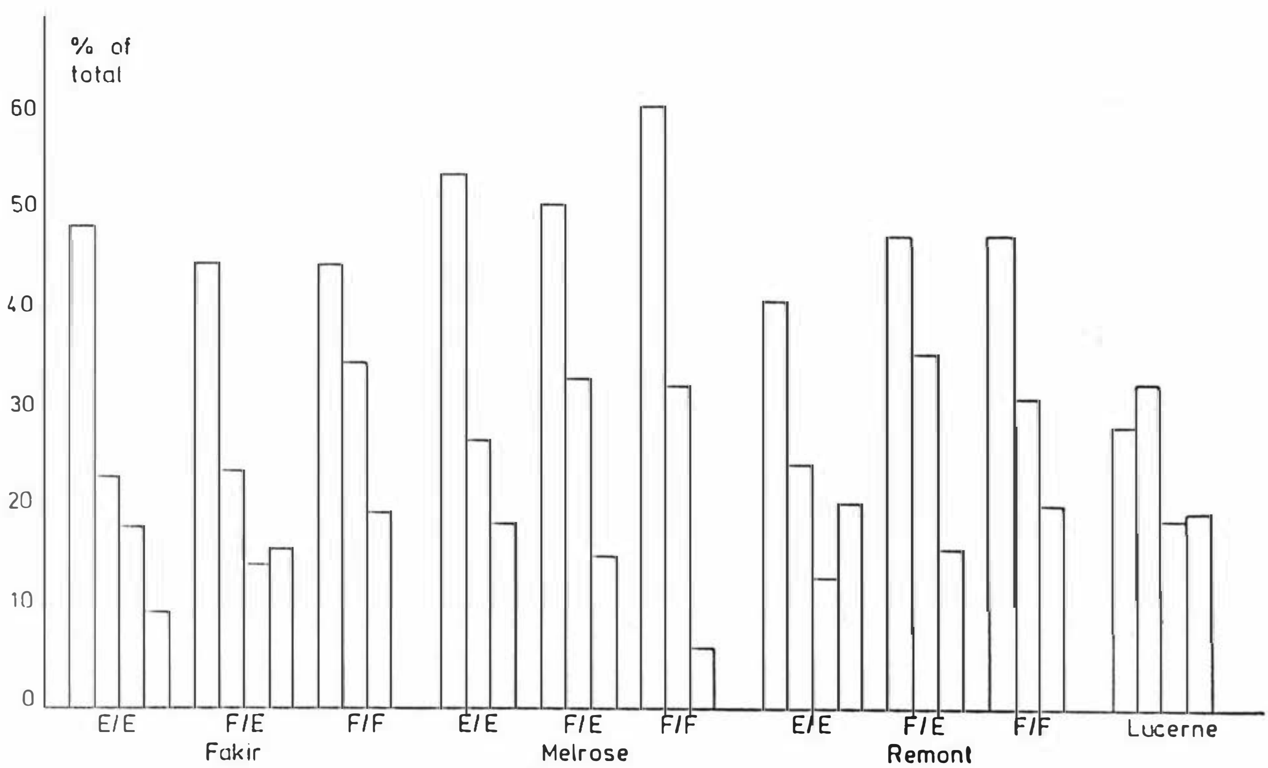


Fig.4.4b 1981-82 % distribution of total dry matter yield for each harvest



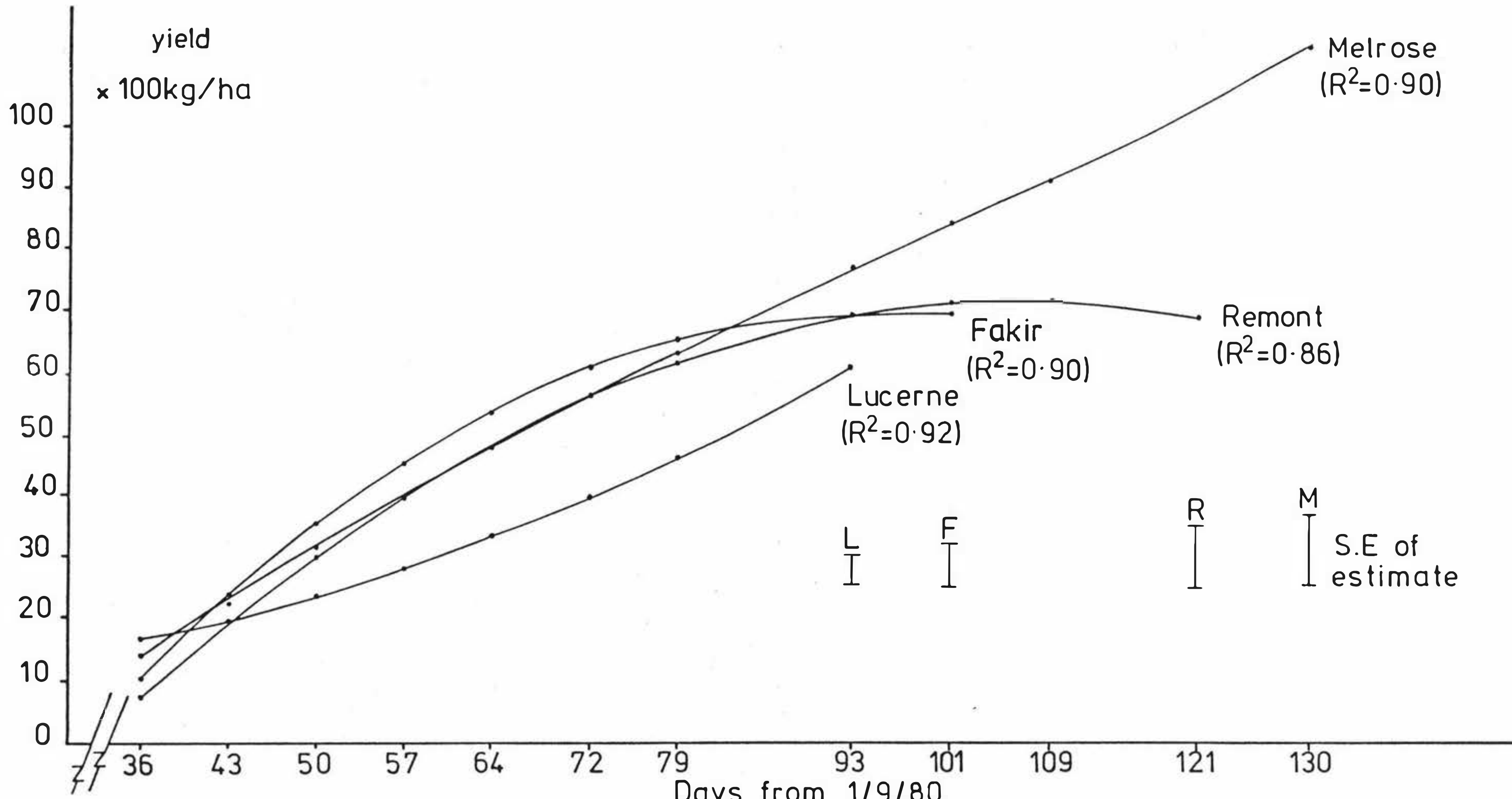


Fig.4.5 Dry matter yield (fitted)-quadratic polynomial

4.3.3.2 Leaf Dry Matter Yield

With the total dry matter production curves (Fig 4.5) indicating a decline for two of the cultivars with maturity, the examination of leaf dry matter yield (Fig 4.6) proves interesting. The three sainfoin cultivars show a marked decline in leaf dry matter considerably before full flowering is reached.

The decline for Melrose, which is not evident in the total dry matter production curve, indicates that production of stem and flowers was of sufficient magnitude to offset the leaf loss of about 1000kg D.M./ha.

When leaf as a percentage of total dry matter production is examined in Fig 4.7, it can be seen that the contribution of leaf to the total progressively declined over the growth period. Melrose was the most extreme, with less than 20% of the final harvest material being leaf. The sainfoin cultivars displayed a similar rate of decline when slopes were compared (Steel and Torrie, 1960).

There is no indication that lucerne was even reaching a plateau of leaf production (Fig 4.6). The rate of decline in leaf as a percentage of the total herbage was smaller for lucerne than for sainfoin, despite an early season problem with aphids.

4.3.3.3 Leaf Area Index

The fitted curves for LAI (Fig 4.8) followed much the same pattern as those observed for leaf dry matter yield (Fig 4.6). Melrose had the

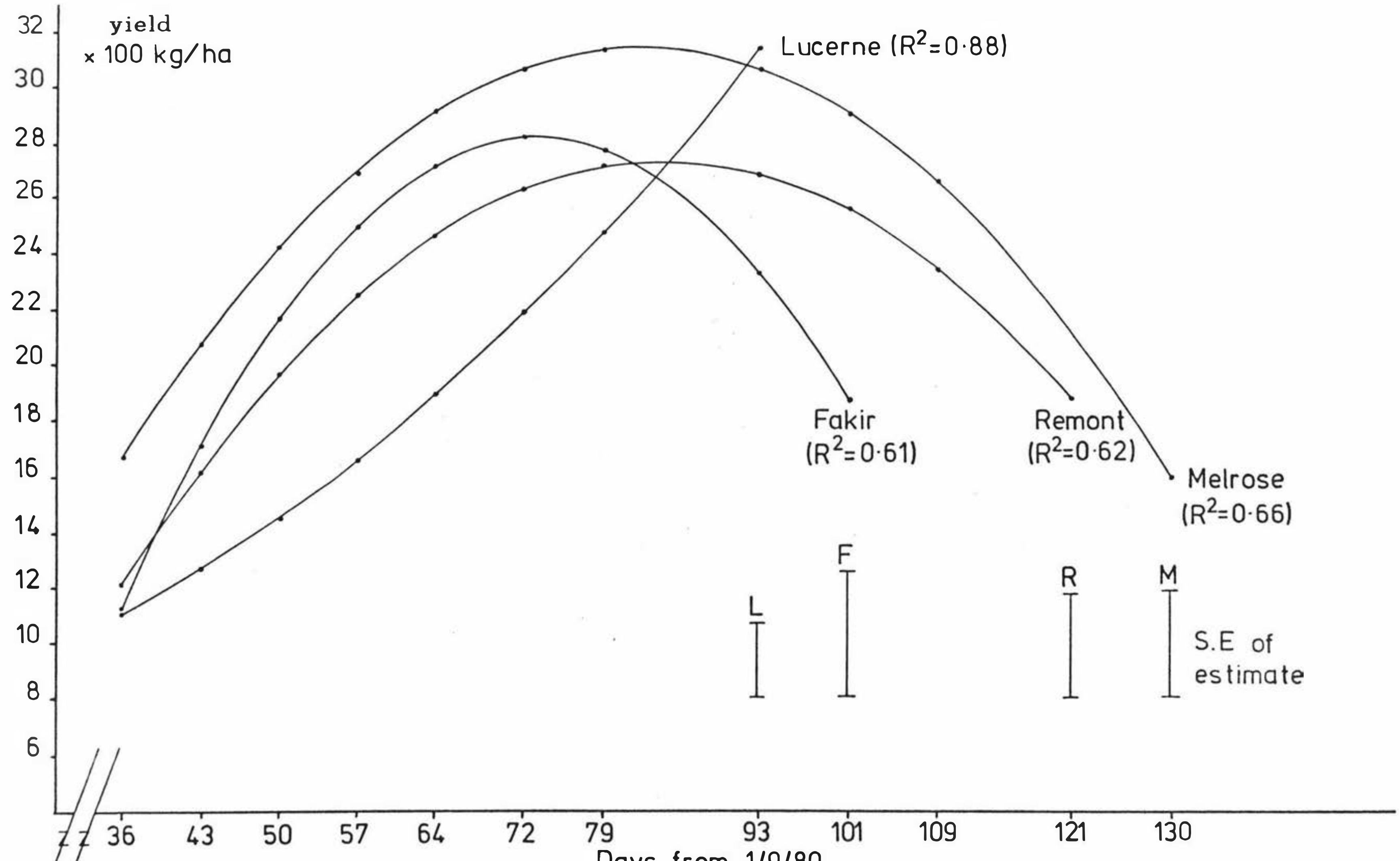


Fig.4.6 Leaf-dry matter yield [fitted quadratic polynomial]

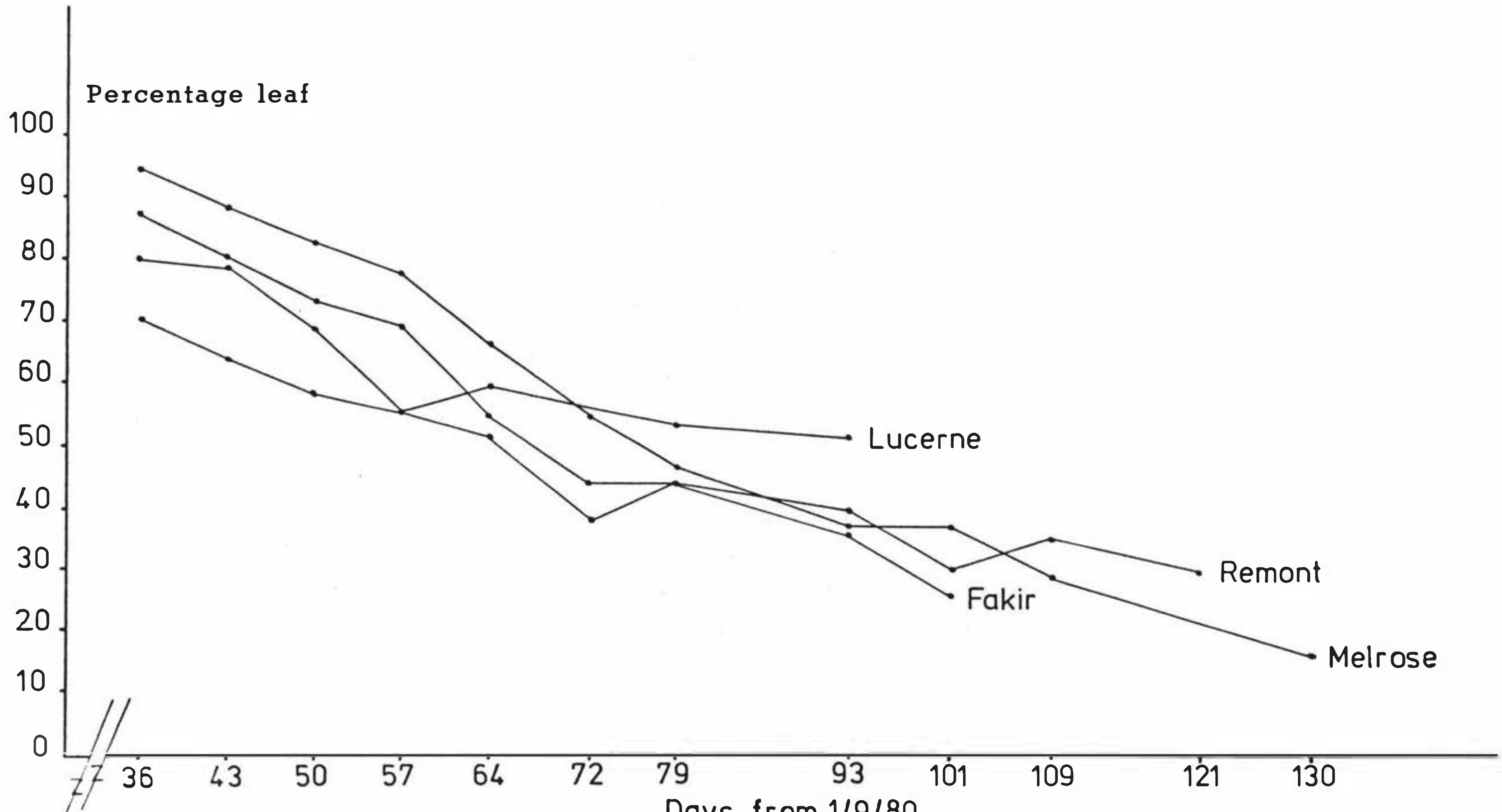


Fig.4.7 Leaf as a percentage of total dry matter over a single growth cycle

highest LAI throughout the growth period and Fakir reached higher values than Remont but declined more rapidly. The maximum LAI was reached well in advance of the final harvest. In the case of Melrose about 60%, or 80 days of the time period to full bloom had elapsed, while for Fakir and Remont the figure was about 70%, or 72 and 86 days respectively. Lucerne showed no indication of having reached a peak when it was harvested.

It is also of note that Fakir and Remont were cut at the E/E stage prior to achieving maximum leaf area, while Melrose had already reached its maximum about nine days before the E/E harvest.

While data is only presented for a single growth cycle, the patterns for other harvests and the second season were similar, i.e. sainfoin tended to have LAI's in the range 0.8-4, while lucerne was always superior. The same decline and instability with maturity was observed in the 1981-82 season.

4.3.4 Single Plants

While every attempt was made to isolate the individual plants that were measured during the 1980-81 season, it became evident that in some cases there was some overlap with neighbouring plants. However, as this problem was applicable to all cultivars and each treatment was represented by 30 replicates (individual plants), its influence on absolute and relative values was thought to be minor.

As individuals were being monitored it was of interest to examine any correlations between the first and second harvests, especially for yield and numbers of stems produced. Table 4.3 shows that while many of the

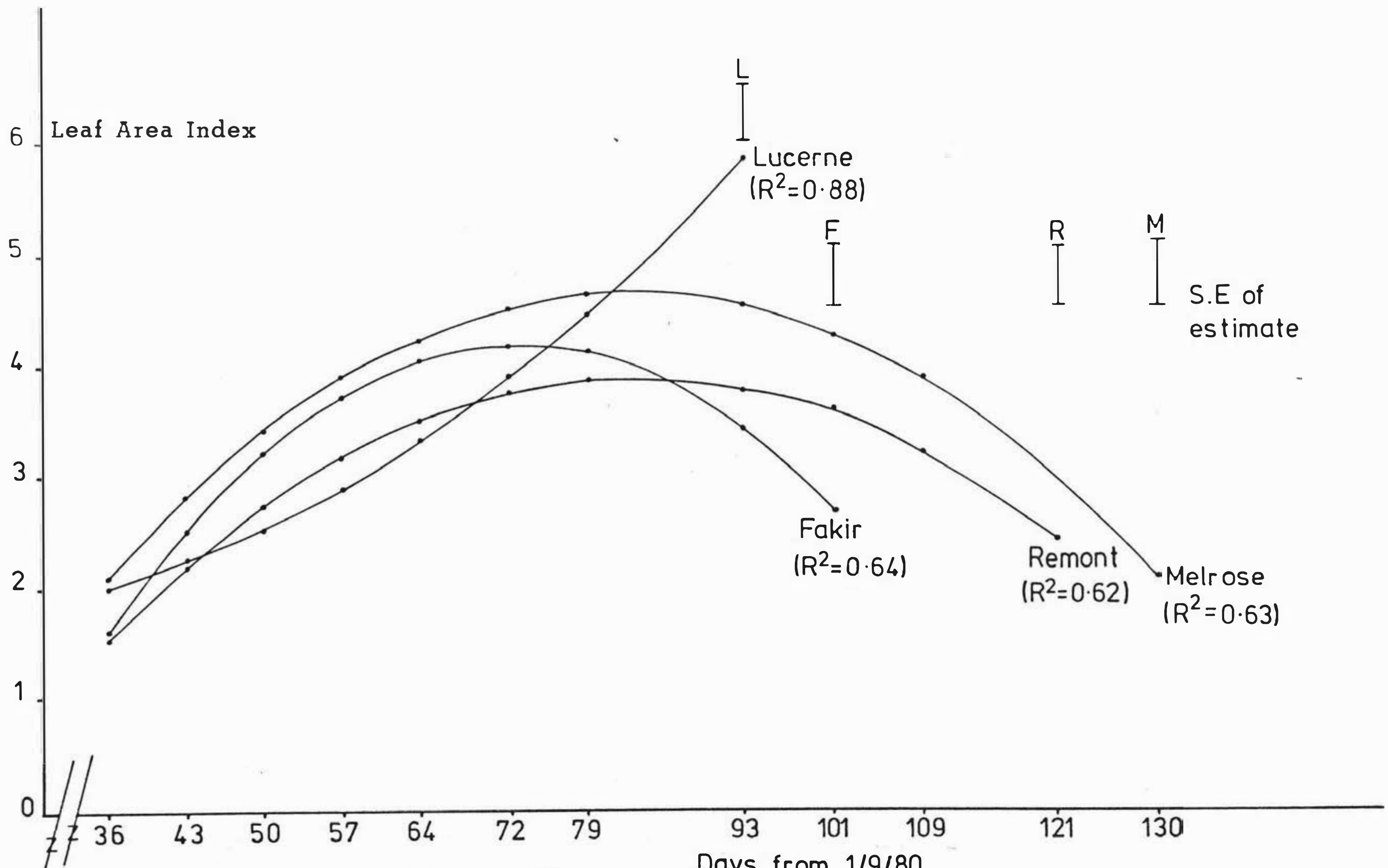


Fig.4.8 Leaf Area Index (fitted polynomial)

regressions for yield were significant the correlations were sometimes quite poor. However, where there was a complete set of data for Fakir, the correlations between the first and second harvests, for yield in particular, show an improvement where the plants have been left to either F/E or F/F stage before cutting. Examination of stem numbers gave little evidence of a relationship between numbers of stems produced in the spring growth phase and subsequent regrowth (see also Appendix 3).

Table 4.3

Predictive equations for either yield (Y) or stem number (S) over two harvests (1 = first; 2 = second) in the 1980-81 season

Fakir	Equation	R^2
E/E	Y1 = 2.48 + 0.41 Y2	0.36
	S1 = 4.53 + 0.30 S2	0.08 (ns)
F/E	Y1 = 3.53 + 1.22 Y2	0.54
	S1 = 4.47 + 0.31 S2	0.09 (ns)
F/F	Y1 = 3.23 + 1.49 Y2	0.74
	S1 = 1.37 + 0.58 S2	0.35
Melrose		
E/E	Y1 = 4.59 + 1.34 Y2	0.38
	S1 = 5.86 + 0.18 S2	0.02 (ns)
Remont		
E/E	Y1 = 3.08 + 0.69 Y2	0.77
	S1 = 3.21 + 0.69 S2	0.32

All regressions significant ($P<0.05$) except those marked ns.

Due to the extent of data collection on the single plants only a brief summary of the more pertinent data is presented in Table 4.4. It can be seen that while the three cultivars started off with about the same numbers of stems on each plant, there were losses between the bud stage (E/E) and full flowering, especially for Melrose. The losses would seem to be the result of the death of stems that failed to elongate and support flowers. While analysis is not presented here, any attempts to relate stem numbers with stem weight generally resulted in very poor correlations ($R^2 < 20\%$). The actual size of the stems at both harvest times indicated a clear order, with Melrose producing much larger stems than either Fakir or Remont. Remont showed a much smaller increase in stem weight between the two harvests than the other cultivars.

The number of nodes on the main stem (Table 4.4) provided an indicator as to the number of potential leaf sites. This is important as it was observed that sainfoin almost universally produced only one developed leaf per node. When other leaves were present they were only 1-2cm in length, where a developed leaf was often 20-30cm long. The ranking of numbers of nodes in terms of superiority was Melrose, Remont and Fakir, but all sainfoin was poor when compared to lucerne, which had about 20 per stem. Lucerne was also noted for producing more than one leaf per node.

The decline in specific leaf area with time meant that the leaf morphology of the new leaves was altering, or that thicker leaves with relatively less surface area had a better survival rate. The differences between cultivars at a given time may in part reflect climatic differences

Table 4.4

Single plant data from first harvests in the 1980-81 season (mean ± S.E.)

		Fakir	Melrose	Remont
Number of stems/plant				
	E/E	6.6 ± 0.5	6.0 ± 0.5	6.9 ± 0.4
	F/F & F/E	6.0 ± 0.4	4.5 ± 0.3	5.3 ± 0.3
Wt/single stem (g/stem)				
	E/E	0.40 ± 0.04	0.97 ± 0.09	0.78 ± 0.07
	F/E & F/F	1.23 ± 0.12	2.72 ± 0.20	1.06 ± 0.08
Number of nodes on main stem				
	F/E & F/F only	7.7 ± 0.2	10.6 ± 0.3	8.4 ± 0.3
Specific leaf area (cm^2/g)				
	E/E	163.8 ± 2.3	146.7 ± 2.6	144.0 ± 1.4
	F/E & F/F	132.9 ± 2.5	110.8 ± 4.2	125.7 ± 2.2
Leaf area/plant (cm^2)				
	E/E	433 ± 25	579 ± 40	445 ± 26
	F/E & F/F	366 ± 23	224 ± 20	271 ± 19

during the growing period as much as being a unique feature of the cultivar. This was especially noticeable in the case of the mature Melrose as the weather conditions in the lead-up to harvest were exceptionally poor and leaf loss was high.

Leaf area per plant is the sum of a number of factors such as number of stems, number of nodes and area of each leaf. Variation in any of these can alter the total leaf area as can the rate of production of new leaf tissue and loss of old. Both number of stems, and specific leaf area decline with time, which may have contributed to the observed losses for all cultivars. However, losses of the magnitude observed for Melrose, and to a lesser extent Remont, in the 1980-81 season must be attributed to leaf shedding.

4.3.5 Rosette Phase

The data for providing an estimate of the size and structure of the overwintering plants were obtained from plants in the dug quadrats. Analysis of production was done on a per unit area basis, as well as a per plant basis. Any confounding of the relationship between plant numbers and size was regarded as minimal as the mean number of plants dug up for each treatment were not significantly different. While examining plant numbers it is of interest to note the consistently poor ability with which plant numbers are estimated without actually digging the plants up (Table 4.5). This was due to the crowns of individual plants overlapping and giving the appearance of a single structure.

Table 4.5

Comparison between visual estimates of number of plants and actual numbers dug in quadrats (plants/m^2)

	Field count	Dug quadrat	Ratio
Fakir	74.4	190.0	2.55
Melrose	75.9	224.4	2.96
Remont	75.9	190.0	2.50
Lucerne	68	246.7	3.63

As the only significant difference that occurred for the sainfoin cultivars was that Remont had a greater crown mass per plant than either Fakir or Melrose (0.94g/plant vs 0.72g/plant), only sainfoin and lucerne comparisons are examined in Table 4.6. The data is presented on a per hectare basis to provide a ready comparison with the total harvested dry matter in a given season.

Table 4.6

Total mass (kg D.M./ha) of plant components from quadrats excavated in autumn 1981

	Leaf	Crown	Taproot	Crown & Taproot	Total
Sainfoin	1232	1497	1499	2996	4288
Lucerne	984	2812	5359	8171	9155
Sainfoin as a % of Lucerne	125	53	28	37	46

Both sainfoin and lucerne maintained comparable amounts of leaf on the overwintering plants. These leaves are compact due to the lack of any internode elongation of the supporting shoots and are responsible for the rosette appearance.

Of particular note was the much smaller size of the perennial structures (crown and tap root) of sainfoin compared with lucerne. Observations of the plants during dissection were that the crown and tap root were also more robust and healthy for lucerne.

4.3.6 Plant Disease

While the sainfoin cultivars and treatments showed no significant treatment differences it is of importance to consider the magnitude of the scores (Table 4.7). An overall mean of 3.18 indicated considerable vascular discolouration and damage, or partial necrosis of all the tap root tissue.

Table 4.7

Crown-root rot intensity scores (1 = min; 5 = max) from autumn 1981

	Treatment			
Cultivar	E/E	F/E	F/F	Mean
Fakir	3.46	3.20	3.16	3.27
Melrose	3.18	2.86	3.22	3.10
Remont	3.25	3.13	3.20	3.19
Mean	3.30	3.06	3.19	3.18
Lucerne	-	-	-	1.34

Treatment mean S.E. = 0.08 (n.s. treatment effect)

Chi-square analysis of the disease distribution data for sainfoin resulted in the patterns being regarded as similar for all cultivar and treatment combinations. Therefore, only the highly significant comparison between sainfoin and lucerne is presented in Fig 4.9. Of particular note in Fig 4.9 is that 77% of all lucerne plants sampled were disease free, while only 7% of sainfoin plants were similarly clear of disease. Lucerne also had no plants with a score of 4 or 5, whereas 37% of sainfoin plants were in these two categories and thus displayed extensive tissue decay.

4.3.7 Final Plant Density and Distribution

As a large number of individual quadrats were assessed, the significance of treatment effects was to some degree dependent on whether these were used as individual scores or meaned for each of the three replicates. The results in Table 4.8 do tend to indicate that the E/E treatments result in relatively less surviving plants than the other treatments.

Table 4.8

Final plant density scores (1982)
(One score unit is approximately 10 plants/m^2)

	Treatment			
	E/E	F/E	F/F	Mean
Fakir	1.66	2.40	1.76	1.94
Remont	1.64	2.31	2.60	2.18
Melrose	1.82	3.13	3.14	2.70
Mean	1.71	2.61	2.50	2.27
Lucerne	-	-	-	5.0

Treatment mean S.E. = 0.40 (n.s. treatment effects)

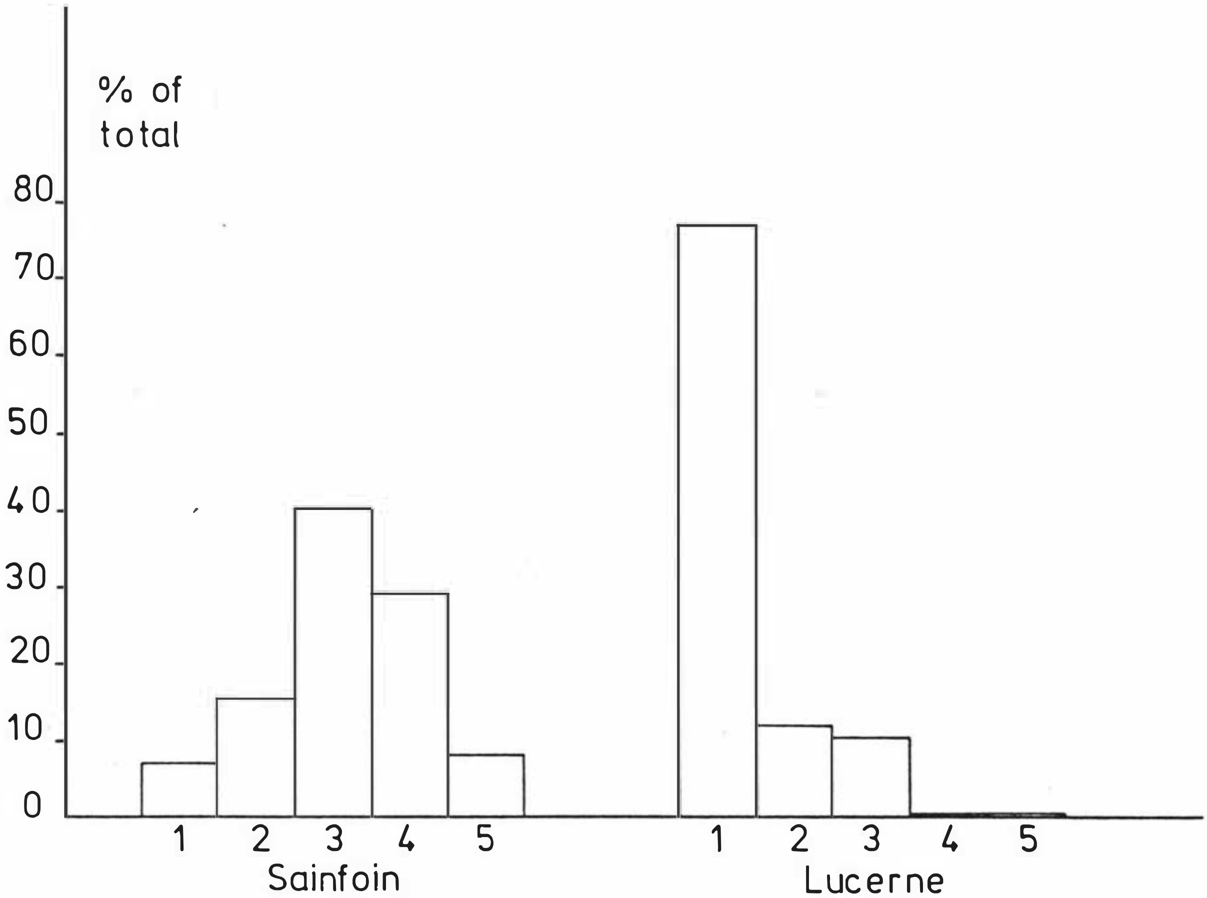


Fig.4.9 Distribution of disease scores (1-clear — 5-extreme)

When the distribution patterns of plant density over the plot areas were examined, chi-square tests indicated that the distribution of surviving plants was not the same for either all the treatments separately, or for cultivars when grouped. The histograms shown in Figs. 4.10a and 4.10b illustrate the patterns.

For all cultivars the E/E treatment resulted in the majority of the quadrats being poorly populated (Fig 4.10a). The distribution of the quadrats in the F/E and F/F treatments tended to be more normal, but with a bias towards lower values.

With the results of the cultivars grouped (Fig 4.10b), it becomes clear the Remont and Fakir were being dominated by poorly populated quadrats. Melrose still had a much greater proportion of quadrats that had in excess of 50 plants/m^2.

The total area of lucerne survived in a relatively uniform manner resulting in a mean score of 5 ie. very little evidence of mortality having an effect on production.

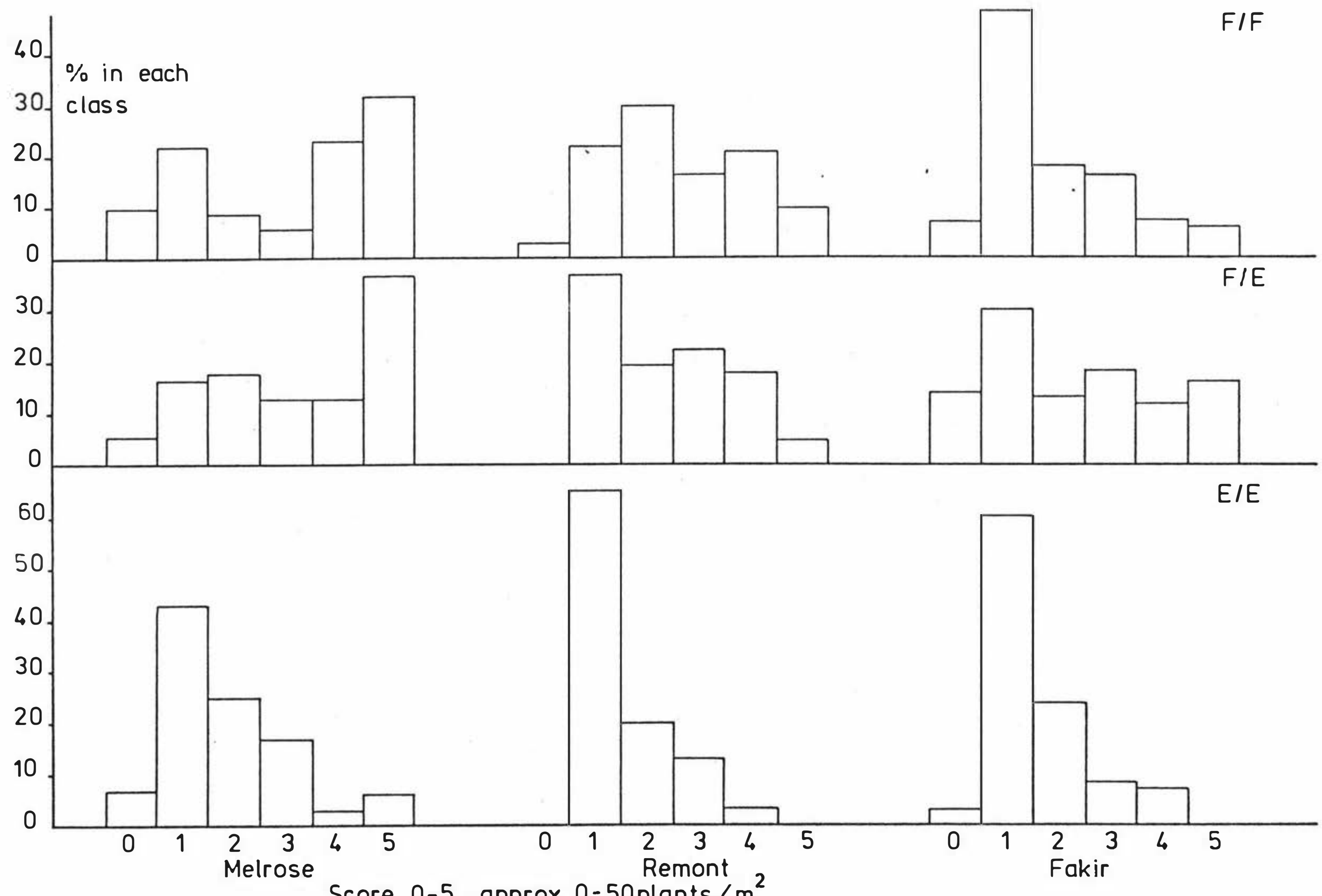


Fig.4.10a Final plant distribution-all treatments

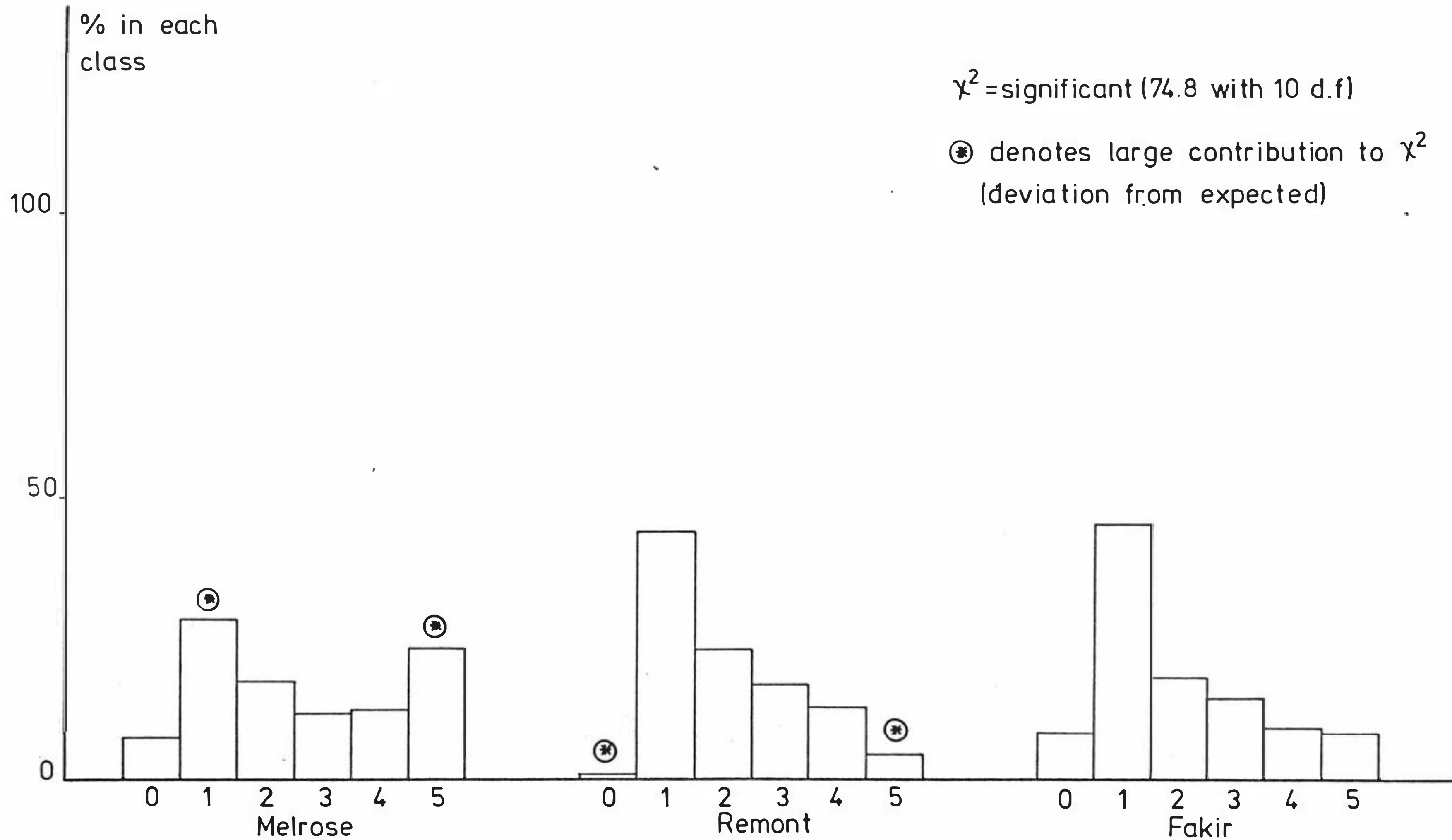


Fig.4.10b Final plant distribution - cultivar effect

4.4 Discussion

The progress of the field experiment was marked by an increased variability of all recorded data. As various factors seemed to contribute to this eg. poor plant survival aggravated by early cutting and plant disease, commentary will require some subjective judgement based on field experience and observations as stastical support was limited.

As a general note, the cultivars performed to expectation in terms of their relative flowering times and ability to regrow. Remont was more disappointing than Melrose or Fakir as it was originally anticipated that it would display greater vigour and production than was evident.

The dry matter production for all sainfoin cultivars was below that of lucerne growing under the same conditions. Melrose performed better than either Fakir or Remont but only achieved about 80% of the lucerne yield over the two growing seasons, a performance that was in agreement with other New Zealand work on sainfoin (Percival and McQueen, 1980). A key contribution to this poor relative performance was the decline in production in the 1981-82 season when growing conditions were good enough to permit lucerne to produce about 15 tonnes DM/ha. Only one sainfoin treatment (Melrose F/E) exceeded 10 tonnes DM/ha, and the overall mean for all sainfoin treatments was only about 8.5 tonnes DM/ha. Possible reasons for this will be developed in discussion, but the low yields from the cultivars used in this experiment would seem to be a handicap to further development work.

While some minor differences were apparent between the cultivars eg. Fakir active earlier in spring; Melrose producing slightly larger stems, the unifying feature was the early onset of reproductive development, and production of at least fifty percent of the season's dry matter at the first harvest. Distribution of production throughout the season is of particular importance to practical management and all sainfoin cultivars were most productive in late spring and early summer. Early harvesting did seem to permit some balancing of production through the season, but not to the same extent as lucerne. None of the sainfoin cultivars appeared to have the ability to develop a new generation of shoots prior to harvest, which resulted in the delay in regrowth while these established after cutting. Cooper and Watson (1968), suggested that this development would naturally occur if plants were left to produce mature seeds, unlike the situation with lucerne of new shoots developing at about the time that flowering commenced. When plants were cut at the E/E stage, the more rapid onset of regrowth would appear to have been derived from residual, unelongated shoots below harvest height. If plants were left to grow to full flowering it would seem that this group of shoots dies out through the combined stress of shading and competition for assimilates.

There was a general lack of response by sainfoin to the cutting treatments. If this were taken to mean that sainfoin was not particularly sensitive to the pattern of cutting, then applied field management could be flexible. However, if this is true, then much of the variation that was apparent was due to interactions with climate and other environmental variables. An example of this was provided by Fakir which produced two and four harvests in 1980-81 and 1981-82 respectively, from the E/E treatment. The difficulty with this type of genotype-environment interaction is that it requires experimentation over a greater number of sites and/or years to validate management considerations.

With all sainfoin, a confounding factor was the rapid, and often unpredicatable onset of flowering, particularly for the cycles of regrowth after the spring growth. There was not a clear separation into vegetative and reproductive phases of growth and this resulted in a variable number of well developed floral stems. The apparent dominance that these exerted on the plant may explain why poor correlations were obtained between the stem numbers of plants over two growth cycles. It would seem that if dominance was established early, then a lesser number of stems developed in that cycle and the remainder of the stems elongated in the subsequent cycle. The development of the remainder is supported by the constant mean total production of 12-13 stems per plant (Appendix 3). This type of behaviour by all sainfoin may well have contributed to the overall variability and to the lack of clear effects from the cutting treatments.

Where cutting treatment appeared to usefully highlight other genotype-environment interactions was for leaf production in the 1980-81 season, and to a lesser extent in 1981-82. If plants were left to reach full flower there appeared to be the potential for leaf losses of the order of 1000 kg/ha. This type of shedding was not evident for the early cut material, the faster maturing cultivar Fakir, or lucerne. From a nutritional viewpoint, this may not be a serious problem as the stem is reasonably digestible and voluntary intake by ruminant animals apparently remained high, even when stemmy material was offered (Baker et al., 1952; Parker and Moss, 1981). However, if this material could be retained the productive potential of sainfoin would be improved. This may require careful monitoring of mature stands if the leaf loss is stress related.

Another cutting interaction may be with disease severity. Even though there was no strong evidence to support a higher disease incidence for the E/E treatments, they were cut more often and so subject to a greater impact. If grazing animals were used directly, these weakened plants would have to survive the effects of treading and grazing and the potential for crown tearing (Ditterline and Cooper, 1975), resulting in plant destruction, would be increased.

Plant distribution showed clearly that sainfoin stands did not just diminish in a uniform manner, but also that plant loss was patchy, whereas with the lucerne plots, the losses appeared to be more evenly distributed. Early cutting seemed to cause an increase in the problem for all cultivars, but generally the cutting treatments did not seem to effect Melrose to the same extent as Fakir or Remont. Using lucerne as an example, it has been shown that productive stands can contain as few as 15 plants/m^2 (Palmer and Wynn-Williams, 1976), but with sainfoin the lack of uniformity would cause problems with weed ingress into the stand and localised competition. Also, and most importantly, the decline in population of sainfoin occurred over only two full growing seasons, and not the longer time periods that are commonly associated with lucerne.

When the information on plant disease is considered in conjunction with final population estimates, it would seem that sainfoin has some real problems with perennial performance. Disease is also regarded as a major contributing factor to the poor longevity of certain lucerne stands (Dunbier and Easton, 1982), and as that is the case, then the severity noted for all sainfoin cultivar and treatment combinations should be regarded seriously. Combined with a relatively small tap root structure, the disease might reasonably be assumed to have marked effects on the ability of the plant to transfer both nutrients and assimilates. This may well have occurred in the 1981-82 season when sainfoin did not seem to be able to respond to the good growing conditions. Evidence suggests that it may be possible to select for resistance to this type of disease complex (Auld et al., 1977) but there was a marked lack of resistance evident in the cultivars sampled in this experiment.

While the relative merits of leaf proportion may be argued from a quality viewpoint, it is clear that the potential for leaf loss shown in the 1980-81 season can add a further stress to sainfoin by drastically reducing the photosynthetic surface of the plants, and perhaps exacerbating the effect of the root disease by not maintaining an adequate supply of assimilates to the root system. A delining specific leaf area and stem losses contributed to the overall reduction in leaf area, but the major cause would seem to have been leaf shed from the surviving stems, particularly for plants left to full flowering. The LAI never reached that observed for lucerne, about 5 (Brown et al., 1972), before the decline occurred. During the decline phase the plants were rapidly increasing in size as the stems elongated and developed flowers, and so the demand for carbon was being met from a decreasing leaf area. However, some consideration should be given to the stems, which until a relatively advanced stage of maturity remained quite green and succulent. As they may well contribute to the photosynthetic pool, it was of interest to examine the potential surface area presented by the stems (Table 4.9).

Table 4.9

Potential stem surface area (cm^2)

diameter (cm)	height (cm)	area	no. stems	area/plant (cm^2)
0.5	50	78.5	5	392.5
0.25	50	39.3	5	196.5
0.5	70	110.0	5	550.0
0.25	70	55.0	5	275.0

Assumption: The stem is a reasonable approximation of a cylinder, therefore its surface area can be calculated by dh, where d = stem diameter and h = height. The values for diameter and height are based on the size range of stems found on early to mid-flowering plants. In many instances these values would be conservative.

While it is arguable as to the exact proportion of stem presented to incoming radiation at any given stage of the day, it was obvious that even when conservative estimates of stem area were calculated, the area was of the same magnitude as that observed for the leaves. Therefore there could well be some value in assessing the photosynthetic capacity of sainfoin stems.

Even though no specific measurements were taken, two other features about sainfoin and its management were indicated by this study and are worthy of brief mention. The first was the formation of the overwintering rosette which appeared to have a dual function of providing the starting stem population for the subsequent spring growth, and a photosynthetic surface to meet the plant's maintenance respiratory requirements during the winter period. It was clear that this structure was much smaller for all sainfoin than for lucerne, and so it would seem likely that any interference, by either grazing or weed competition, could well effect later growth and even plant survival.

The second feature for consideration was irrigation. From the outset, it was decided that the logical place for sainfoin was under natural rainfed conditions, as if the cost of irrigation was to be supported, then lucerne was still indicated as being most profitable (Ditterline and Cooper, 1975). Despite this, in the second season (1981-2), some water was applied to the plots, mainly after each harvest. Even with this application, and the relatively moderate conditions experienced in that season, sainfoin formed a rosette in early autumn while lucerne still displayed active growth. This would indicate that factors other than moisture may have been limiting. Examination of the effect of daylength may provide some indication as to the control of rosette formation.

In concluding it can be seen that sainfoin, particularly the later maturing cultivar Melrose, can produce reasonable quantities of dry matter in the late spring and early summer. All three cultivars appeared to be relatively insensitive to the cutting treatments even though the repeated early cutting may have increased the rate of stand decline, partly through the effects of an unspecified crown-root rot complex. It was also evident that mature sainfoin plants could easily shed a significant portion of leaf under conditions of stress. It may be that if sainfoin germplasm could be obtained with a much greater degree of distinction between the vegetative and reproductive phases, then management interactions may be more obvious. Whether this is advantageous would depend on the degree of flexibility that could be obtained by using a range of cultivars with different flowering times. When compared with lucerne under the same overall growing conditions, only Melrose could be seriously considered for further attention in terms of production, but all sainfoin displayed serious problems with perennial performance as indicated by plant survival after only two full growing seasons.

CHAPTER 5: PATTERNS OF GROWTH AND ASSIMILATE PARTITIONING IN TWO CULTIVARS OF SAINFOIN (*Onobrychis viciifolia*)

5.1 Introduction

For a perennial legume like sainfoin to function effectively there must be translocation of assimilates to the roots for

a) formation of dry matter during the growth of roots and nodules

b) respiration of nodulated roots

c) the carbon source for formation of fixation products which ultimately leave the root in the transpiration stream

d) maintenance of the plant during its dormant phase and perhaps for reutilisation during the period immediately after defoliation.

The first three points have been well documented and to a certain extent quantified for annual legumes *e.g.* Pate, (1979), and also managed perennial ryegrass swards (*Lolium perenne*) *e.g.* Parsons and Robson, (1981). The last point is somewhat less clear and has been the subject of controversy, the basis of which centres around the problem of whether the reserves, a term that May (1960) regards with scepticism, are utilised as a respiratory substrate or as structural components of the new generation of shoots.

This issue has been clarified to some degree by the use of ^{14}C labelling of the assimilate pool. This has proven a useful technique for indicating the pattern of movement and utilisation of assimilates in other perennial legumes such as lucerne (Hodgkinson, 1969; Pearce et al., 1969) and Lotus pedunculatus (Sheath, 1978). In all of these cases this information has been helpful in interpreting why the plants have certain characteristic growth and regrowth patterns. This information is valuable when considering management as, if assimilates do show accumulation or depletion phases, then untimely harvesting may mean that the plant is left with a poor or inadequate reserve base on which to function and commence regrowth e.g. Deregibus et al., (1982).

For this study, the use of ^{14}C labelling had a two-fold objective and that was to

a) identify at what stage the plant appears capable of translocating assimilate to the roots and thus enable consideration of potential interactions with various harvest strategies. [DISTRIBUTION]

b) determine to what extent a pool of assimilates in the root system at a given harvest time might be utilised for regrowth. [REDISTRIBUTION]

Associated with the ^{14}C work there was also an opportunity for closer examination of the growth of two of the cultivars used in the field study. This meant that data could be gathered to both support, and add to, the information base already begun with the field results. Of particular interest and importance was the chance to consider the regrowth of defoliated plants under conditions that had no apparent environmental limitations.

5.2 Experimental

5.2.1 Plant Preparation

Individual plants of the two sainfoin cultivars, Melrose and Fakir, were grown from seed in planter bags (140x140x420mm) filled with sand. Each bag had a mixture of potassic superphosphate and trace elements incorporated into the top 10cm just prior to sowing in February 1982. During the first three weeks of growth the plants were watered lightly and frequently. On three occasions the plants were also liberally watered with dilute suspensions of the *Rhizobium* strain 3157 (NZPS 301). Top watering was continued beyond this stage, but as the capillary matting under the pots became more effective as the plants developed, the frequency was reduced to about once or twice per week.

All plants were maintained in the glasshouse through autumn and winter as healthy, vegetative rosettes. During this period insect pests, mainly white fly and aphids, were controlled using dichlorvos and acephate (systemic), and there were also prophylactic applications of the systemic fungicide benomyl.

5.2.2 Distribution Experiment

For the distribution experiment the tops of 50 plants of each cultivar were cut back to about 3cm. The defoliated plants were placed in a controlled climate room at the Plant Physiology Division, D.S.I.R., Palmerston North on the 30/8/82. Seven sequential harvests were taken for each cultivar approximately achieving a reasonably even harvest interval from early growth to about the anticipated time of flowering. At each harvest, six plants of each cultivar were treated with ^{14}C and 24 hours later the roots were washed free of sand and the plants dissected. An untreated plant was also harvested on each occasion to determine if there was any plant within-room transfer of ^{14}C. Overall this gave seven groups of plants that had been treated with ^{14}C at different stages of development, to use in determining if the pattern of assimilate movement might have peaks or troughs that could interact with the timing of harvests.

5.2.3 Redistribution Experiment

For the redistribution experiment 60 plants of each cultivar were cut to about 3cm and placed in another controlled climate room on the 9/8/82. These plants were all grown through to the stage when most plants were showing developed florets. This took 36 days for Fakir and 43 days for Melrose. At this point 48 plants of each cultivar were treated with ^{14}C, and cut back to about 3cm 24 hours later, along with 8 untreated control plants. Sequential groups of plants were then taken starting from the time of treatment, to determine the course of events through the regrowth period.

During the period in the controlled climate room all plants were subject to identical conditions. Basically they were a simulation of the long days and warm growing conditions experienced in the Palmerston North region during late spring and early summer (see Appendix 4 for more complete operating conditions). The plants were on a automatic watering system in the rooms and were also given regular waterings with a nitrogen-free nutrient solution. The three week gap between placing the two lots of plants in the rooms was to meet logistic requirements of the facilities.

5.2.4 ^{14}Carbon Labelling Procedure

Batches of radioactive $^{14}CO_2$ were generated by reacting aqueous $Na_2{}^{14}CO_3$ with 2N H_2SO_4 prior to each treatment time. Sufficient $^{14}CO_2$ was generated to provide 50 μCi for each plant. The gas was stored in an air column which was maintained at atmospheric pressure with adjustable mercury columns, thus permitting consistent subsample volumes and activities. Immediately prior to treatment, individual plants were supported in a light, wooden frame and covered with a large Mylar bag. This bag was then sealed to the pot and the $^{14}CO_2$ was injected into the air-space surrounding the plant. Circulation was provided by a combination of a bellows action of the bag and direct pumping with a large syringe. Based on the size of the bag, and conservative estimates of the photosynthetic rates of the plants it was estimated that the plants would usually take less than one minute to deplete all the CO_2 in the bag and so a treatment period of about three minutes was chosen. All treatments were conducted outdoors for safety reasons and were always conducted between 11am and 3pm. This meant that the plants had already been subject to several hours of light in the growth rooms and that the light intensity during treatment was also high.

When the plants were harvested, they were removed from the growth rooms and all soil washed from the root systems. Plants were then immediately stored in a darkened cool room at 3° C and dissected as soon as possible. Once dissected all plant material was dried in a vacuum oven (2mm Hg, 40° C, 16hr), weighed and stored in sealed containers for subsequent analysis. The components that were kept separate were flowers, floral buds, stems, leaves, tap root, fibrous roots, crown material and vegetative buds from the crown.

For the ^{14}C analysis, the plant material was finely ground and subjected to a wet combustion procedure similar to that of Shimshi (1969). About 50mg of plant tissue was placed into a screw top jar into which a smaller vial of CO_2 absorbent solution (5mls of 10% ethanolamine + 10% ethanol in water) was placed. About 20ml of cold saturated chromic acid ($K_2Cr_2O_7$ + 18N H_2SO_4) was then poured onto the plant sample and the jar sealed with the rubber lined screw lid. The jars were then placed in a water bath at about 45°C for 3.5 hours during which time occasional gentle agitation ensured that any clumps of material were dispersed to permit complete oxidation. After at least an hour's cooling period a 0.5ml sample of the absorbent solution was taken and added to 10ml of scintillation cocktail. This was a mixture of toluene and p-terphenyl, Triton X-100 detergent and water in the ratio of 20:10:3 (Laing and Christeller, 1976). Radioactive measurements were made using a Beckman 8000 liquid scintillation counter. Each sample was counted for 10 minutes. Correction for blank activities and a quench factor were used to give disintegrations per minute (d.p.m.).

Data were analysed by comparing relevant components across harvests using one-way analysis of variance. Also a recoding procedure was used where the hypothesis that the accumulation of ^{14}C was in direct proportion to the mass of a given component was tested. This was seen as a way of examining the data for any indication of sink strength. If more replicates for each harvest had been available a regression approach could have been used to test the relationship. If the total percentage of ^{14}C for a given component was less than its percentage contribution to the total plant mass, then a score of -1 was used, and if the activity was greater than that suggested by the component mass the score was 1. Contingency table analysis was then used to test the hypothesis of whether the observed number of components with a greater or lesser accumulation of ^{14}C differed. The two situations that were considered were with all components together and when grouped into top, or harvested material, and residual material.

5.3 Results

5.3.1 Introductory Remarks

The timetable of events (Fig 5.1), displays clearly the harvest schedules in terms of days of growth and percentage of elapsed time for the distribution and redistribution experiments. The percentage of elapsed time provides a common scale over which the two cultivars can be compared, when examining patterns of development.

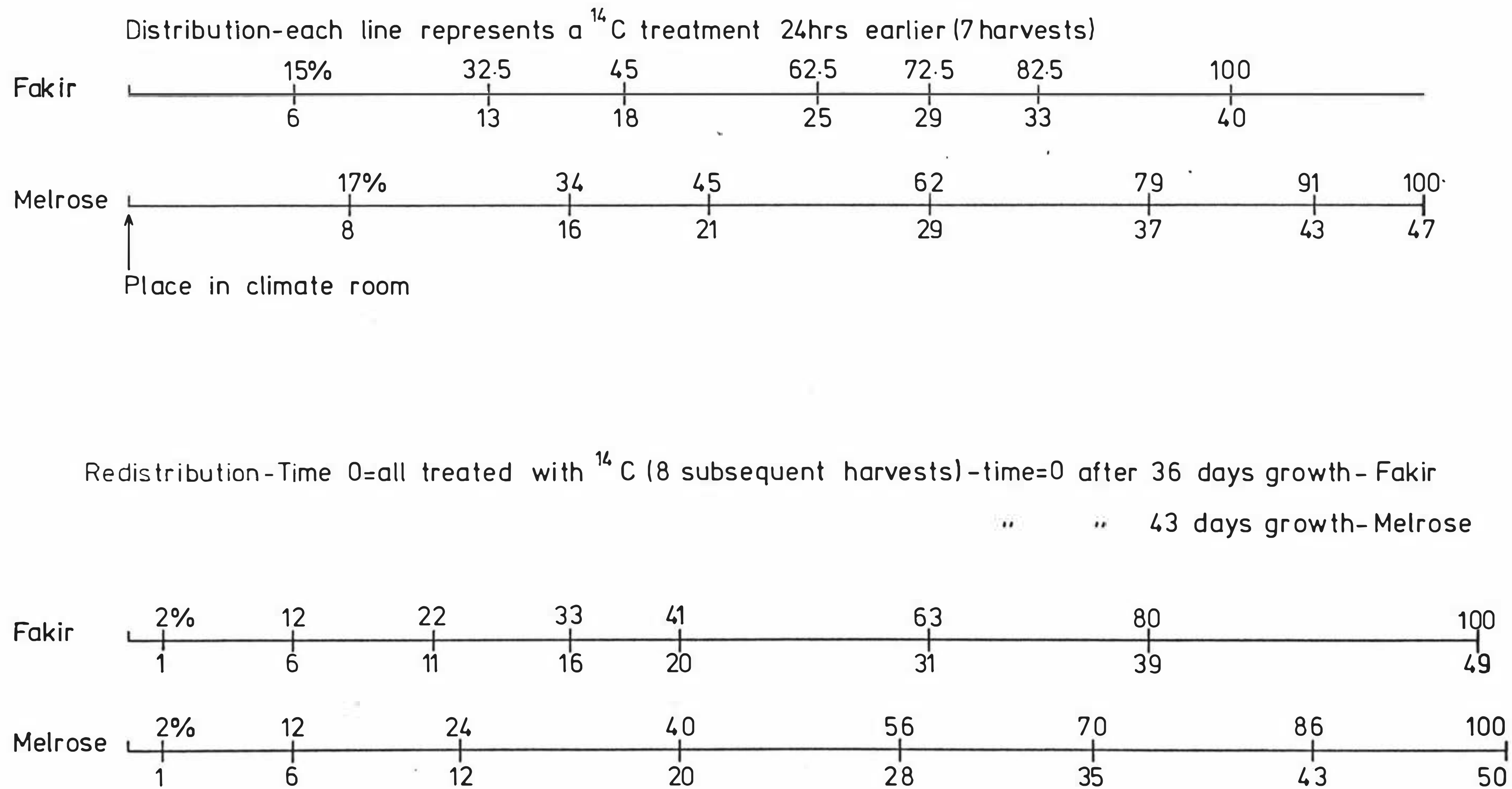


Fig.5.1 Timetable of harvests (upper scale=% elapsed time; lower scale=number of days)

It should be noted that any reference to growth or primary growth, and regrowth, refer to the plants from the distribution and redistribution experiments respectively.

The rate of growth and development was slightly more rapid than predicted from the field experiment but this was attributed to the constant growing conditions of the controlled climate rooms rather than any alteration in the pattern of growth. Only one plant from both experiments failed to flower in the period immediately after introduction from the glasshouse. This indicated that the autumn-winter conditions in the glasshouse, and the photoperiod in the controlled environment rooms (14 hours), permitted floral initiation and development.

One element to note is that as the two experiments were run concurrently, a decision had to be made about when to treat and harvest the primary growth from the plants in the redistribution experiment, before the full cycle of sampling was completed for the distribution experiment. As a result of this, the treatment for the redistribution was about the same timing as the sixth, of the seven distribution treatments and harvests.

As anticipated from the field work, the regrowth period was a few days longer and the between plant variability tended to be greater, than for the primary growth phase. For example, the final sampling time for the redistribution experiment was delayed by the slowness of all plants in achieving a minimum stage of floral development.

5.3.2 Growth and Regrowth Patterns

Dry weights for individual components for the growth and regrowth cycles are graphed in Fig 5.2 for Melrose, and Fig 5.3 for Fakir. Any subsequent reference to tops indicates the harvestable portion of the plant i.e. leaf, stem and flower, while the residual material can be regarded as the crown, tap and fibrous roots and any vegetative buds that may have been present.

While some of the data e.g. top mass, as shown in Fig 5.4, could have been fitted by regression there appeared little advantage in doing so considering the relatively parallel nature of growth for both Fakir and Melrose. Also due to the variability of the plants and the limited replication, none of the components measured at the final harvests in both growth and regrowth phases could be regarded as significantly different between cultivars.

Time taken to reach 50% of the final dry matter production is suggested as a useful "index" in this study to permit comparisons between the two cultivars and to provide an indication of the pattern of plant development and is shown in Fig 5.4. What became clear was that for the primary growth phase, about three-quarters of the growth period elapsed before 50% of the top production was achieved, and that this time increased by about 10% in the regrowth phase. In both cases Fakir developed earlier than Melrose even though final yields were not significantly different.

The other general feature of the growth and regrowth cycles was that top regrowth for Fakir represented about 40% of the primary growth, while

Fig. 5.2 Component dry weight (g/plant) for Melrose primary growth and regrowth

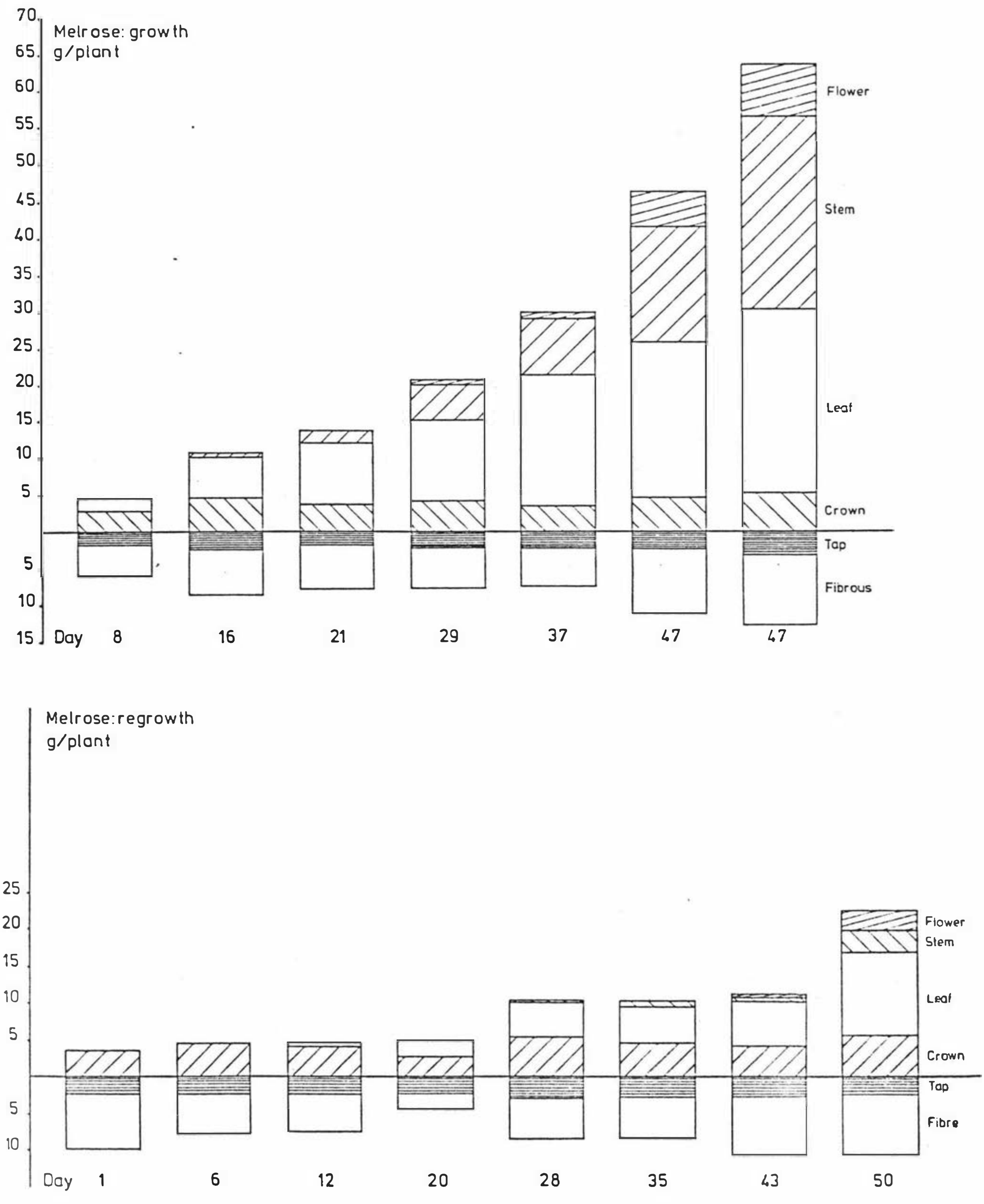
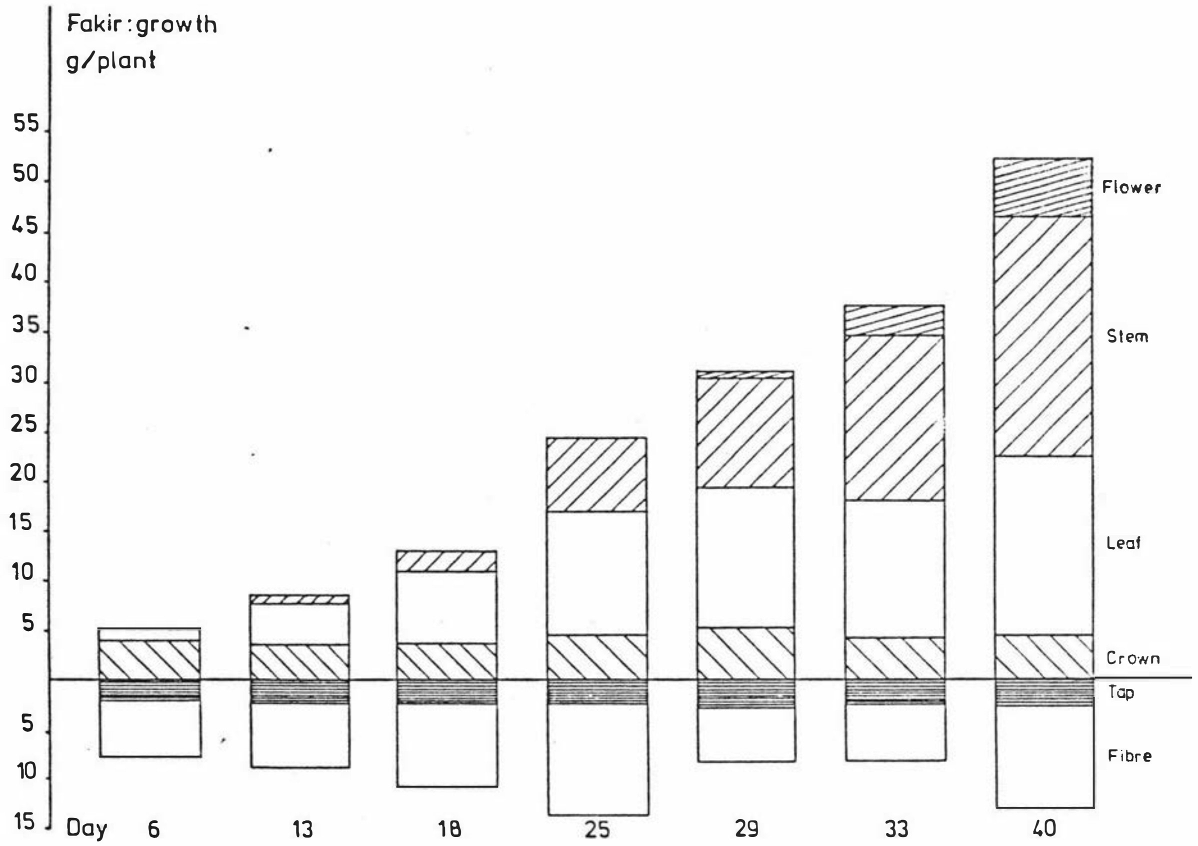
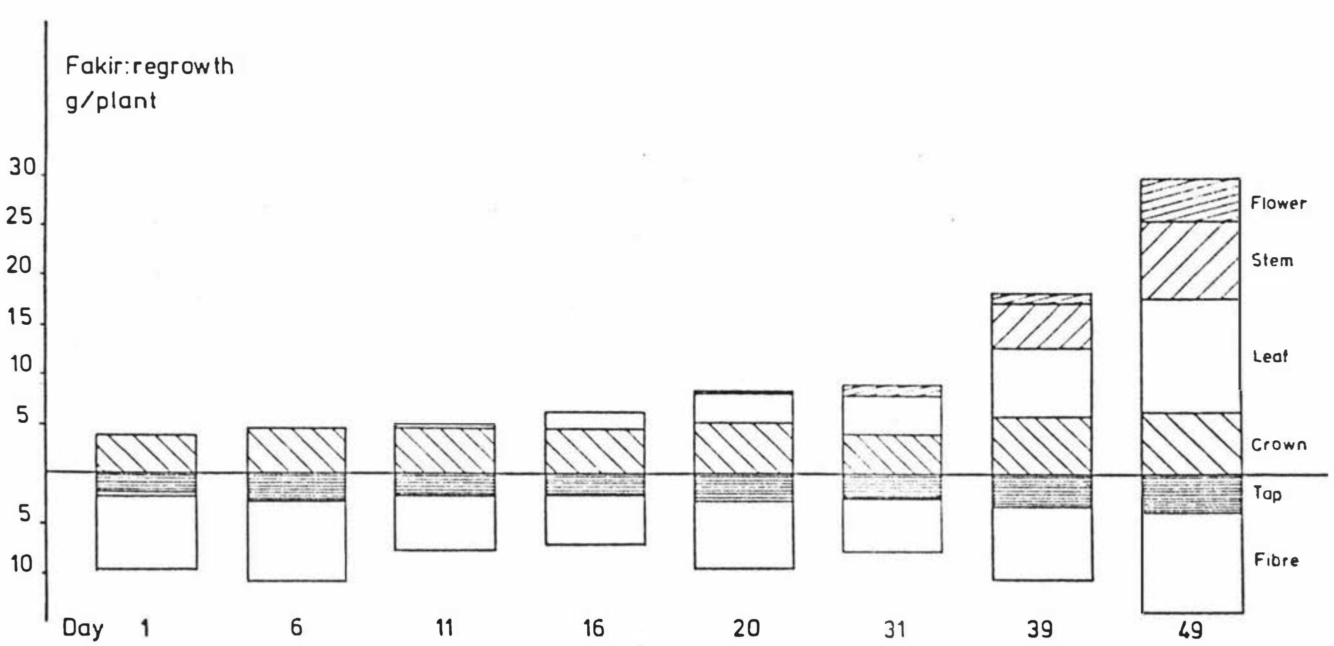


Fig.5.3 Component dry weight (g/plant) for Fakir primary growth and regrowth





for Melrose regrowth reached only 29% of the primary growth. The growing conditions in both cases were identical.

Figure 5.5 shows the development of leaf area was similar for both cultivars. The apparent advantage of Melrose for the primary growth period was not significant. As with the decline exhibited for top growth, leaf area of the regrowth only reached about 44% of the primary for Fakir, and about 37% for Melrose.

Examination of flower bud and flower numbers (Fig 5.6), and their weights (Figs 5.1 and 5.2), reveal that sainfoin, at least as indicated by the variable cultivars used here, does not have distinct vegetative and reproductive stages. While microscopic examination was not used, it was clear that there were flower buds present for the same duration as any vegetative growth for both the growth and regrowth cycles. The number were similar for both cultivars, and even though Fakir appeared more prolific earlier in the regrowth phase, the final difference was not significant. Weights follow the same pattern of development and represent about 12% and 15% respectively of the top mass in the growth and regrowth stages.

When the root material is examined from both Figs 5.2 and 5.3, it can be seen that the root mass varied in terms of both accumulation and depletion over a relatively short time span. However, as several of the results for both tap and fibrous root weights were extremely variable and non-significant, only the more general features will be considered.

Fig 5.7 is indicative of the general pattern of growth for both tap and fibrous roots and so these weights were not graphed separately. Even though the patterns were similar, the range of change was much greater for fibrous root than tap root weight. This can be readily seen from Figs 5.2 and 5.3. The common features of the growth period would seem to have been a recovery of root mass over the first two weeks, as the plant recovers from the harvest prior to placement in the controlled environment, and an increase in root mass when the plant approached reproductive maturity. The basis of the peak and then decline for Fakir root mass between 25 to 29 days (Fig 5.3) was not clear, even though it coincided with a rapid increase in numbers of inflorescences and a comparatively stable period of leaf development (Fig 5.3 and Fig 5.7).

During the regrowth period there appeared to be a definite loss of root mass, reaching a maximum after two to three weeks. Melrose would seem to have been more affected than Fakir, with relatively large losses of both tap and fibrous root mass, particularly around day 20 (Fig 5.2). Once leaf area commenced to build up, the recovery in root mass resulted in the plants achieving a final root mass similar to that of the primary growth phase.

Basal or crown buds are considered separately (Table 5.1), as their contribution to the overall plant mass was minor. These buds were examined on the basis that they were likely to provide the next generation of shoots. In retrospect some of the material assigned the status of basal shoots would appear to have been nothing more than the protective stipule material of unelongated nodes. While this would not have much effect on the weight, it may have contributed to the fluctuations in counts, particularly in the regrowth phase. Despite this shortcoming it was very clear that there was a relatively large pool of basal buds that appeared to

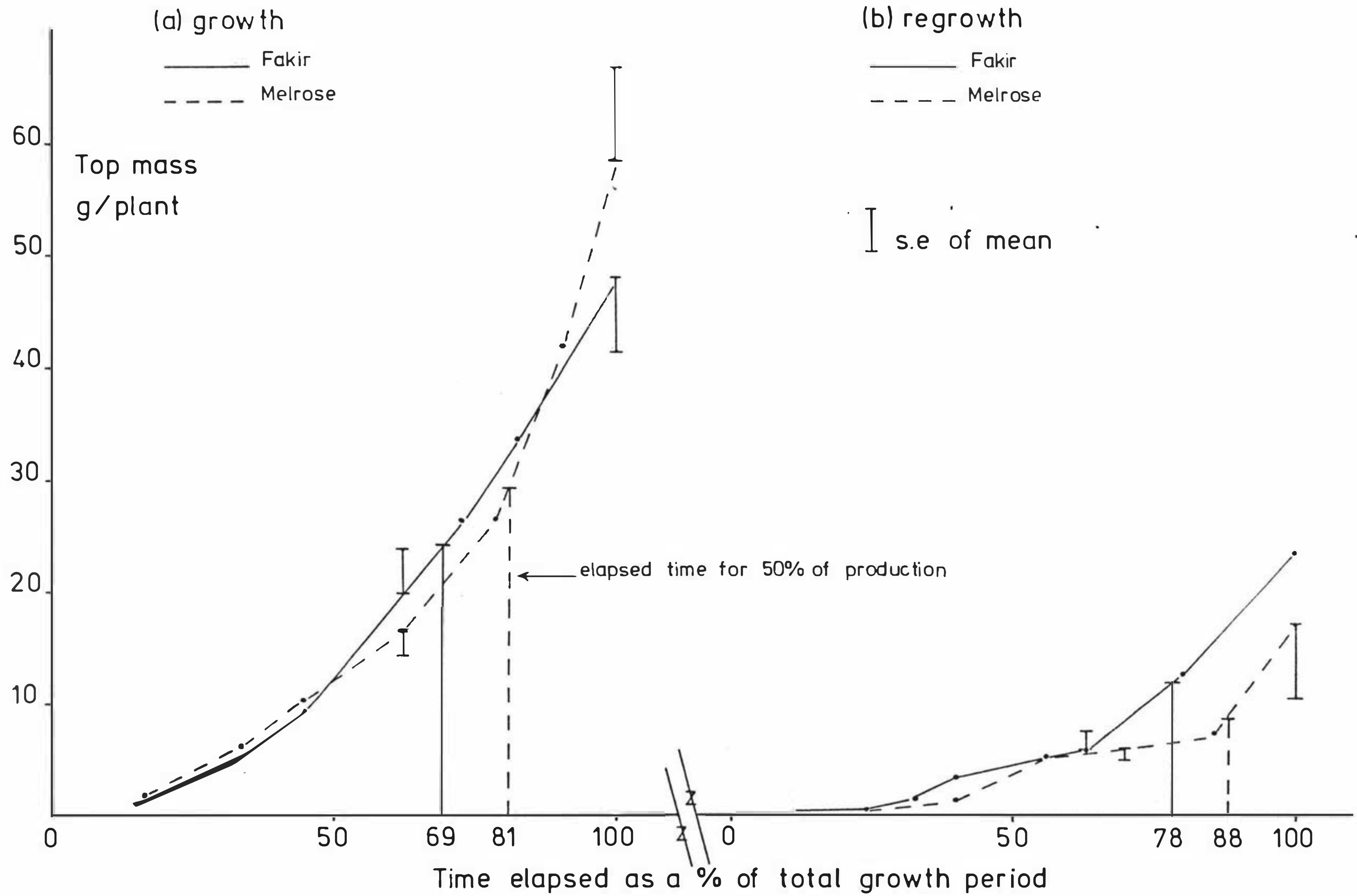


Fig.5.4 Top mass (g/plant) for Fakir and Melrose primary growth and regrowth

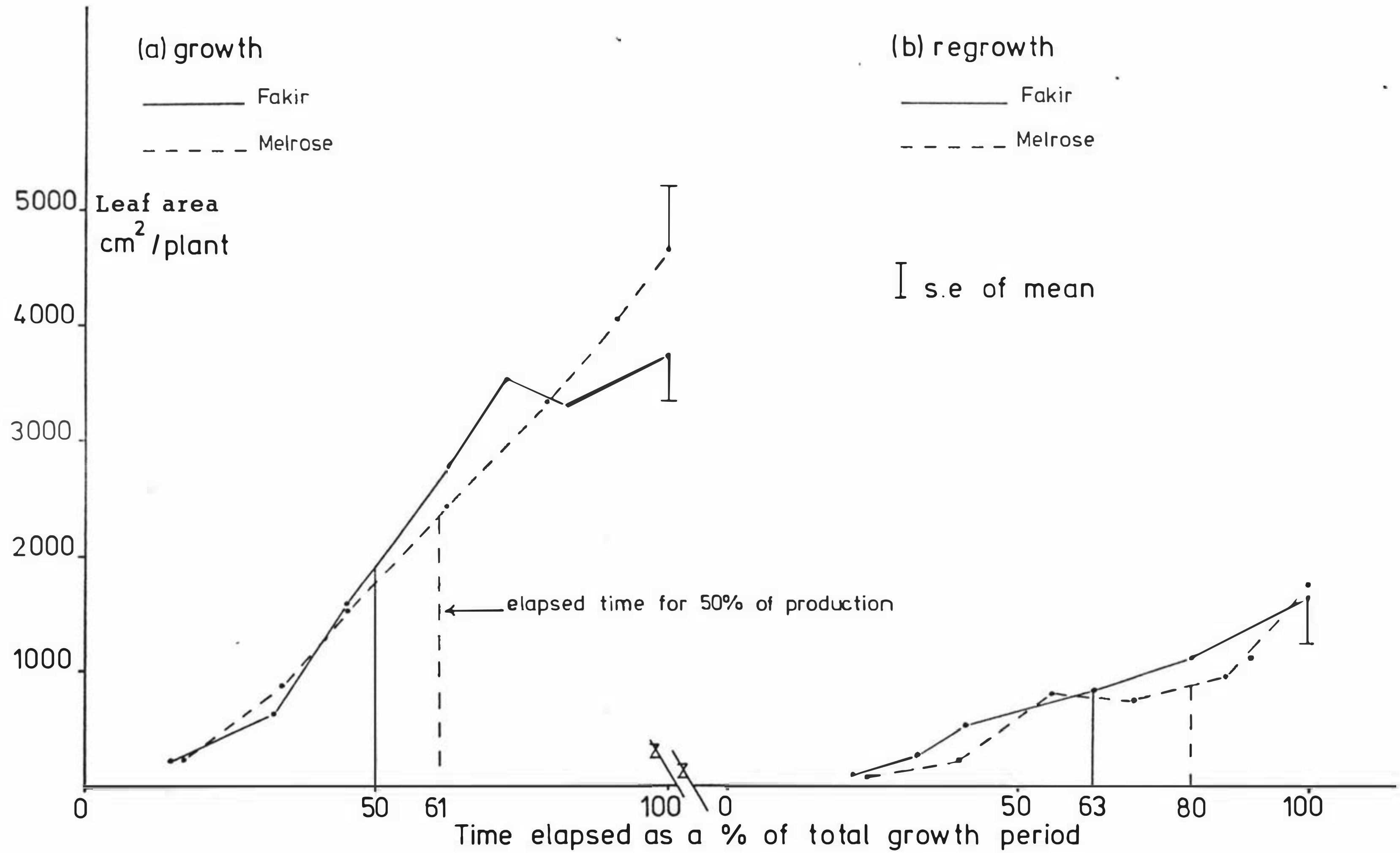


Fig.5.5 Leaf area development (cm^2/plant) for Fakir and Melrose primary growth and regrowth

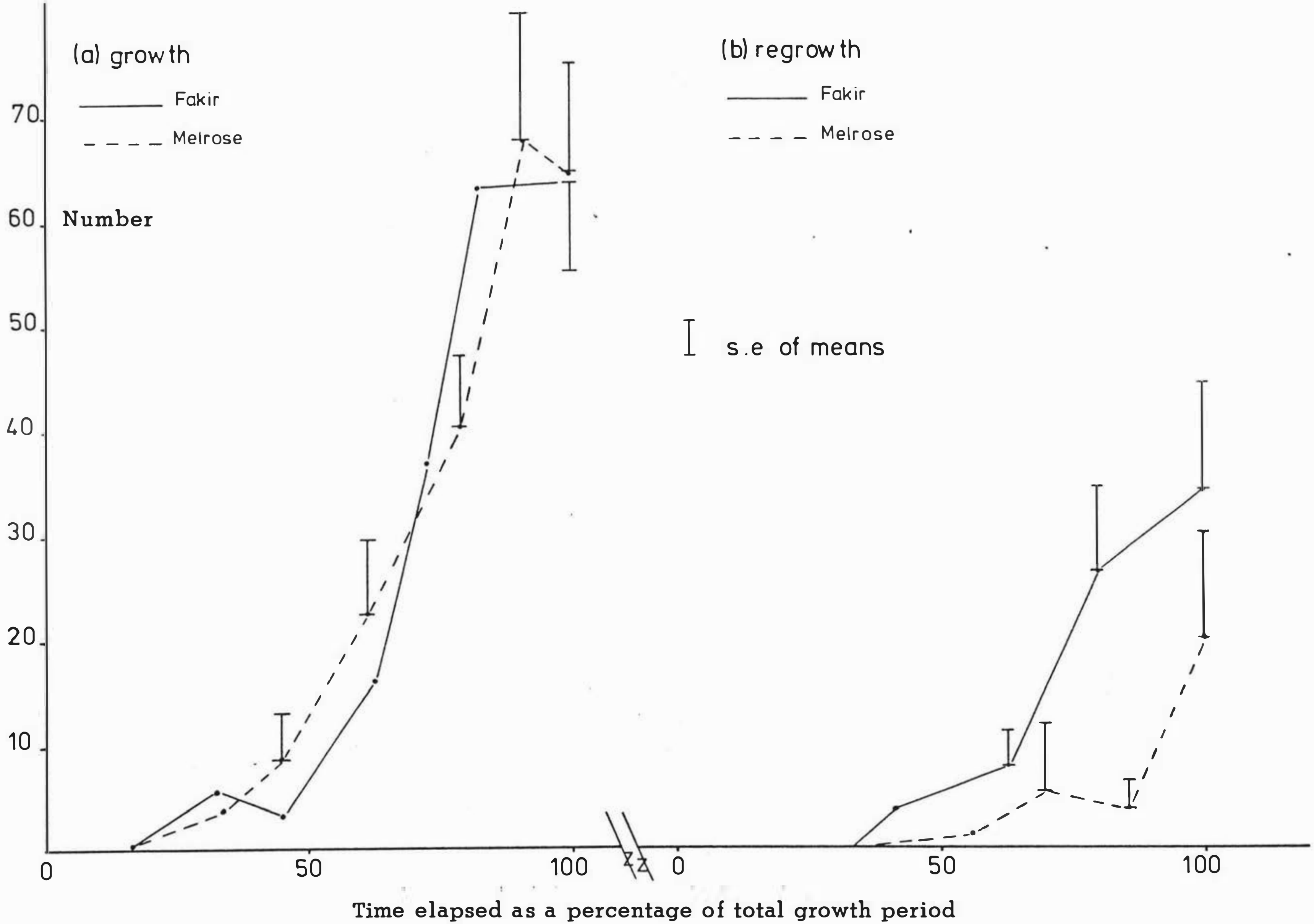


Fig.5.6 Numbers of flower buds and flowers

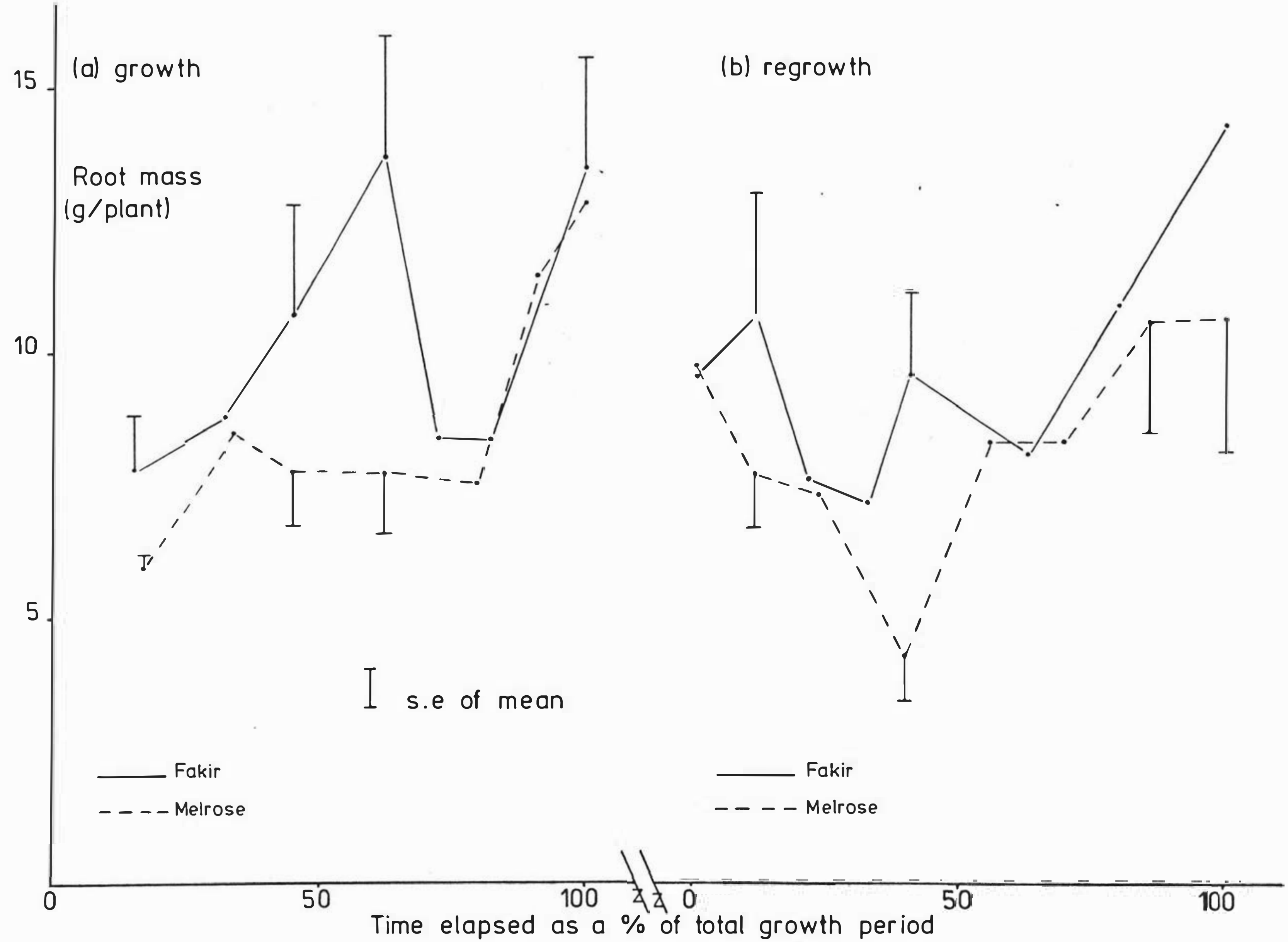


Fig.5.7 Total root mass (g/plant) for Fakir and Melrose primary growth and regrowth

form in the growth period and again during regrowth. Of this pool, only about 10 stems for Fakir and 9 for Melrose actually showed any elongation during regrowth. The other feature of importance was that few, if any, of the individual buds had shown development beyond extension of 1-2mm when the regrowth phase commenced.

5.3.3 Distribution

Table 5.2 provides an indication of the differences in ^{14}C uptake with time. Apart from the first one or two harvests, where there was a temporary error with the experimental technique, there is a general increase in activity of treated plants as they increase in size.

Table 5.1 Numbers and weights of basal buds.

GROWTH

Melrose			Fakir		
Day	N°/Plant	Wt (mg/plant)	Day	N°/Plant	Wt (mg/plant)
8	0	0	6	1.6	17
16	1.9	4	13	2.9	13
21	6.3	31	18	1.9	6
29	8.7	43	25	8.9	27
37	15.3	59	29	12.1	53
43	20.9	106	33	24.7	70
47	16.7	43	40	29.3	97
sign level	*	n.s.		***	**
S.E. mean	3.5	26		3.4	15.6
LSD (5%)	10.1			9.8	45

REGROWTH

1	4.7	11	1	0.8	3
6	27.4	146	6	23.4	131
12	23.1	127	11	24.6	167
20	14.4	83	16	30.6	249
28	25.0	131	20	17.4	137
35	14.9	63	31	10.7	54
43	17.3	139	39	17.3	53
50	18.1	89	49	18.0	149
sign level	n.s.	n.s.		n.s.	*
S.E. mean	3.7	24		3.0	23
LSD (5%)					66

Table 5.2

Mean counts per plant for each harvest (d.p.m.).

	Melrose	Fakir
H1	21585	9646
H2	11341	4596
H3	4564	5642
H4	5507	6439
H5	6852	7833
H6	9096	6588
H7	12845	10821
S.E.	877	695
LSD (5%)	1718	1362

When the individual components were examined for the percentage distribution of ^{14}C (Figs 5.8 and 5.9), it is clear that the leaf was the major location of the ^{14}C twenty four hours after treatment. Over the final two harvests, the stem was also a large sink for the labelled assimilates. While the developing inflorescences formed a sink of increasing size, there was no evidence to suggest that they were accumulating unusual amounts of ^{14}C, but it should be remembered that no seed formation occurred in the controlled environment facilities.

The higher activity of the crown tissue at the first harvest for both cultivars was thought to have resulted from the dissection technique which left the rapidly developing, but unelongated, stems and young leaf material below harvest height.

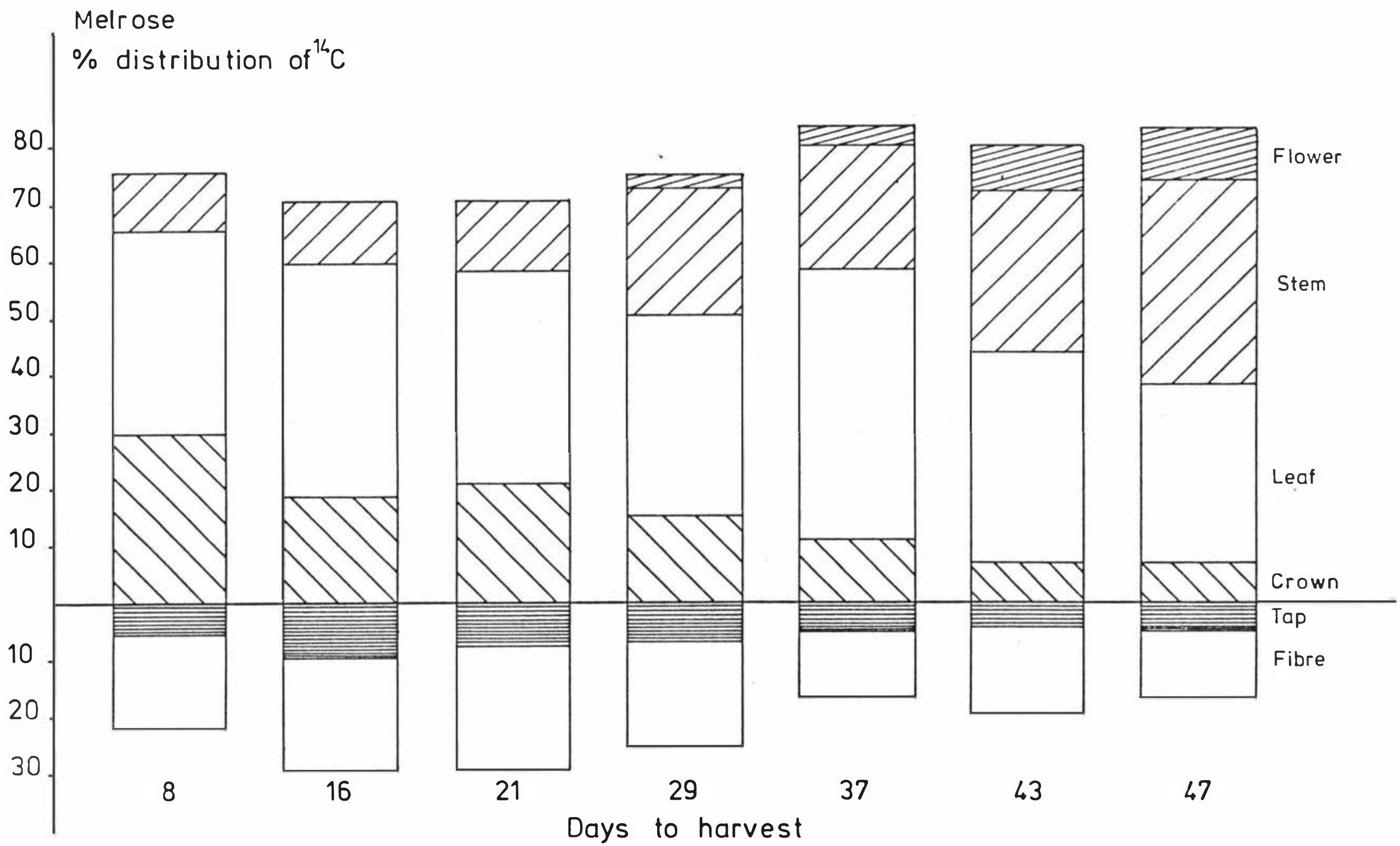


Fig. 5.8 Distribution of ^{14}C in Melrose over seven consecutive treatment periods (Distribution Experiment)

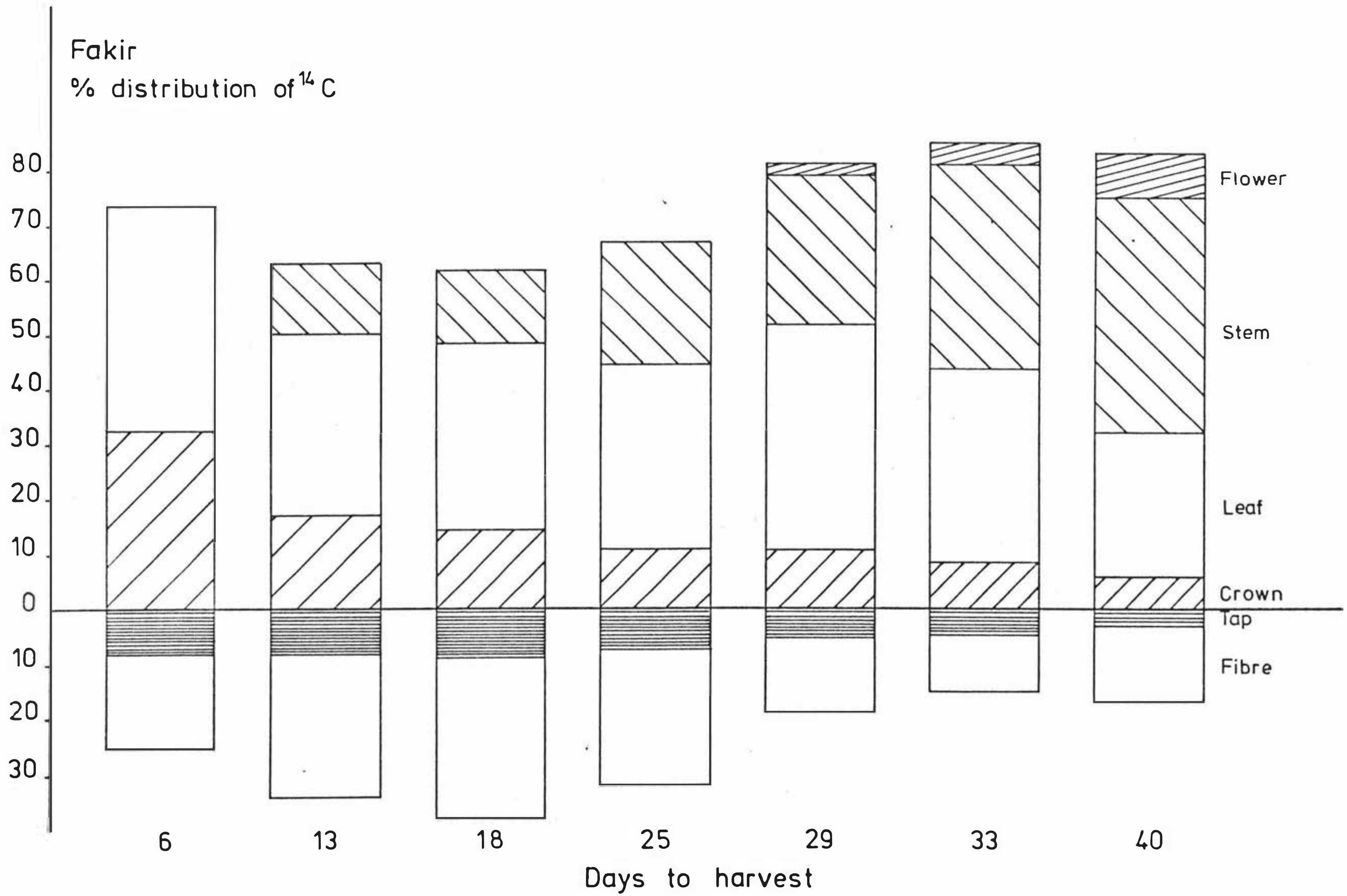


Fig.5.9 Distribution of ^{14}C in Fakir over seven consecutive treatment periods (Distribution Experiment)

The proportion of labelled assimilates going to the root system reached a peak of about 30% of the total about 3 weeks after growth commenced, and then declined to be less than 20% at the final harvest (Fig 5.8 and Fig 5.9). The proportion going to the fibrous roots was the main contributor to the observed changes for the below ground fraction, as the results for the tap root were relatively constant.

Basal bud data was not included due to the very small sample sizes involved, and no evidence of any disproportionate accumulation of ^{14}C.

Due to the considerable range in the weights of individual components it was regarded as useful to examine the relationship between component dry weight and accumulation of ^{14}C on the basis of the hypothesis that ^{14}C might be directly related to dry weight (Section 5.2.4).

Tables 5.3a and 5.4a showed the observed and expected values for the number of components that might have been either exceeding, or not achieving the hypothesis of equality of distribution in relation to component dry weight when all components from the harvests were considered together. A summary of the data is presented as Tables 5.3b and 5.4b.

Table 5.3 Contingency Table for Fakir.

(More/Less = Greater or less accumulation of ^{14}C than based on dry weight).

a) All components

b) Components grouped as top - flower, stem, leaf.
residual - bud, crown, tap, fibre.

a)

	More		Less	
	observed	expected	observed	expected
Flower	18	12	9	15
Stem	22	15.5	13	19.5
Leaf	32	18.6	10	23.4
Bud	16	8.9	4	11.1
Crown	10	18.6	32	23.4
Tap	7	18.6	35	23.4
Fibre	6	18.6	36	23.4

$Chi^2 = 73.4$ ($p<0.005$)

b)

Top	72	46.2	32	57.8
Residual	39	64.8	107	81.2

$Chi^2 = 44.4$ ($p<0.005$)

Table 5.4 Contingency table for Melrose

(More/Less = Greater or less accumulation of ^{14}C than based on dry weight).

a) all components

b) components grouped as top - flower, stem, leaf.
residual - bud, crown, tap, fibre.

a)

	MORE		LESS	
	observed	expected	observed	expected
Flower	25	14.3	5	15.7
Stem	26	16.7	9	18.3
Leaf	21	20.1	21	21.9
Bud	11	5.7	1	6.3
Crown	17	20.1	25	21.9
Tap	9	20.1	33	21.9
Fibre	8	20.1	34	21.9

$Chi^2 = 60.9$ ($p<0.005$)

b)

Top	72	51.1	35	55.9
Residual	45	65.9	93	72.1

$Chi^2 = 29$ ($p<0.005$)

It is immediately clear that for both cultivars there was a significantly greater proportion of the ^{14}C remaining or located in the top of the plant (leaf, stem and flower), than might have been predicted on the basis of dry matter distribution. While for Fakir all three components displayed this trend, Melrose leaf conforms closely to the proportional hypothesis. Conversely the residual components, particularly the tap and fibrous roots, did not accumulate ^{14}C in nearly enough cases to satisfy the hypothesis.

The basal buds appeared to accumulate more ^{14}C than expected for both cultivars, but the absolute values involved were very small and the numbers of this component measured were somewhat less than other components.

5.3.4 Redistribution

As the exact time of treatment was based on most plants displaying some degree of floral maturity i.e. expanded florets, and this experiment was being run concurrently with the distribution experiment, the time of treatment meant that Fakir had a primary growth period of 36 days and Melrose, 43 days. These differences appear partly responsible for certain notable features.

Firstly, the plants used in the redistribution experiment displayed an apparently lower starting mass than might have been expected from the final harvest of the distribution experiment (Fig 5.7). However, by examining the root development in the overlapping period it can be seen that the roots were rapidly gaining weight and so differences could have easily occurred within a few days.

While the smaller root mass at the start of the redistribution experiment may not present problems with subsequent growth, it does indicate the source of a key problem in this experiment. Over the period of the last two distribution harvests the absolute uptake of ^{14}C increases (Table 5.2), while the proportion entering the root system was declining (Figs 5.8 and 5.9). Therefore by treating and harvesting the redistribution plants earlier than the final harvest stage for the distribution experiment, the uptake of ^{14}C would have been lower than if delayed for the extra days. This, in combination with the rapid decline in root mass over the first 15-20 days after defoliation, led to a very poor and irregular recovery of ^{14}C.

As a result of this no ^{14}C data were considered to be useful from the redistribution experiment and so any discussion will necessarily centre on the dry weight data alone.

5.4 Discussion

The first major point to emerge from the controlled environment study was that even under relatively ideal conditions for growth, both cultivars displayed poor regrowth. As expected, Fakir appeared to produce slightly less top growth than Melrose during the primary growth stage, but more during regrowth. These results tend to support the hypothesis generated from the field study that the poor regrowth is not just a function of the immediate supplies of water and nutrients, but rather a function of plant development as mediated by temperature and daylength. More specifically it would seem that the sainfoin plants require the stimulus of declining daylengths.

This work also clearly demonstrated that for what was the equivalent of a spring and subsequent growth cycle, there can be little or no differentiation into separate vegetative and reproductive phases. Coupled with the early development of inflorescences, which suggested initiation in the pre-experimental, short-day conditions, was their prolificacy. The results of this may be two-fold, with the flower buds or reproductive shoots exerting strong apical dominance over further basal bud development and secondly, being a large sink for assimilates if they are permitted to develop and undergo fertilisation. The former situation is suggested by Sheely (1977) as a result of the early reproductive development, and supported in this work by the poor development of basal shoots as well as the almost complete absence of any stem branching. The magnitude of the sink formed by the inflorescences was indicated by their mass prior to any fertilisation being about 12-15% of the top growth, and the fact that they appeared to gain assimilates at a greater rate than dry matter increase might have suggested. If the total carbon and other assimilate requirements for nectar flow, which is known to be considerable (Howes, 1945), and seed production for which Ditterline and Cooper (1975) regard 1400kg/ha as possible are considered, it can be seen that sainfoin has a large investment in regeneration via seed.

Leaf growth in the controlled environment conditions was rapid and did not display the same plateaus or declines that were evident in the field studies (Section 4.3.3.2). This may have been due to a combination of a more stable and continuous light environment, a continuous water supply and not the same level of within-canopy shading by either other sainfoin plants or weeds. The result of this was that the leaf area developed by these plants should be somewhere near the potential for these cultivars of sainfoin. Therefore, if inadequacies in growth are directly related to leaf area, they might be expected to be exaggerated under field conditions. For the primary growth stage leaf development occurred almost as soon as the plants were placed in the controlled environment rooms. However, with the regrowth stage there was a delay of between two and three weeks before almost any leaf growth was observable. Therefore during this phase the plant must have been totally reliant on stored assimilates for respiration of the remaining tissue and to meet the demands of leaf development, at least until photosynthetic competence was achieved.

When the root material was considered it was immediately obvious that the greatest part was the comparatively fine, fibrous material which displayed limited secondary thickening and also supported the nodules. Even though the tap root was clearly distinguishable it was difficult to reconcile observations with any of the literature that suggests it is a large and extensive structure e.g. Spedding and Diekmahns, (1972). While the distribution of root mass was irregular there did appear to be definite periods of accumulation and depletion. These would seem to be subject to the effects of both competing sinks and the leaf area and photosynthetic efficiency. The former was indicated by the limited transfer of ^{14}C-labelled assimilates to the root system, and the latter by the abrupt decline in root mass in the regrowth phase when there was no remaining leaf tissue. The decline over that period was due to a combination of death and decay, as evidenced by the sloughing off of fine roots and the collapse of many nodules, and the respiratory demands of the plant for survival and leaf growth. Butler et al., (1959) have also shown the rapid and widespread browning of roots and nodules in defoliated red clover

(*Trifolium pratense*), a species that also tends to display poor vigour in regrowth.

The presence of a moderate number of basal buds that produced little or no growth, and had only limited metabolic activity in terms of ^{14}C accumulation suggested some form of inhibition of development. This may be, as already mentioned, due to the rapid establishment of apical dominance by those stems that elongate and carry flowers, both in growth and regrowth cycles. Support for this comes from Cooper and Watson (1968), and the field observations in this study on sainfoin, where some of the basal buds eventually elongate when flower and seed development is advanced, indicating some reduction or breakdown in apical dominance. Another possibility is that these buds, or the majority of them, actually require the stimulus of a combination of short-days and lower temperatures before development will occur. If that is the case, the buds would develop in the rosette phase and form the basic population for the subsequent spring regrowth.

The basic objective of the ^{14}C treatments was to examine the potential interaction between assimilate movement and management, in terms of cutting time and perhaps frequency. While the discussion of the redistribution phase will be of necessity, speculative, the information of dry matter build-up did provide an indication of why regrowth might be poor.

The first feature that was particularly clear was that from the ^{14}C uptake and distribution pattern, the roots represented a limited sink for assimilates over the spring growth period when compared to the leaf and stem tissue. Floral structures too, placed an increasingly important demand on the current assimilates. However, this experiment only measured where the ^{14}C was finally found. It was quite possible that movement of assimilates to the roots and re-export as nitrogen compounds from the nodules, as well as the respiratory requirements for synthesis of these compounds and maintenance of root tissue, means that a greater proportion of ^{14}C may have involved the root system. Recent studies by Witty et al., (1983), would suggest that sainfoin has a relatively high demand for respiratory carbon for both maintenance and nitrogenase activity when compared with other legumes such as lucerne and white clover. Circumstantial support for a demand of this sort comes from the fact that except for a minor supplement as seedlings, the plants involved in this experimental work received non-nitrogenous fertiliser for the duration of the programme. Over this time all plants displayed well developed nodules and gave no indication of nitrogen deficiency. Even when these factors are considered the root system showed a tendency to increase in size towards floral maturity. When this feature is considered with the relatively late accumulation of total top dry matter it can be seen that the time period available for some form of harvest strategy that combines the best of both conditions is extremely limited.

When regrowth is considered a range of problems become apparent. Of particular importance is the lack of leaf area remaining after harvest to generate any assimilate to meet the plant's immediate growth and respiratory requirements. From this work and the field experiment (Section 3.2.2), any attempt to improve this by increasing cutting height is somewhat limited by the few leaves that develop on each stem and the wide internode spacing.

If no active leaf tissue remains, then by default, the plant must mobilise reserves of some description to provide an energy source until photosynthesis is re-established. The combination of this demand, and the removal of the flow of assimilates to the root system, results in the extensive death and decay of feeder roots and nodules. This tissue is then replaced at a later stage in the growth cycle thus using assimilates that may potentially have been available for foliage production. While this cycling of the root tissue is not uncommon in forage legumes (Butler et al., 1959; Cooper and Watson, 1968), it does seem particularly disruptive in sainfoin. Root tissue loss of the extent observed will not only interfere with nitrogen fixation, which might be expected to cease with no direct energy source, but also effect the uptake of water and nutrients. Therefore it would not seem unreasonable that even mild stresses e.g. water shortage, coinciding with defoliation in the field, could effect the plants survival ability.

A further consideration at this point is that the sloughed off material is an ideal medium for pathogenic organisms. The field trial has shown that sainfoin appears extremely susceptible to root rotting organisms (Section 4.3.6), and this may have been in part due to this supply of organic material to the soil with each harvest.

Differences between the two cultivars, Fakir and Melrose, were mainly limited to the more obvious features. Fakir was earlier flowering than Melrose and tended to have better regrowth in comparison with primary growth. This may have been partly due to a less pronounced loss of root tissue after harvest, and a generally greater root mass than shown for

Melrose. Greater replication than was used in this work would be necessary to clarify these points.

Finally, while many of the vegetative characteristics seem very prone to the effects of defoliation, it is interesting to note that the plants still invest in development of moderately large numbers of flowers. Presumably, if the seasonal conditions were sufficiently good, these would reach maturity and set seed.

In concluding the controlled environment studies permitted a range of useful comparisons and contrasts with the field studies. This enabled a closer definition of some of the parameters that might be controlling the growth and regrowth in sainfoin. While the ^{14}C data was more limited than anticipated when establishing the experiments, it still provided a useful method with which to consider the pattern of movement of assimilates in what is sainfoin's main period of production.

CHAPTER 6: A MULTIVARIATE APPROACH TO THE EXAMINATION OF VARIABILITY IN SAINFOIN (*Onobrychis viciifolia*)

6.1 Introduction

From the data and discussion of both the field trial and the controlled environment study it is quite clear that sainfoin plants display a high level of heterogeneity. The effects of this will be dependant on the objectives of a given experiment. It may present problems in that it reduces the sensitivity of more conventional field trials, or it may be most useful in providing flexibility to a plant breeder.

The first point is often alluded to in reviews and comparisons of work (e.g. Fortune and Withers, 1980) where there is a lack of consistency of trends between experiments. The work of Rumball (1982), on plant introduction trials provides a useful example of using patterns of variation to form certain general groups of lines from a germplasm collection.

In this study this approach is taken further with the use of multivariate statistical techniques to examine variability in a more orderly and less subjective manner. There is nothing new in the theoretical and conceptual basis to multivariate analysis but with the increasing availability of computer services the facilities now exist to undertake the large scale numerical manipulations that are often involved.

In this work only two techniques were used, factor analysis and K-means clustering. This permitted the examination of the relationships between the measured variables and also to see whether there is some basis for grouping the individuals into definable lines. The overall objective of this numerical approach was to examine the variability, simplify the complex array of data collected, and to generate hypotheses on how to make effective use of the reduced data.

6.2 Experimental

The detailed outline of the plant propagation procedure was presented in Section 5.2. The plant material that provided the data for this part of the programme was obtained from the plants involved in the redistribution experiment. When the plants had all been treated with ^{14}C at about floral maturity, all top material was removed after 24 hours and dissected. Thus for each of the two cultivars, Fakir and Melrose, there was an information, matrix of 56 individuals by 8 variables. The variables were flower number, floral bud number, total dry weight of flowers and floral buds, number of stems, total and individual stem weight, total leaf weight and specific leaf area.

6.2.1 Data Analysis

The data were examined using factor analysis and K-means clustering. The computer programmes used were part of the BMDP statistical package (Dixon et al., 1981) on a PRIME 750 computer system.

Factor analysis involves extracting factors from the correlation matrix with the aim of reducing the number of variables to a more manageable number of factors without any loss of information. The underlying assumption is that a smaller number of factors exist that can reproduce exactly the correlations in a larger set of variables. There are a number of methods of extracting factors and these will vary with the computer programme used and personal preference, rather than rigid statistical demands. In this experiment a principal components procedure with subsequent varimax rotation was used. The aim of the rotation was to achieve a simple structure by maximising the important factor loadings while minimising the others. Ultimately for each case the set of variables can be replaced by a more limited set of factor scores and these examined for deviations from the mean. The factor scores are derived from the values of each of the variables multiplied by the standard scores for each variable of the individual cases. This is in effect the same as using the factor structure as an equation similar to multiple regression to arrive at simple descriptors for each case.

The K-means clustering procedure is an iterative process of allocating various cases (individual plants) to a number of groups based on a criterium of minimising within groups sums of squares. The separation of cases is based on a multivariate distance measure, in this case the Euclidian distance. The exact number of clusters can be defined or based on a descriptive F-test that compares the between-cluster mean square to the within-cluster mean square. All data were initially standardised to z-scores (standardised normal deviates) to reduce all attributes to a scale of comparable range, so that the recording scales do not automatically permit the dominance of certain variables.

6.3 Results

Of the eight principal components shown in Table 6.1, only the first two are maintained for subsequent analysis as these account for about 76% of the overall variance. In this case the first factor explained a little more than 50% of the variance while the second factor adds a further 25%. The exact number of factors used is the result of fairly empirical techniques and in many computer programmes a factor with an eigenvalue (variance explained) of less than one is discarded. Low values usually represent either error variance or influences which affect only one or few of the variables. While not shown here, the extraction of a third factor separates out SLA which is relatively poorly defined by the first two factors.

Table 6.1

Complete factor structure for Fakir and Melrose after principal component extraction.

	FAKIR		MELROSE	
Factor	Variance Explained	% of Total	Variance Explained	% of Total
1	4.33	54.1	4.29	53.6
2	1.76	76.1	1.81	76.2
3	0.95	88.0	0.79	86.1
4	0.57	95.2	0.66	94.3
5	0.23	98.1	0.24	97.2
6	0.08	99.1	0.14	99.0
7	0.04	99.6	0.04	99.6
8	0.03	100.0	0.05	100.0

Table 6.2 illustrates both the factor structure after the initial extraction and after the varimax rotation procedure. It is immediately apparent that the rotation maximised certain of the variables and these have been underlined in Table 6.2 to provide emphasis. On the basis of this, Factor 1 might be regarded as a later maturing, vegetative descriptor, while Factor 2 has a strong loading for flower number and weight. Any reference to maturity was based on the comparative numbers of flowers and buds, rather than an index or scale based on flowering dates. The variable weight/stem seems to be associated with both factors. Both cultivars displayed a marked similarity with their overall structure.

While the rotated result made interpretation a little easier, the same result could be derived from the unrotated factors. If they are examined, it can be seen that the first factor might be regarded as a general descriptor of plant size and structure, while the second was a bipolar factor with opposite loadings on the "reproductive" and "vegetative" attributes. This emphasises the fact that rotation was not altering the relationships but was a rescaling procedure.

Table 6.2

Loadings of the variables on the first 2 factors after both direct extraction and varimax rotation.

FAKIR

	1		2	
Variable	Direct	Rotated	Direct	Rotated
Flower No.	0.62	0.18	-0.70	0.90
Bud	0.90	0.78	0.02	0.45
Flower wt	0.74	0.33	-0.58	0.88
Stem	0.70	0.84	0.45	-0.02
Stem wt	0.90	0.95	0.36	0.16
Leaf wt	0.84	0.97	0.50	0.03
Wt/Stem	0.70	0.57	-0.04	0.40
SLA	-0.31	0.07	0.64	-0.71

MELROSE

Variable	Direct	Rotated	Direct	Rotated
Flower No.	0.49	-0.06	0.80	0.94
Bud	0.90	0.77	-0.08	0.45
Flower wt	0.67	0.14	0.70	0.96
Stem	0.68	0.86	-0.52	-0.03
Stem wt	0.91	0.89	0.25	0.32
Leaf wt	0.77	0.94	-0.54	0.00
Wt/Stem	0.76	0.54	0.13	0.54
SLA	-0.60	-0.39	-0.17	-0.48

Table 6.3 provides a general summary of the five clusters formed for Fakir. Five clusters were chosen in each case to provide a balance between descriptive power and an unnecessary number of small clusters. The following notes provide a brief description of the clusters.

Table 6.3

Fakir clusters and character means (weights = g/plant).

	1	2	3	4	5	variable grand mean
Flower No.	14.6	5.4	6.2	3.5	0.3	4.9
Bud No.	46.3	40.2	70.6	30.3	19.3	34.3
Flower wt	5.9	2.5	4.1	1.7	0.4	2.3
Stem No.	9.5	13.8	15.0	7.3	7.7	9.1
Stem wt	11.6	12.5	24.0	8.4	5.7	9.9
Wt/Stem	1.3	0.9	1.6	1.2	0.7	1.1
Leaf wt	9.4	12.2	17.9	7.8	6.7	9.0
SLA	228	282	249	227	287	252
Top mass	26.9	27.2	46.0	17.9	12.8	21.2
Cases	10	5	5	19	17	56

Cluster 1: a group of earlier maturing plants that have a large number of well developed flowers.

Cluster 2: these plants tend to have a greater than average number of small stems.

Cluster 3: these plants are later maturing than those in Cluster 1 but still show reproductive prolificacy as well as strong vegetative development.

Cluster 4:} both these clusters are similar and the plants tend to
Cluster 5:} be at, or below the grand mean for most attributes. Cluster 5 does, however, incorporate plants with high SLA's and few other distinguishing features except for a constantly poor value for the other variables.

Table 6.4

Melrose clusters and character means (weights = g/plant).

	1	2	3	4	5	variable grand mean
Flower No.	31.0	0	4.6	1.3	2.6	3.1
Bud No.	64.0	11.6	67.0	20.7	48.2	37.8
Flower wt	11.5	0.2	4.0	1.0	2.1	2.1
Stem No.	10.0	6.8	17.2	7.2	13.8	11.1
Stem wt	11.2	1.6	21.5	3.9	9.4	8.0
Wt/Stem	1.1	0.3	1.3	0.6	0.7	0.7
Leaf wt	9.6	5.8	19.3	7.5	12.8	10.8
SLA	198	330	205	241	234	241
Top mass	32.3	7.6	44.8	12.4	24.3	20.9
Cases	2	5	5	19	25	56

Table 6.4 provides a general summary of the five clusters formed for Melrose. The following notes provide a brief description of the clusters.

Cluster 1: only two plants well separated from the other clusters by their degree of floral development and maturity.

Cluster 2: these plants are below average for most attributes but have a particularly high SLA.

Cluster 3: this group is well above avarage for all attributes except SLA. All plants have a large number of well developed stems which provide sites for both leaf and floral development, and this is reflected in the respective values for leaf weight and bud number.

Cluster 4:} two more general clusters where attributes are
}
Cluster 5:} distributed either slightly above (Cluster 4), or below (Cluster 5), the grand mean values.

Total top mass of the plants in each cluster is shown in Tables 6.3 and 6.4, but was not used as a variable in the clustering procedure. This value was provided as an indicator of harvestable production from various clusters.

As the two cultivars showed similar patterns with the type of factors and clusters formed it was important to consider any differences that may have occured between them. Table 6.5 shows that Melrose produced more stems than Fakir and more leaf mass at or near maturity. The smaller average stem size on the Melrose plants is balanced by the greater number with the result being that both cultivars have a similar stem mass. It should be noted that Melrose was harvested about 7 days later than Fakir and this may have contributed to its advantage in leaf production.

Table 6.5

Comparisons between Melrose and Fakir where differences occur. ($p<0.05$)

	Fakir ($\pm$S.E.)		Melrose ($\pm$S.E.)	
Stem No.	9.08	(0.48)	11.07	(0.64)
Wt/Stem	1.07	(0.05)	0.69	(0.05)
Leaf Weight	9.04	(0.53)	10.85	(0.61)

Table 6.6

Factor scores for individual cases associated with some of the clusters.

Cultivar	Cluster	Case	Factor Score 1	2
Melrose	1	6	-0.62	5.12
		25	-0.57	2.27
	3	16	2.06	0.05
		40	2.97	-0.53
		48	2.30	0.54
Fakir	1	5	0.07	2.27
		41	-0.73	1.98
		47	-0.01	1.76
	3	22	2.06	0.79
		33	3.67	-1.34
		35	1.86	-0.02

Table 6.6 provides some indication of the comparative outcome of the two techniques. It can be seen that the factor scores do give an indication as to the cluster arrangement that was revealed by cluster

analysis. Cluster 1 in both cultivars are plants with well developed flowers and so they also rate high factor scores for Factor 2. Conversely the plants indicated in Cluster 3 are late maturing and have above average vegetative development and so they score highly for Factor 1.

6.4 Discussion

The object of this examination of data associated with two sainfoin cultivars was not to make definitive statements about individual performance, but rather to examine patterns of variation that exist, and how these may be usefully exploited. While it may be argued that many of the patterns observed, for the way in which both variables and cases tend to group, could have been formed on an intuitive basis from examination of means, variances, and correlations, the multivariate techniques permit a formalisation of this approach. It should however, be emphasised that the overall success of these and other multivariate techniques are very dependant on a good understanding of the strengths and weaknesses of the techniques. In this way these methods are the same as the more familiar univariate statistics (Pearce, 1969).

Given these introductory perspectives, the first point of importance is that for the factor analysis there has been a data reduction from 448 (56x8) elements to 112 (56x2) elements, and for the K-means clustering from the same start to 40 elements (5x8) and the 8 grand mean values. While data reduction and summary are not confined to multivariate techniques, these procedures do permit a rapid and structured examination of the data. In this case the examination will be confined to some general aspects of the results and one or two more specific points which might be regarded as hypothesis generating.

Of particular interest is the extent of variation that occurs within both of the named cultivars. While it is accepted that an open pollinated population will be variable (Simmonds, 1979), cultivar development usually involves some narrowing of the distribution. This is especially so for characters such as flowering time which tend to be quite amenable to selection i.e. character heritability is high. However, in this study, both techniques use degree of floral development and maturity as key criteria on which to differentiate plants. Fakir and Melrose produce distinctive clusters (Cluster 3), of later maturing plants that have strong vegetative development in terms of leaf and stem, as well as the potential for reproductive yield as indicated by the high number of developing floral buds. Cluster 1 for both cultivars are plants that show strong early floral development, and only average vegetative yield. Factor analysis tends to result in similar grouping patterns as the factors retained weight for either late maturing, vegetative attributes or early floral development. This range of flowering time also means that under field conditions the choice of a single occasion when all plants are at or near some optimum point of development is highly improbable.

It is the Cluster 3 plants that would seem to offer some avenue for gain as they appear to have the combination of good vegetative yield, total harvestable yield and the potential for seed production, if factors other than flower numbers are not limiting. The first features are the essential basis of a forage plant while the latter would ensure ready dissemination of any improved material.

While this study is not extensive enough to confirm or deny the hypotheses developed elsewhere (e.g. Pearce et al., 1969; Straley et al., 1972), that modification of SLA may effect photosynthetic efficiency and therefore production, it is of interest to examine Cluster 5 (Fakir) and Cluster 3 (Melrose). Both these clusters have plants with well above average SLA's that show poor performance for all other characters, especially leaf mass. Therefore some caution may be necessary with the suggestion of Sheehy and Popple (1981) that leaf area index of sainfoin might be improved by selecting for higher SLA.

Plants with poor flower and bud development, as for example those in Melrose Cluster 2, tend to be well below average in terms of stem number. While this may reflect a general lack of vigour, it also illustrates the importance of stem numbers in providing the necessary sites for both flower and leaf development.

It is obvious that further ideas can be generated from the data, but to attempt excessive analysis or conclusions from the limited population would to some extent be an abuse of the techniques. Therefore, the next step would be to examine other populations to see if their variation conforms with the general patterns observed here. Also select groups of plants that have been indicated as having certain key attributes, such as a high number of stems and a later than average flowering time, might be studied in greater detail. If the two cultivars examined in this study are regarded as a representative subset of a larger population, probabilities of finding certain combinations of attributes could also be derived giving some indication of how possible gains might be without resorting to hybridisation programmes. To establish soundly based breeding programmes would also require heritability estimates of the various desirable traits (Varga, 1968; Simmonds, 1979).

In conclusion, if there is reasonable access to computing facilities the multivariate techniques offer the opportunity to readily examine the structure of a population and the interactions amongst measured variables. This can reveal certain patterns that can be directly useful in selecting plants and also provide the basis for further hypothesis generation. For the relatively limited sainfoin data base used in this study, groupings of plants were formed that were consistent with observations from the field e.g. later flowering time and higher yield. However, the magnitude of the differences that occured between the groupings of plants was revealing, and offers considerable scope for population improvement if the differences are shown to be consistent on a larger sample.

CHAPTER 7: GENERAL DISCUSSION

In the field and controlled environment studies reported in this thesis sainfoin was examined to determine factors that were likely to influence the pattern and extent of growth when subject defoliation. Different cultivars were used to provide variation in flowering time and expected regrowth ability. Without attempting to preempt unbiased evaluation of sainfoin in light of this work, it is important that due recognition be given to the fact that the germplasm base for the experiments was quite narrow, and that the local environmental conditions were favourable for lucerne production.

The study was undertaken on the basis that sainfoin was a highly desirable forage legume in terms of its feeding value to ruminants. However, a forage legume must also possess a number of other attributes that are prerequisites for successful commercial adoption. The plant must be easy to establish, must readily form an effective symbiotic relationship, be highly productive under a range of management treatments, be persistent, have a good pest and disease resistance, and ultimately produce enough seed to enable cheap dissemination to users (Rogers, 1975; Humphreys, 1981; Vickery, 1981). By considering these various aspects with reference to the results and observations reported in this thesis, it should become clear where the relative strengths and weaknesses of sainfoin appear to lie.

Sainfoin proved to have the ability to germinate rapidly and to have early growth rates that were comparable with lucerne. Apart from some additional lime for pH adjustment and the use of a specific inoculum, as sainfoin was a new crop to the area, the procedures for establishing sainfoin were conventional and did not require special equipment. The ready establishment of sainfoin has also been noted in other experiments (Smoliak et al., 1972; Cooper, 1977; Smoliak and Hanna, 1977), and is often attributed to the large seed size of sainfoin and its ability to grow rapidly at relatively low temperatures. The drawback to the large seed size is that the recommended sowing rates to achieve a similar starting population to other legumes is high (Spedding and Diekmahns, 1972; Fortune and Withers, 1980). Currently this makes sainfoin expensive to establish (Scott, 1979; Doyle et al., 1984), but this might be expected to change if seed were more widely grown and available.

While work on plant establishment and early growth is in general agreement, there is considerable divergance concerning the successful development of a symbiotic relationship. Even though this study did not involve direct measures of nodule function, plants sampled from the field over the duration of the experiment displayed large numbers of nodules. An indication of their activity was provided by the presence of leghaemoglobin and the fact that even the vigorous plots of sainfoin displayed no visible symptoms of nitrogen deficiency after three seasons growth. The only additional nitrogen added was 20kg/ha of nitrogen as urea at the start of the trial as a precaution in case nodulation proved ineffective. Plants established in coarse sand for the controlled environment study received no nitrogen over about six months and also appeared to have successful nodule formation and function. This does not assist in considering why the work of Ditterline and Cooper (1975), and Hume (1981) has highlighted problems with both nodulation and nitrogen fixation in relatively young plants that have not been subject to defoliation. Without acetylene reduction data to support the argument for successful function, the only procedure followed in this work that resulted in its apparent success was careful culture of a selected strain of Rhizobium, followed by its application to the seed and at least one watered-on application after seedling emergence. It is an important problem and needs further attention to clarify factors contributing to the instability of the symbiotic relationship.

Sainfoin displayed the potential to be highly productive in given cultivar and treatment combinations but in all cases was bettered by lucerne. This was best illustrated by Melrose which yielded about 20,000 kgDM/ha over the two main growing seasons. While this may present a barrier to immediate commercial utilization, the advantage of lucerne was only of the order of 20%, which seems to represent a comparatively small gain for a long and intensive breeding history. For the local environment, some immediate gains may be possible if later flowering material is chosen as all cultivars flowered earlier (10 - 20 days) than lucerne in the main spring, early summer growth period. During this time, sainfoin was capable of growth rates in excess of 100kg/ha/day and so if these could be extended by even a week or two, higher yields would seem possible. Perhaps more important than absolute yield, when considering direct grazing, is the seasonal yield distribution. In the field experiment (Chapters 3 and 4) it was shown that sainfoin generally displayed slow regrowth and limited yield potential after early January, or about mid-summer. As the field experiment was dependant on the prevailing seasonal conditions, it was also of considerable importance to note that under the "ideal" controlled environment conditions, regrowth was also poor and much less vigorous than the primary growth. This type of situation has been observed by others (Koch et al., 1972; Meyer, 1975; Percival and McQueen, 1980), and is a likely problem if sainfoin is required to feed animals for the duration of the summer period.

In order to regrow, a plant needs a number of active meristems at the base or in the axils of old shoots and leaves, a supply of energy from either residual leaf tissue or from some form of reserves and the ability to translocate the reserves or current assimilate to the active meristems. In the case of sainfoin there would appear to be a deficiency in all these areas for the management techniques used in this study. While basal buds were evident on sainfoin these did not show any appreciable development until well after cutting which tends to indicate strong apical dominance, especially when the only report of significant basal shoot development came from Cooper and Watson (1966), for plants that had already set seed. In this study a further indication of this dominance was the rapid regrowth and development of occasional single stems after cutting, that appeared to inhibit all other plant growth. More axillary buds may have been available if the cutting height had left more stem material but, as discussed in Chapter 4, the sainfoin cultivars used had large internode distances. Therefore to leave more than one or two elongated nodes behind would have meant using a cutting height in excess of 12cm and so leaving a large proportion of unharvested dry matter.

The cutting height used also resulted in very little, or no leaf area remaining after harvest to supply current assimilate when the plants were cut either at floral maturity or late in the season. Some of the early spring cuts left rosette material below the cutting height and in these cases regrowth was not delayed. The reasons for this would appear to have been a combination of the leaf area and a number of developed but unelongated shoots in the first growth cycle. After a cutting stimulus these shoots elongated to provide a second cycle of growth. If the stand was not cut until maturity many of these shoots would appear to succumb to the stresses of shading and general competition with the parent plant.

While the recovery of ^{14}C from the regrowth phase was not successful, the circumstantial evidence indicated by the large loss of root mass after defoliation, without a balancing gain in top growth, was that sainfoin displayed little ability to supply meristems with translocated reserves. The extent of root loss, which did not appear to be due to any other factors other than the cutting treatment, also raises the question of sainfoin's ability to supply the plant with water and nutrients in the period immediately post harvest, particularly if this period coincided with climatic stress. It would seem reasonable to assume that replacement of root tissue requires the use of current assimilate and will therefore be in competition with the new shoots e.g. Harris, (1978). The end result of this is that both the rate of regrowth and the final quantity of material is diminished.

Therefore to achieve the goal of high productivity it will be necessary to clarify the relative influence of the morphological and physiological constraints. The former will involve gathering more information on the ability of different genotypes and species of sainfoin to produce new shoots during the growing season, and also to maintain some leaf area in the lower canopy that can remain after harvest. This may be achieved by finding and using material that has a number of active lower nodes due to branching or prolific stem production. Physiological factors may well influence the number of potential stems in a given season e.g.

initiated in the preceding autumn or winter, and the internal distribution of assimilates to the various components. It may be that sainfoin does not have the potential to fit the lucerne type model of regular and predictable growth cycles (Janson, 1982), in which case the management will always be constrained by a short, but productive growing season.

However, sainfoin has shown that it has a further weakness and that is poor persistence. While it was not possible to identify a single source of this problem it would seem reasonable to include plant disease, annual development of some plants i.e. failure to develop a second successful cycle of vegetative shoots, and stress due to cutting treatments as expressed, for example, by the loss of root material. The later factor may be of importance if it was providing the disease organisms with a quantity of suitable substrate for growth and proliferation. Poor persistence should not in itself cause any direct management problems if it is predictable such as with annual pastures. But it is essential such a critical factor is defined as it does introduce extra cost of possible regular replacement or alternatively, the need for the plant to have the ability to naturally reseed. As this work concentrated on sainfoin as a perennial, the problem raised was at what point the stand would become uneconomic. This would depend on the number of remaining plants and their distribution, and the likely replacement species in the declining stand. While in this work weeds were controlled by both chemicals and hand-weeding, indications were that had this control not been practised, weed ingress would have been rapid. The distribution and density effects are more difficult to assess as the stands of sainfoin were so varied that it was not possible to correlate yield and plant density. Indeed, in a commercial situation, the poor distribution of the plants in the stand would probably be enough reason to remove the stand, even if production was coming from the surviving patches of plants.

Until now the discussion may have tended to cast sainfoin in an unfavourable light when some of its apparent deficiencies are considered. However, it is a high quality forage legume that has given yields of the order of 10,000 kg DM/ha in a full seasons growth under management conditions that may have provided a useful experimental structure, but may also have been less than favourable to sainfoin. No consideration was given to its annual performance and even though floral prolificacy was noted, no attempt was made to let the plant form seed. For many plants there are comparatively clear indicators of long term survival strategies, and seed production is often associated with annuals (Harper, 1977). It may be that sainfoin is still performing more like its annual relatives (Simmonds, 1979), and so this avenue needs further exploration both in terms of the physiology of the plant and its place in productive pastures.

Sainfoin does contain condensed tannins in its leaf tissues and it would seem reasonable to expect that these complex molecules have a cost both in carbon skeletons and the energy required for their synthesis. However, the benefits that they confer on the plant are enough that other legumes are being rigorously examined for the possibility of incorporating genes for this attribute by the most advanced techniques of genetic manipulation available (Anon, 1984). It would be interesting to consider the gains that might be possible with sainfoin if it were to receive some of this attention.

Finally, sainfoin obviously has a large and relatively untapped gene pool that gave some indication of the potential that may be possible when examined under controlled environment conditions (Chapter 6). If this material were assembled and examined for attributes like branching, node formation, late flowering, limited floral mass and freedom from disease then gains would be inevitable. The close focus on management areas as attempted in this work tends to preclude the examination of a large collection and so the answer may lie in treating sainfoin strictly as a new introduction. In this way it may be evaluated for its own worth as either an annual or a perennial, but not as an assumed substitute for some other species like lucerne.

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Appendix 1: Rainfall and evaporation data for duration of field trial. From Grasslands Division D.S.I.R. Palmerston North, about 1 km from the experimental site.

Date	Rainfall (mm)	Evaporation (mm)	Balance
Oct 79	96.9	84.1	12.8
Nov	105.0	146.5	-41.5
Dec	114.1	166.7	-52.6
Jan 80	60.5	157.3	-96.8
Feb	35.7	149.5	-113.8
Mar	137.0	90.4	46.6
Apr	96.8	43.2	53.6
May	32.4	39.2	-6.8
Jun	56.6	19.5	37.1
Jul	88.0	24.8	63.2
Aug	94.5	44.6	49.9
Sep	122.8	74.6	48.2
Oct	129.6	100.2	29.4
Nov	113.5	115.6	-2.1
Dec	54.7	128.8	-74.1
Jan 81	15.5	202.7	-187.2
Feb	68.2	144.8	-76.6
Mar	58.3	117.1	-58.8
Apr	43.0	85.9	-42.9
May	135.2	44.2	91.0
Jun	121.6	18.3	103.3
Jul	109.2	28.0	81.2
Aug	57.6	32.1	25.5
Sep	98.7	73.7	25.0
Oct	43.5	88.6	-45.1
Nov	33.8	125.0	-91.2
Dec	93.8	165.6	-71.8
Jan 82	70.4	183.7	-113.3
Feb	69.2	143.0	-73.8
Mar	81.4	110.2	-28.8
Apr	11.2	64.3	57.3
May	80.7	44.0	36.7

Appendix 2: Polynomial equations for describing plant growth in the field experiment (1980 - 81).

Cultivar	Variable	constant	x	x^2	R^2
Melrose	D.M.	-36.2	1.51	-0.0003	0.90
	Leaf	-14.6	1.11	-0.007	0.66
	LAI	-3.27	0.19	-0.001	0.63
	lnD.M.	1.23	0.05	-0.0002	0.93
Remont	D.M.	-74.3	2.74	0.013	0.86
	Leaf	-18.7	1.09	-0.006	0.62
	LAI	-3.31	0.17	-0.001	0.62
	lnD.M.	0.24	0.08	-0.0004	0.94
Fakir	D.M.	-82.7	3.21	-0.02	0.90
	Leaf	-36.8	1.78	-0.01	0.61
	LAI	-5.79	0.27	-0.002	0.63
	lnD.M.	-0.64	0.12	-0.0007	0.93
Lucerne	D.M.	10.33	-0.05	0.006	0.92
	Leaf	7.22	0.01	0.003	0.88
	LAI	2.14	-0.03	0.0007	0.88
	lnD.M.	1.34	0.04	-0.0002	0.91

D.M. = Total dry matter yield of tops.
Leaf = Total yield of leaf only.
LAI = Leaf area index.
lnD.M. = Natural log transform of total dry matter yield of tops.

Appendix 3: Stem numbers per plant from single plants harvested in the 1980-81 season. (Only for treatments that were harvested twice.)

	Numbers of Stems		
Treatment	First cut	Second cut	Total
Fakir E/E	6.6	6.9	13.5
Fakir F/E	6.4	6.2	12.6
Fakir F/F	5.7	7.4	13.0
Remont E/E	6.9	5.3	12.1
Melrose E/E	6.9	6.0	12.9

Appendix 4: Controlled Environment Rooms (Plant Physiology Division, D.S.I.R. Palmerston North) - Conditions for experiments outined in Chapter 6.

This facility enabled close control of the preset variables over the duration of the experiement. All conditions were continuously monitored and no problems were experienced.

	Day	Night
Temperature: ± 0.5°C	20	13
Humidity: ± 5%RH	65	80
VPD: mb	8.2	3.0
Lighting:	14 hour photoperiod	
Light intensity: Wm^{-2} PAR	151	

VPD = Vapour pressure deficit
PAR = Photosynthetically active radiation (400-700nm)

The changeover in day/night conditions was programmed to take two hours and the lights went off (day/night), and came on (night/day) half way through the changeover period.

Carbon dioxide levels were monitored in both rooms and for most of the experiment were 330 ± 20 ppm.

All plants were connected to an automatic watering system.

The lighting system used in each room was 4 x 1000W Sylvania "Metal-arc" high pressure discharge lamps, and 4 x 1000W Philips tungsten iodide lamps.