AN INVESTIGATION INTO THE EFFICIENCY OF NITROGEN FIXATION IN SAINFOIN (*ONOBRYCHIS VICIIFOLIA* SCOP.)

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ABSTRACT

Earlier reports have indicated that growth of the forage legume sainfoin (*Onobrychis viciifolia* Scop.) is limited by its capacity to fix adequate quantities of N_2.

Symbiotic N_2 fixation and development of sainfoin up to the flowering stage was studied under glasshouse conditions. Growth and development of plants that were dependent solely on fixed N_2 for their N requirements, were compared with plants supplied with abundant combined (nitrate) N. The effect of a low rate of combined N on symbiotic N_2 fixing activity and plant growth was also investigated.

From an early stage, plants dependent on symbiotic N_2 fixation had lower relative growth rates than plants supplied with combined N, indicating that the N_2 fixing system of sainfoin was not capable of providing enough N to meet the requirements of the plant, or that N_2 fixation required an energy input greater than that for the assimilation of mineral N.

The mode of N nutrition was found to influence the dry matter distribution in sainfoin to a greater extent than reported for most other legumes. Plants dependent on symbiotic N_2 fixation allocated a substantially greater proportion of dry matter to root and nodule growth and consequently had lower top:root + nodule ratios than plants provided with combined N.

Sainfoin was found to produce abundant nodules, and had a relatively high nodule weight in relation to total plant weight, compared to other legumes. Specific nodule activity, however, was found to be relatively low, and possible reasons for this are discussed.

For plants dependent on symbiotic N_2 fixation, total plant N, and hence N_2 fixation appeared to be the major factor limiting plant growth. Evidence was obtained which indicated that the N_2 fixing system of sainfoin may be relatively inefficient. The observed ratio of C_2H_2 reduced:N_2 fixed, was higher than the theoretical ratio, and appeared to be high relative to other legumes, which suggested possible wastage of energy by the N_2 fixing enzyme. The addition of a low rate of combined N had the effect of immediately reducing $N_2[C_2H_2]$ fixing activity, and the combined N appeared to substitute for, rather than supplement, symbiotic N_2 fixation, further indicating an inefficient symbiotic N_2 fixing system.

Leaf area ratio was found to be lower in sainfoin dependent on N_2 fixation than reported values for other N_2 fixing legumes; this suggests that sainfoin is less efficient at intercepting photosynthetically active

radiation. Leaf area was highly correlated with total plant N, and there was evidence that this link was via energy supply to the symbiotic N_2 fixing system. Thus leaf area may have been limiting N_2 fixation and hence total plant N.

Overall, a mutual dependence between the ability of the root nodules to fix N_2 and the ability of the leaves to supply energy was indicated. There was evidence that both of these factors may play a role in limiting the growth of sainfoin, relative to other more productive legumes, such as lucerne.

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INTRODUCTION

There is some interest in growing the forage legume sainfoin (*Onobrychis viciifolia* Scop.) in New Zealand at present. It is thought that it could play a similar role to that already played by lucerne (*Medicago sativa* L) particularly in providing forage for livestock under dry summer conditions. Sainfoin has two advantages over lucerne, in that it has non-bloating properties (e.g. Cooper *et al.* 1966) and it is not subject to some of the major pests of lucerne (e.g. Hanna *et al.* 1977a; Lance, 1980). Its major disadvantage at present, is that its yield potential appears to be substantially lower than that of lucerne in many instances (e.g. Spedding & Diekmahns, 1972). However, it is thought that sainfoin could possibly be useful as a bloat preventing crop to supplement the diet of ruminants in certain circumstances.

Problems in establishing a satisfactory stand of sainfoin and maintaining it after defoliation have been widely reported. The nitrogen fixing ability of sainfoin is reported to be insufficient to provide for the nitrogen requirements of the plant (e.g. Koter, 1965a), and nitrogen deficiency symptoms have been observed on inoculated sainfoin at an early stage in the development of the crop (Sims *et al.* 1968; Roath & Graham, 1968; Schneiter *et al.* 1969; Meyer, 1975; Smoliak & Hanna, 1975) despite plants being abundantly nodulated (Burton & Curley, 1968).

Up to the time of this project there had been little work specifically investigating nitrogen fixation and nodulation in sainfoin, and possible causes of its poor nitrogen fixing performance. The aim of this project was to investigate the nodulation and nitrogen fixation of sainfoin in relation to overall plant development, and to compare the performance of plants dependent on symbiotic nitrogen fixation with those supplied with abundant mineral nitrogen.

CHAPTER 1

REVIEW OF LITERATURE

For convenience, the review of literature is divided into three main sections, dealing with sainfoin, nitrogen fixation and the acetylene reduction technique.

1 SAINFOIN

1.1 AGRICULTURAL POTENTIAL OF SAINFOIN

1.1.1 YIELD

Comparisons of sainfoin (*Onobrychis viciifolia* Scop.) with lucerne (*Medicago sativa* L.) are probably inevitable, and provide a useful frame of reference in the evaluation of sainfoin (Hanna & Smoliak, 1968). If sainfoin is to be utilised as a hay or pasture crop, even if only under specific environmental conditions which favour its growth, it is logical to place considerable emphasis on its yield performance in comparison to lucerne, which is one of the highest yielding forage legumes over a wide range of temperate environments (Hanna & Smoliak, 1968).

Yields of sainfoin in pure stands have generally tended to be lower than those of lucerne (Hanna & Smoliak, 1968; Murray & Slinkard, 1968; N.I.A.B. 1974; Spedding & Diekmahns, 1972; Hanna *et al.* 1975; Rogers, 1976; Hanna, 1977) and red clover (*Trifolium pratense* L.) (Spedding & Diekmahns, 1972). The annual yield of sainfoin is often in the order of 20 - 30% lower than lucerne (Spedding & Diekmahns, 1972; Melton, 1973; Hanna *et al.* 1975; Rogers, 1976). Hanna & Smoliak (1968) state that even the better yielding strains of sainfoin are not likely to out-yield lucerne, where the latter is well adapted, especially where two or more cuttings can be obtained. Forage yields of sainfoin are frequently inferior to those of lucerne, where seasonal rainfall is adequate, due to poor regrowth and plant vigour (Meyer, 1975).

There are, however, a number of situations where sainfoin has out-yielded lucerne. Murray & Slinkard (1968) report dry matter yields of sainfoin (one cut type) similar to those of lucerne, in Idaho under dry summer conditions. This result was due to the early growth and relatively high first cut yields of sainfoin. Carleton *et al.* (1968b) report that, for irrigated hay, Eski sainfoin (a one cut type) yielded more than, the same as, or less than lucerne, depending on location and year. Sainfoin generally yielded more than lucerne at the first cut and less at the second, and appeared to have a comparative advantage where conditions enabled only one cut per year. Roath (1968) found that under dryland conditions, hay yields of sainfoin compared favourably with lucerne, red clover and cicer milkvetch (*Astragalus cicer* L.) except on acid soil. Smoliak & Hanna (1975) grazed subirrigated sainfoin with sheep over five years. Over the five years sainfoin yielded slightly higher than lucerne, with both yielding substantially higher than cicer milkvetch. Kozyr (1948) reported higher hay yields from sainfoin than lucerne, and attributed this to drought endurance and resistance to pests.

Thus, although sainfoin yields are often reported to be lower than the better forage legumes such as lucerne, yields may surpass those of lucerne under particular environments to which sainfoin is better adapted. This, considered together with the desirable nutritional and non-bloating characteristics of sainfoin (Sections 1.1.2 and 1.1.3) may make it a desirable forage legume under certain circumstances.

Reports on the performance of sainfoin with companion grass species are conflicting. A companion grass can tend to increase total yields and decrease weed invasion (Bland 1971). Sainfoin is found to be competitive with grasses when planted in mixtures (Dubbs, 1968). Dubbs (1968), Roath (1968) and Hanna *et al.* (1977b) report good yields of sainfoin grown with a range of grasses, and found that sainfoin grass mixtures tend to yield higher than grass alone but in most cases, not higher than sainfoin alone. It has been found that sainfoin performs better in mixtures with bunch grasses, rather than rhizomatous grasses. The legume component tends to be reduced when the latter grasses are present, resulting in a lower quality forage (Hanna *et al.* (1975). Hanna *et al.* (1977b) measured forage yields of sainfoin/grass and lucerne/grass mixtures and found the legume component yield to be consistently higher in the lucerne/grass mixtures. Cooper (1972) found that sainfoin/grass mixtures were less productive than sainfoin/birdsfoot trefoil (*Lotus corniculatus* L.) mixtures. Sainfoin has been found to perform better in alternate rather than mixed row seedings with grass (Hanna *et al.* 1977b) or birdsfoot trefoil (Krall *et.al.* 1971).

Thus, grass may be sown along with sainfoin to decrease weed invasion and increase stand longevity, but this is at the expense of decreased forage quality, and usually results in slightly decreased total yield.

To summarise, sainfoin often does not compare favourably, in terms of yield, with some of the more commonly grown forage legumes such as lucerne. However, there are certain situations, such as on dry, free draining, high pH soils (Section 1.5.1) in which the performance of sainfoin may be superior to that of other forage legumes. The potential also exists to improve yields of sainfoin by plant breeding. South Australian workers appear to have selected an acceptable cultivar within the space of two to three years (Lance, 1980). Sheehy & Harding (pers.comm.) suggest that sainfoin yields would benefit from a selection programme aimed at increasing its specific leaf area (and hence leaf area ratio) and rate of leaf growth (section 1.4).

Sainfoin also has certain attributes, which may make it a more desirable crop, than for example lucerne, even at the expense of reduced yield. Three of these attributes are its non-bloating characteristic, its very high nutritional value (discussed in sections 1.1.2 and 1.1.3) and its resistance to some lucerne pests, such as the alfalfa weevil (*Hypera postica*) (Eslick, 1968; Hanna *et al.* 1977a) and the spotted alfalfa aphid (Lance, 1980).

1.1.2 NON BLOATING CHARACTERISTIC

Bloat is a problem of ruminant animals in which a persistent foam develops in the rumen, in amounts sufficient to prevent the animal from belching the large amounts of gas formed therein (Wright & Reid, 1974). Bloat is a particular problem in New Zealand because of the large number of ruminant animals grazing legume based pastures. Bloat occurs most frequently in New Zealand, in dairy cattle, in which it is a common disorder. Up to 90% of the herds in a particular district may experience bloat, and most animals in an individual herd may be affected (Reid, 1976). Deaths, however, are not usually high (as a result of preventative measures) with regional average death rates seldom higher than 2% (Reid, 1976). As at 1976 the annual cost of materials alone for bloat prevention could be as high as 10% of the value of a good dairy cow, or 4% of the probable annual gross income from the cow.

Soluble plant proteins have been implicated as surfactants responsible for the persistent foam that develops in the rumen of animals suffering from boat (Gutek *et al.* 1974; Jones & Mangan, 1977). McArthur & Miltimore (1964) indentified the foaming agent in lucerne as an 18-S protein. They stated that the 18-S protein was probably chloroplast lamellae, and that it was found in all legumes, but may vary qualitatively because of physical characteristics.

Three common forage legumes which cause bloat are lucerne, white clover (*Trifolium repens* L.) and red clover (McArthur & Miltimore, 1969). There are, however, some forage legumes which do not cause bloat. These include sainfoin and birdsfoot trefoil (*Lotus corniculatus* L.). Cooper *et al.* (1966) found sainfoin to have the lowest foam formation of twenty seven legume species that were evaluated for bloat potential.

Non bloating legumes such as sainfoin, have been found to contain protein precipitating substances called condensed tannins or flavolans (Reid, 1976). The soluble dietary protein of green leaves forms insoluble complexes with tannins, which are stable over the pH range 3.5 to 7.0, which includes the rumen pH range of 5.6 to 6.8 (Jones & Mangan, 1977). These complexes break up in the more acid conditions (pH 2.5) of the abomasum (Jones & Mangan, 1977). A complete absence of soluble protein has been observed in the rumen of cattle grazing pasture species that contain flavolans (Jones & Mangan, 1977). The flavolans serve a dual purpose in that they also protect dietary protein from deamination by rumen bacteria and thus have a beneficial effect on N metabolism (Jones & Mangan, 1977). Sainfoin is found to be highly palatable to herbivores, in contrast to other species containing tannins, in which they have been reported to cause reduced palatability (Jones *et al.* 1976). Sarkar *et al.* (1976) report that the flavolan composition of sainfoin is such that nutritive value is high, unlike some other flavolan containing legumes.

Reasons other than flavolan content have also been cited as being responsible for the non bloating characteristic of forages such as sainfoin. McArthur and Miltimore (1969) state that non bloating legumes, including sainfoin, were found to contain low levels of the bloat causing 18-S protein relative to bloat causing legumes.

Howarth *et al.* (1978a) found that the mesophyll cells in non bloating legumes were more resistant to mechanical rupture than those from bloat causing legumes (without cell rupture, the major foaming agents, which are intracellular, would not be released from the cells, and foam formation would not occur). Howarth *et al.* (1978a) state that the presence of flavolans, and the resistance of cells to rupture are two separate and complimentary explanations for the bloat safe nature of sainfoin.

Pectin methyl esterase (PME) activity has also been linked to stable foam formation on ruminants (Rumbaugh, 1972). Sainfoin was found to have the lowest level of PME activity (zero) of the four forage legumes tested.

Howarth *et al.* (1978b) hypothesised that colloidal sized fragments of chloroplast membranes may act as nucleation sites for bubble formation during the onset of pasture bloat. They cited evidence to support this hypothesis, and stated that sheep fed sainfoin had lower concentrations of suspended chloroplast fragments in their rumens than sheep fed other forage legumes.

1.1.3 NUTRITIONAL VALUE

Sainfoin is reported to be a very nutritious forage (Shain, 1959; Schneiter *et al.* 1969; Krall *et al.* 1971) which is highly palatable to all classes of livestock (Hanna *et al.* 1975).

Sainfoin is reported to contain higher levels of N-free extract, total digestible nutrients and phosphorus than lucerne, similar levels of ether extract, and lower levels of crude protein, crude fibre, total ash and calcium (Baker *et al.* 1952; Carleton *et al.* 1968a; Jensen *et al.* 1968; Schneiter *et al.* 1969; Smith *et al.* 1974; Ditterline & Cooper, 1975). It is found that sainfoin provides a very good balance of protein and total available energy (Carleton *et al.* 1968a; Ulyatt *et al.* 1977). Kaldy *et al.* (1979), scored sainfoin and lucerne in terms of protein quality for non ruminants. Scores were 68 and 71 respectively, compared to 100 for the ideal (whole egg) protein.

Sainfoin is reported to be similar, in terms of digestibility, to lucerne (Jensen *et al.* 1968; Chapman & Carter, 1976) and red clover (Osbourn *et al.* 1966). The leaves of sainfoin have been found to be less digestible than those of lucerne, but the stems more so (Ulyatt *et al.* 1977). In terms of animal live weight gain, sainfoin appears to be as food as, or better than, other forage legumes. The efficiency of utilisation of metabolisable energy for liveweight gain has been found to be higher for sainfoin than lucerne, white clover or subterranean clover (*Trifolium subterraneum* L.) (Ulyatt *et al.* 1977). Forage consumption, feed conversion (kg of forage consumed per kg of live weight gain) and live weight gain were similar in beef cattle fed either sainfoin or lucerne (Jensen *et al.* 1968). Sainfoin fed to beef cattle is found to give superior weight gains and a better feed to beef conversion than grasses or grass/ladino clover (*Trifolium repens*) mixtures, at the expense of decreased stocking rate (Krall, 1968; Wilson, 1976). Young lambs allowed unlimited grazing have been found to make better growth on sainfoin than any other grass or legume tested (Spedding & Diekmahns, 1972). Krall *et al.* (1971) found that sainfoin produced superior daily weight gains and more beef per acre than ladino clover, or grass fertilised with N. Newman (1968) compared rates of growth of pigs fed a diet containing 3% of ground sainfoin or lucerne hay and found that average daily gains were not significantly higher with the sainfoin containing diet.

Sainfoin is reported to be very palatable, and to be superior to lucerne in this regard (Chapman & Carter, 1976). Osbourn *et al.* (1966) found the voluntary intake of sainfoin by sheep to be greater than for lucerne or red clover, and Smoliak and Hanna (1975) found that sheep preferred to graze sainfoin rather than lucerne or cicer milkvetch (*Astragalus cicer* L.).

The maximum yield of sainfoin is achieved at late bloom. Koch *et al.* (1972) found that little was lost, in terms of quality, if sainfoin was not harvested until late bloom. Lignification occurs before flowering, and the proportion of lignin remains nearly constant thereafter. The stems of sainfoin have high digestibility despite their coarse appearance (Koch *et al.* 1972), and this probably accounts for the fact that the nutritive value of sainfoin does not decline with increasing maturity to the same extent as for other forage legumes. However, changes in the chemical composition of the whole plant, as it matures, are brought about principally by changes in the leaf to stem ratio (Baker *et al.* 1952).

Sainfoin seed has been evaluated as a source of protein for monogastrics. Ditterline (1974) found it to contain 36% crude protein and to have an essential amino acid composition similar to soybean meal. Weanling pigs fed sainfoin seed as a diet supplement wasted more feed and gained less weight than pigs fed soybean meal (Ditterline, 1974; Ditterline & Cooper, 1975). Weanling rats fed diets with sainfoin seed or soybean meal as the protein sources showed similar average daily gains, feed consumption and feed conversion (Ditterline, 1974; Ditterline & Cooper, 1975). Sainfoin seed did have an advantage in that the trypsin inhibitors it contained did not increase pancreas size like those in soybean meal, which usually has to be treated to overcome this problem (Ditterline, 1974).

1.1.4 CONCLUDING COMMENTS - AGRICULTURAL POTENTIAL

If yields of sainfoin can be raised to an acceptable level it could become an attractive proposition for the dryer areas of New Zealand with suitable soils (section 1.5.1). The non bloating characteristic and high nutritional value may compensate for the higher yields which may be able to be obtained from other crops.

1.2 AGRICULTURAL HISTORY OF SAINFOIN

The genus *Onobrychis* comprises 80 to 100 species of plants native to southern Europe, northern and western Africa, and western Asia (Whyte *et al.* 1953). Sainfoin (*Onobrychis*) species have been part of native pastures in the eastern Mediterranean for as long as 6,000 years (Hely & Offer, 1972). *Onobrychis viciifolia*, originating in central and southern Europe, and temperate Asia, is the most important agricultural species. It is used in central Europe, Mediterranean countries and Great Britain as a hay and pasture plant (Whyte *et al.* 1953). Shain (1959) states that sainfoin has been used in parts of the U.S.S.R. for over 1000 years and that it was transferred to western Europe about 400 years ago. Sainfoin appears to have been first cultivated in France, the first definite record, according to Vianne, being in 1582 (Piper, 1924). Sainfoin was grown in Germany in the 17th century but not in Italy until the 19th century (Piper 1924). The spread of sainfoin over Europe led to the profitable cultivation of much dry, calcareous land (Piper, 1924).

It is thought that sainfoin came to England from the continent, particularly since the name sainfoin, meaning healthy hay, is of French origin (Bland, 1971). The writings of Jethro Tull in 1733 indicate that sainfoin must have been widespread and popular in England at this time (Spedding & Diekmahns, 1972). Sainfoin cultivation was widespread in England in the 18th, 19th and early 20th centuries (Bland, 1971; Spedding & Diekmahns, 1972) but the area grown now is almost negligible (Spedding & Diekmahns, 1972). The demise of the crop in Britain over the last 60 years has been attributed to its lack of ability to respond, as well as alternative fodder crops, to the changing requirements of British agriculture (Hutchinson, 1965). Sainfoin is still a very important crop in parts of Europe and according to Shain (1959), is more important than lucerne or red clover in many parts of the U.S.S.R.

Piper (1924) stated that sainfoin had never attained agricultural importance in the U.S.A., although often tested, and went on to say that, on suitable soils, its culture might become profitably established. The lack of interest in sainfoin in the U.S.A. has been attributed to the crop being tested under conditions (such as low pH, high rainfall and frequent irrigation, see section 1.5) to which it was not well adapted in comparison to lucerne (Eslick, 1968; Jensen & Sharp, 1968). Also, factors such as its non bloating charactertistic may have been overlooked and its coarse appearance may have led to an impression of low palatability (Eslick, 1968).

Renewed interest in growing sainfoin in North America has resulted from the need for a dryland forage, and a substitute for lucerne on irrigated land during periods of heavy lucerne weevil infestation (Ditterline & Cooper, 1975).

The introduction of well adapted genotypes, e.g. Eski & Remont in the U.S.A., and Melrose & Nova in Canada, has also made sainfoin a more attractive proposition (Ditterline & Cooper, 1975; Hanna *et al.* 1977a; Hanna, 1980).

1.3 MORPHOLOGICAL DESCRIPTION

Sainfoin (*Onobrychis viciifolia* Scop.) is a member of the Fabaceae family.

Sainfoin is a slightly pubescent, long lived perennial herb (Whyte *et al.* 1953; Spedding & Diekmahns, 1972). From a branched crown arise numerous erect, ribbed, hollow, branched stems, which are decumbent at the base, and which may grow to about 1 metre in height (Piper, 1924; Percival, 1943; Andreev, 1963; Schneiter *et al.* 1969; Spedding & Diekmahns, 1972). Leaves are borne on long petioles, and are pinnately compound with 5 to 14 pairs of oblong leaflets and a terminal one (Percival, 1943; Spedding & Diekmahns, 1972). The number of leaflets per leaf decreases acropetally (Thomson, 1951a).

The flowers, which are rose coloured and papilionaceous, are borne on dense, erect racemes. These are carried on long, erect axillary stalks (peduncles) which enable maximum exposure for pollination (Piper, 1924; Percival, 1943; Carleton & Weisner, 1968; Spedding & Diekmahns, 1972). Flower and pod maturation begins at the base of the inflorescence and proceeds upwards (Schneiter *et al.* 1969). Inflorescences may consist of 5 to 80 flowers, and each flower has the capability of producing a single seed. A plant may have 5 to 40 stems each having 3 to 5 inflorescences (Carleton & Weisner, 1968). The flowering of sainfoin is indeterminate, but is more determinate than lucerne (Carleton & Weisner, 1968).

Seeds are borne singly in brown indehiscent pods. The pods are bilaterally symetrical and almost semi-circular in side view, with a straight ventral and a curved dorsal suture. On the sides are networks of prominent vascular ridges, often projecting from which are spines (Piper, 1924; Thomson, 1951b; Spedding & Diekmahns, 1972). The true or milled seed is kidney shaped, with the hilum situated about the middle of the concave edge. The seed colour tends to be olive to brown, or black (Thomson, 1951b; Spedding & Diekmahns, 1972). Sainfoin seeds tend to be larger than those of other forage legumes. The weight of 1000 seeds was found to be approximately 21.5 g unmilled and 15.5 g milled (Thomson, 1951b). In comparison lucerne seed weighs approximately 2 g per 1000 seeds (Schneiter *et al.* 1969).

Sainfoin has a thick (up to 5 cm diameter) tap-root which normally extends to a depth of 1 to 2 metres, but up to 10 metres (Piper, 1924; Whyte *et al.* 1953; Andreev, 1963; Spedding & Diekmahns, 1972). The roots of sainfoin may penetrate to depths even greater than lucerne in open, dry subsoils (Percival, 1943). The root system has a few main branches and numerous fine laterals (Spedding & Diekmahns, 1972). Sainfoin is reported to have a better developed root system with twice as many laterals as lucerne (Kozyr, 1948; Kalugin, 1950; Massaudilov, 1958).

Root nodules occur mainly on the fine lateral roots, but a few also occur on the jeuvenile tap-root (Spedding & Diekmahns, 1972). The nodules are large (3 x 6 mm approximately), wedge shaped, orange-white in colour, possess a subterminal meristem (Wittmann, 1968; Spedding & Diekmahns, 1972), and tend to be formed in clusters (Schreven, 1972).

The early development of the plant is described briefly by Thomson (1938) and Percival (1943). After the kidney shaped cotyledons reach the soil surface, foliage leaves with various numbers of leaflets are produced. The first foliage leaf is usually simple, the second and third, trifoliate, and the later leaves pinnately compound. Short lateral branches are formed and the plant forms a rosette which tends to be more prostrate in the one cut or 'common' type than in the multi-cut or 'giant' type.

Sainfoin can be classified into two taxonomically indistinguishable types, a one cut or 'common' type and a multi-cut or 'giant' type, according to its growth behaviour after about the six leaf stage (Thomson, 1938).

In the one cut type, stem elongation is limited during the establishment year. Flowering usually first occurs in the second year, and occurs once a year (Spedding & Diekmahns, 1972). Stems tend to be shorter and leaflets smaller than in the multi-cut type (Spedding & Diekmahns, 1972).

The multi-cuttype is said to be shorter lived than the one cut type (Spedding & Diekmahns, 1972). Stem elongation and flowering occur on the establishment year (Thomson, 1938). After cutting, the multi-cut type again sends up flowering stems (Thomson, 1938; Spedding & Diekmahns, 1972). Stem elongation is found to take place in the upper internodes, with the first four to six remaining short. Lateral buds develop in the axils of the lower leaves, and stems either elongate, or remain short, producing a tuft of leaves at the base of the plant (Thomson, 1938). In the axils of the upper leaves, either inflorescences or branches may be produced (Thomson, 1938).

1.4 GROWTH PATTERN

Sainfoin is easy to establish. The seeds germinate readily and produce vigorous seedlings that grow rapidly (Hanna *et al.* 1977a). Seedling weight is highly correlated with seed size. However, for a given increment in seed size in sainfoin, the associated cotyledon area increase is less than for lucerne or birdsfoot trefoil (Carleton & Cooper, 1972). Sainfoin is a rapid developing legume, the seedlings of which are more aggressive than those of birdsfoot trefoil or cicer milkvetch (*Astragalus cicer* L.) in mixed culture (Smoliak & Hanna, 1977). Sainfoin seedlings may have an initial advantage over the other two legumes because of a relatively large seed and cotyledon area. It is thought that this advantage would persist throughout seedling growth (Smoliak & Hanna, 1977). Cooper and Fransen (1974) looked at energy relationships and the photosythetic contribution of the cotyledons during early seedling development. Growth was dependent on seed stored carbohydrate during the first seven days of growth (Cooper & Fransen, 1974). The store of substrate was not adequate for normal first leaf formation and expansion, which appeared to depend on cotyledonary photosynthesis. Once stored reserves have been used, photosynthesis by the cotyledon is of major importance to early seedling growth (Cooper & Fransen, 1974). The decrease in photosynthetic contribution of the cotyledons was in proportion to their decrease in area as a proportion of total leaf area, and was only 18% of the total when the seedlings were 19 days old. Smoliak *et al.* (1972) found that lucerne had a greater mean relative growth rate (RGR) and net assimilation rate (NAR) than sainfoin, indicating that lucerne seedlings grew more rapidly during the ten week period of their test. Mean LAR (LAR = leaf area ÷ total dry weight) was slightly higher for sainfoin than lucerne, as were top and root weights. Accumulated leaf area was also higher for sainfoin than lucerne (Smoliak *et al.* 1972). Mean NAR and RGR values for cicer milkvetch were intermediate between those of sainfoin and lucerne. The important difference between sainfoin and lucerne appeared to be in terms of NAR, with the higher NAR of lucerne enabling a higher RGR despite having a lower leaf area. However differences between these species in terms of seedling top growth were found to be reflected in yields throughout the first year, with sainfoin being highest yielding, followed by lucerne and cicer milkvetch.

Sainfoin begins to grow in the spring before most other perennial legumes (Hanna *et al.* 1977a). A description of the early development has been provided in section 1.3, as has a discussion of the classification of sainfoin into one and multi-cut types according to its growth behaviour and propensity to flowering. The one cut type has a winter requirement for flowering and remains prostrate in its first year, producing one crop of herbage towards the end of the growing season (Bland, 1971; Spedding & Diekmahns, 1972). In subsequent years it grows vigorously, sending up stems and flowering once only (Bland, 1971). The large bulk of herbage produced at this time, in association with flowering, is normally cut and fed, or conserved. The regrowth, or aftermath, which is prostrate and much lower yielding, is normally grazed (Thomson, 1938; Bland, 1971).

The multi-cut type flowers on its first year and flowers two or more times per year (Thomson, 1938; Thomson, 1951a; Bland, 1971). In association with flowering it produces a sufficient bulk of erect growing foliage for cutting twice or more each year, and the aftermath of the final cut can be used for grazing (Bland, 1971). The multi-cut type is probably more appropriate where cutting and conservation is the primary object, as it is a more rapid and luxuriant grower, it flowers earlier than the one cut type, and it will produce two or more heavy crops per year (Percival, 1943).

Thomson (1951a) found that, in the seeding year, a multi-cut type of sainfoin yielded much higher than a one cut type at the first cut (cut when the multi-cut type was in full flower), but that yields for the remainder of the season were similar. In the second year, yields for the one cut type were greater under both frequent (cut monthly) and non frequent (cut when the multi-cut type flowered) defoliation regimes. The multi-cut out yielded the one cut type at the second cut (in the second year). This could have been associated with a higher proportion of leaf (compared to total top) relative to the one cut type at the time of the first cut (Thomson, 1951a). The higher proportion of leaf could have been due to the more luxuriant growth of fresh leaves and shoots from the base of the multi-cut type at the late flowering stage, or to the fact that the multi-cut type had more leaflets per leaf, shorter internodes on the flowering stems and therefore more leaves per unit length of stem.

Cooper (1972) compared growth and development of the one cut variety Eski, with the two cut variety Remont, during their second season of growth. There was an initial period of leaf formation after which leaf area appeared to increase as a result of leaf expansion. This was followed by a period of stable leaf area index (LAI). Crop growth rate increased with increasing LAI, and was highest during the period of stable LAI. This period coincided with the time of most rapid increase in stem weight, and with the period of restoration of root carbohydrate reserves used in spring growth. The later maturing Eski had a more rapid RGR than Remont as the season progressed. The higher growth rate of Eski was related to a significantly higher LAR, and its yield advantage was thought to be primarily due to this, particularly as the amount of available radiant energy was increasing with increasing day length (Cooper, 1972).

From the stand point of getting early grazing, Remont would have an advantage, because early on its yields were greater than those of Eski. Both varieties were found to recover rapidly following spring clipping, but the regrowth of Remont was more rapid following a hay harvest or late season clipping (Cooper, 1972). No data were given for regrowth following hay harvest, but it was reported that soil moisture was a major limiting factor to later growth. The ability of a two cut variety to produce a second hay crop would probably be negated to some extent under these conditions.

Sainfoin tends to recover more slowly from defoliation than lucerne, and does not produce as much regrowth (Hanna *et al.* 1975). Sheehy & Harding (pers.comm.) compared the growth patterns of sainfoin and lucerne during a summer regrowth period of 48 days. At the end of 48 days, the amount of herbage produced by sainfoin was approximately 32% less than that produced by lucerne. The main difference was in weight of stem, with leaf weights being similar. LAI's however, were markedly different, with the final LAI of lucerne being over twice that of sainfoin. The higher LAI of lucerne was reflected in higher rates of canopy photosynthesis. The more rapid increase in LAI of lucerne was explained partly by the difference in rate of leaf appearance. Axillary leaves accounted for the higher rate of leaf appearance in lucerne. A contributory factor to the higher rate of leaf appearance was thought to be the greater number of nodes on lucerne plants (24) compared with sainfoin (6-7). Specific leaf area (SLA = leaf area ÷ leaf dry weight) for sainfoin was less than half that for lucerne throughout the growing period. Rates of individual leaf photosynthesis per unit leaf area were similar for the two species, as were rates of dark respiration per unit leaf area and the conductance of leaves to water loss. Thus, it appears that the critical difference between the two species was one of leaf morphology. Lucerne, by using its assimilate to produce a greater leaf area instead of the thicker leaves produced by sainfoin, increased its interception of photosynthetically active radiation and hence its photosynthetic capacity.

The best management for optimum yield of sainfoin seems to be to cut when flowering is well advanced (Spedding & Diekmahns, 1972). Frequent or early defoliation can adversely affect the productivity and stand persistence of sainfoin (Thomson, 1951a; Badoux, 1965; Carleton *et al.* 1968b; Jensen & Sharp 1968; Bland, 1971; Hassell, 1971). Hassell (1971) found that pre-bloom and mid-bloom cutting reduced competitiveness and allowed invasion of weeds. Jensen and Sharp (1968) found that weeds severely invaded frequently cut plots of sainfoin, with those most frequently cut having the most weeds. Carleton *et al.* (1968b) also reported loss of stand accompanied by weed invasion on frequently cut plots. However, if defoliation is delayed to the late bud or early flowering stage, regrowth of sainfoin is satisfactory, and stand longevity is not reduced (Spedding & Diekmahns, 1972; Hanna *et al.* 1975). Hassell (1971) cut sainfoin at the prebloom, midbloom and early pod stages, and found yields to be significantly greater at the early pod stage. Carleton *et al.* (1968a) found that maximum accumulation of dry matter and crude protein in sainfoin occurred at 100% bloom, compared to 2-45% bloom for lucerne.

Thomson (1951a) found that sainfoin yielded lower under frequent (monthly) defoliation, in comparison to less frequent defoliation, such as might be encountered under a hay cutting regime. In the second season of growth, the frequent defoliation regime reduced yields by 63% (multi-cut type) and 50% (one cut type), relative to infrequent defoliation. The difference in performance of the two types can be explained largely in terms of their growth behaviour (Thomson, 1951a). The multi-cut type exhibited a much greater propensity to flower than the one cut type, and its propensity to flowering was modified under conditions of frequent defoliation much less than that of the one cut type. The multi- cut sainfoin produced abundant flowers even under frequent defoliation (Thomson, 1951a). It was thought that the lack of flowering in the one cut type was related to its greater resistance to frequent defoliation and that it utilised relatively more of its root carbohydrate reserves in producing leaves, which yielded a return of carbohydrates. In contrast, the flowers of the multi-cut type yielded no such return, and hence root reserves were more rapidly exhausted. Also a greater tendency to flower can result in fewer leaves at low levels on the plant, and hence destruction of a high proportion of the top when cut, resulting in slow regrowth (Spedding & Diekmahns, 1972).

Under a hay cutting regime, sainfoin seems to produce over half of its total annual production at the first cut (Piper, 1924; Thomson, 1951a; Evans 1961; Jensen & Sharp, 1968). The one cut type of sainfoin tends to produce a greater proportion of its yield by the first cut than the multi-cut type, which will undergo stem elongation and flower a second time (Thomson, 1951a).

Regrowth of sainfoin after cutting is slower than lucerne, and is more adversely affected by frequency of cutting than lucerne (Carleton *et al.* 1968b). The slow regrowth of sainfoin relative to lucerne has been discussed previously in this section, in relation to the work of Sheehy & Harding (pers.comm.). This slow regrowth and sensitivity to frequent cutting may be further explained by the results of Cooper and Watson (1968), who found that total available carbohydrate in the roots of sainfoin were lower and showed less cyclic fluctuation with cutting than in lucerne. Total available carbohydrate remained at low levels until late summer or early autumn. It is hypothesised that, following the use of carbohydrate reserves in early spring growth, regrowth of sainfoin depends primarily on carbohydrates synthesised in existing leaf area (Cooper & Watson, 1968). Sheehy & Harding (pers.comm.) calculated that sainfoin, on average, translocated 9% of its photosynthate to the roots, compared to a figure of 3% for lucerne. They suggest that it is possible that the figure for lucerne could be an under estimate. However, if these figures are approximately correct, the additional photosynthate translocated to the roots of sainfoin is being used for some purpose other than building up root reserves. As discussed in section 1.3 it has been observed that sainfoin has a more highly developed root system than lucerne, and produces a greater weight of nodule tissue. It is also possible that its symbiotic nitrogen fixing system is biochemically less efficient, and thus more energy demanding, than that of lucerne.

There appear to be some features of the morphology and growth pattern of sainfoin which contribute to its apparently poor performance in relation to the more productive forage legumes, particularly lucerne. Sainfoin has been found to have a lower SLA and LAR than lucerne, and hence a lesser ability to produce photosynthate. Also the lack of build up of carbohydrate reserves during regrowth cycles may well contribute to slow initial regrowth after defoliation. As discussed in section 1.5.2, however, the low SLA and slow rate of regrowth may be advantageous under very dry conditions.

1.5 ECOLOGICAL NICHE

The two types of sainfoin, the one cut and multi-cut types are best suited to differing environmental conditions. The one cut type, because it produces a greater proportion of its seasonal herbage production at the first cut (e.g. Thomson, 1951a), is best adapted to an environment with a short growing season or a low mid season moisture supply (Murray & Slinkard, 1968; Cooper, 1972, Carleton *et al.* 1968b). Its slow regrowth is thought to enhance its drought resistance (Koch *et al.* 1972), making it suitable for regions having very dry summers. The multi-cut type, because of its ability to produce two or more hay crops in a single year (e.g. Thomson, 1938), appears best suited to environments with a longer growing season, where more than one cut (or grazing) is desired, and where there is adequate mid season moisture to support vigorous regrowth.

1.5.1 SOILS

Sainfoin is especially adapted to dry, well drained, calcareous soils, containing at least 0.3% CaO (Whyte *et al.* 1953; Spedding & Diekmahns, 1972).

Historically the culture of sainfoin in Europe and Britain has been largely confined to chalky or other calcareous soils, particularly where these are subject to drought (Piper, 1924; Bland, 1971), and sainfoin has been found growing wild on the chalk soils of south-east England (Bland, 1971). Both low pH values, and high concentrations of Al have been found to markedly reduce the growth of sainfoin (and lucerne) in comparison to lupins (*Lupinus* species), serradella (*Ornithopus sativus*) and white clover (Rorison, 1957). Al toxicity was found to be most important in the early seedling stage of growth. It is felt that sainfoin is excluded from certain acid grasslands mainly because of the toxic effect of Al on the soil solution (Rorison, 1965).

Traditionally, sainfoin has been grown on the dry calcareous soils of Russia and Eruope, and has shown promise as a hay and pasture crop in dry locations of USA, South America and South Africa (Whyte *et al.* 1953; Andreev, 1963; Spedding & Diekmahns, 1972; Ditterline & Cooper, 1975). Sainfoin is reported to have good persistence under dry land conditions, but may be less persistent (e.g. less than lucerne) under irrigation (Cooper *et al.* 1968a; Ditterline & Cooper, 1975; Chapman & Carter, 1976). Under dry conditions, sainfoin does respond to irrigation, but does not require it as frequently as, for example, lucerne (Hanna *et al.* 1977a). Sainfoin is found to have the poorest tolerance to flooding of a range of forage legumes, including lucerne (Heinrichs, 1970). The sensitivity of sainfoin to wet soil conditions is probably largely explained by its susceptibility to crown and root rot diseases (Ditterline & Cooper, 1975). Ditterline and Cooper (1975) cite the crown and root rot pathogenic complex as being the most limiting factor to sainfoin production. This problem has generally been associated with the fungus *Fusarium solani* (Mathre, 1968; Ditterline & Cooper, 1975; Auld *et al.* 1976). Gaudet *et al.* (1980), however, have evidence that the causal organisms may be one or more bacteria, rather than a fungal pathogen. Although crown and root rot is found under both dryland and irrigated conditions (Sears *et al.* 1975), the problem seems to be more acute under wetter conditions.

As would be expected from the previous discussion, sainfoin generally does not thrive on heavy clay soils (Spedding & Diekmahns, 1972). However, Bland (1971) points out that establishment can be quite satisfactory on clay soils provided the pH is not low. The importance of moisture level is highlighted by Carleton *et al.* (1968b) who report more frequent loss of sainfoin stands on heavy soils, and particular problems following irrigation. The fact that sainfoin appears to perform best on well drained soils is supported by Kornilov and Verteleskaia (1952) who state that sainfoin is exceptionally resistant to unfavourable environment when grown on sandy soil, with good persistence being observed in the desert climate of Karastan, U.S.S.R.

Jensen and Sharp (1968), and Chapman and Carter (1976) state that sainfoin has considerable tolerance to salinity, whereas Hanna *et al.* (1977a), state that it does not tolerate saline soils. Jensen and Sharp (1968) presented evidence indicating that sainfoin does have good salt tolerance, and the ability to persist in highly saline areas. The other authors did not substantiate their claims with evidence.

Thus, it appears that a number of soil factors, including pH, Al level, moisture conditions and texture, in combination determine whether or not sainfoin will thrive on a particular soil. It appears that adverse soil moisture conditions have their effect via the crown-root rot complex.

1.5.2 CLIMATE

Schnieter *et al.* (1969) stated that sainfoin is more drought hardy than lucerne. The deep root system of sainfoin has been cited as a reason for its drought resistance (Bland, 1971). It has also been suggested (Koch *et al.* 1972) that the slow regrowth of sainfoin, particularly the one cut type, may contribute to its drought tolerance. This idea is supported by Shain (1959) who reports lower drought tolerance from multi-cut sainfoin types. Thus it appears that one cut types of sainfoin might be more suitable for areas with very limited supplies of mid season moisture.

Sheehy *et al.* (1978) found that values for leaf area index and leaf area per unit leaf weight of sainfoin were about half those of lucerne. In addition, as the crops grew, mean daily leaf water potentials were substantially less negative, and mean daily turgor potentials were substantially higher in sainfoin than lucerne. There were no significant differences between stomatal resistances of the crops (Sheehy *et al.* 1978), indicating that leaf area is probably the critical factor. Hence the low specific leaf area (SLA) of sainfoin may be a contributing factor to its ability to tolerate drought conditions and selection for increased SLA to improve photosynthetic performance, as suggested by Sheehy & Harding (pers.comm.) may have the effect of reducing drought tolerance. Percival and McQueen (1980) found that the regrowth after cutting, of sainfoin, appeared to be more adversely affected than that of lucerne by dry conditions. It is not however clear, whether the dry conditions were the cause of the slow regrowth, or whether regrowth was just inherently slow, and would have been slow regardless of soil moisture conditions because of the cultivar under study being a one cut type. Poor regrowth of a one cut sainfoin cultivar has been observed where seasonal rainfall was adequate (Meyer, 1975). Despite its reported draught tolerance, however, sainfoin has not been recommended for dryland areas in Montana or Western Canada where rainfall is less than 300 mm per year (Cooper *et al.* 1968b; Hanna *et al.* 1977a).

Sainfoin is reported to be winter hardy and frost resistant (Schneiter *et al.* 1969; Chapman & Carter 1976) with seedlings and mature plants being highly tolerant of autumn and spring frosts (Hanna *et al.* 1977a). It is thought, however, that sainfoin is less winter hardy than the lucerne varieties recommended for locations having severe winters (Hanna *et al.* 1977a), and Andreev (1963) states that sainfoin will stand heavy frost only if there is good snow cover. Sainfoin is found to have a wide range of winter hardiness according to area of origin (Cooper *et al.* 1968b). Introductions from Russia and Turkey (which would probably tend to be one cut types) had good and fair survival respectively, while introductions from England (which would presumably include some multi-cut types, and would tend to be of Mediterranean origin) showed poor survival. Jensen & Sharp (1968) state that, at the succulent stage, sainfoin is more tolerant to frost than lucerne, and that this should enable it to maintain later growth in the autumn, and commence growth earlier in the spring than lucerne. During the autumn, plants develop a low rosette type of growth that may remain green under snow for most of the winter (Hanna *et al.* 1977a).

Thus, the absolute frost tolerance of sainfoin appears to be lower than that of lucerne, but it is reportedly able to maintain growth under colder conditions than lucerne.

1.5.3 CONCLUDING COMMENTS - ECOLOGY OF SAINFOIN

Sainfoin appears to have some quite specific environmental requirements, particularly with regard to soil moisture and pH. Evans (1961) states that lucerne is better adapted to a wider range of environmental conditions than sainfoin. One cut sainfoin varieties appear to display better survival under dry mid season conditions, and may tend to be more winter hardy than multi-cut varieties. In New Zealand, with a relatively long growing season, and an absence of severe winter conditions, multi-cut sainfoin would likely be the more appropriate type.

1.6 NITROGEN FIXATION OF SAINFOIN

The nitrogen fixing ability of sainfoin is, in many instances, reported to be insufficient to provide for the total nitrogen requirement of the plant.

Nitrogen deficiency symptoms have been observed on inoculated sainfoin at an early stage in crop development (Burton & Curley, 1968; Sims *et al.* 1968; Roath & Graham, 1968; Meyer, 1975; Smoliak & Hanna, 1975). Cooper *et al.* (1968a), growing sainfoin with grasses and other legumes under irrigated pasture conditions, found that sainfoin recovered very slowly following defoliation, and that plants were a light green to yellowish colour characteristic of nitrogen deficiency. Schneiter *et al.* (1969) also report the development of nitrogen deficiency symptoms in sainfoin, and state that this is a very unusual condition for a legume which indicates that the strain of nitrogen fixing bacteria present is inefficient or short lived.

Nitrogen deficiency symptoms have been observed in sainfoin despite plants being abundantly nodulated (Burton & Curley, 1968). The nodules on healthy plants were observed to vary greatly in size and shape, with frequently only the small nodules containing the red pigment, leghaemoglobin, associated with nitrogen fixation. They state that this indicates a very delicate balance between the host plant and its microsymbiont, and dominance by the host. Nitrogen deficiency symptoms have been confirmed by low protein levels found on analysis of chlorotic plants (Sims *et al.* 1968). Growth responses of inoculated field grown sainfoin to added nitrogen have been observed by Sims *et al.* (1968), Jensen and Sharp (1968) and Meyer (1975). On soils containing low levels of nitrate-N, Sims *et al.* (1968) reported yield responses to up to 336 kg per hectare of added N. On a site with higher levels of nitrate-N, the effect of added fertiliser N was less pronounced. Nitrogen deficiency symptoms were manifest early in the season, and then disappeared as the activity of nitrifying bacteria increased, and as the plant root systems developed (Sims *et al.* 1968). Jensen and Sharp (1968) observed responses to nitrogen fertiliser by inoculated sainfoin in central Nevada. N at 112 kg per hectare substantially increased yields in the second year (first harvest year) of the crop. The response was diminished in the third year, and non significant in the fourth year of the crop (Jensen & Sharp, 1968). It was thought that the reduced benefits from nitrogen fertiliser in the third and fourth years may have been related to increased nitrogen fixation by rhizobia in the more mature plants. Forage yields of sainfoin are frequently inferior to lucerne, where seasonal rainfall is adequate for two or more cuttings, because of sainfoin's poor regrowth and vigour (Meyer, 1975). Inoculated sainfoin plants typically showed N deficiency symptoms at Fargo, North Dakota, and it was thought that inferior recoverability and stand persistence could be due in part to its inability to obtain sufficient nitrogen via symbiotic fixation (Meyer, 1975). Meyer (1975) applied N, P and K to a three year old inoculated sainfoin stand. N increased yields for each increment applied, up to 448 kg per hectare. The growth and vigour was enhanced by N fertilisation but stand persistence remained poor for all but the 448 kg per hectare treatment. Fourth and fifth year yields did not compare favourably with unfertilised, inoculated lucerne (Meyer, 1975). Tap roots of plants from 0, 224 and 448 kg N per hectare treatments were examined for nodules. Large pink nodules were observed on most plants. It was thought that the *Rhizobium* inoculant was ineffective, or that the nodules were short lived (Meyer, 1975).

Babian & Karagulian (1959), on two soils, found that sainfoin responded more to added N than lucerne. Koter (1965a) grew sainfoin and red clover in pure sand and applied a range of N levels. The presence of combined N in the medium accelerated the growth rate of both species in comparison to plants reliant solely on symbiotically fixed N_2. Larger responses to N addition were obtained with sainfoin than with red clover. At the highest rate of N, the amount of herbage produced by sainfoin was increased by 112% and that of red clover by 55% compared with plants supplied with no combined N. Sainfoin not supplied with combined N also flowered later than plants supplied with N. The introduction of N into the nutrient solution reduced the nodulation of both species (Koter, 1965a). The increase in yield of nitrogen, above the quantity fixed from the atmosphere by the zero N plants was greater for sainfoin (20 - 33%) than for red clover (7 - 15%).

Koter (1965b) found that low levels of combined N stimulated nodulation and N_2 fixation in sainfoin. N_2 fixation was stimulated by 21 - 28%. High rates of combined N severely checked nodulation and nitrogen fixation, with most of the plant's nitrogen requirements being absorbed from the medium.

Major *et al.* (1979) measured $N_2[C_2H_2]$ fixing activity (N_2 fixing activity as measured by the acetylene reduction technique) in seedlings of sainfoin, lucerne and cicer milkvetch. It was found that the amount of acetylene reduced by lucerne was not as closely related to shoot dry weight as it was for sainfoin and cicer milkvetch. The relationship between nodule weight and top weight was closer in sainfoin than it was for the other two plants (Major *et al.* 1979). This may indicate a more critical relationship between plant performance and N_2 fixing activity in sainfoin than in lucerne. Sainfoin had a greater weight of nodules and a greater $N_2[C_2H_2]$ fixing activity per plant than the other two species, but a lower specific $N_2[C_2H_2]$ fixing activity per nodule weight. Sainfoin had more nodule tissue and a greater acetylene reducing activity per weight of shoot dry matter than lucerne (Major *et al.* 1979). Copley (1972) also reports higher $N_2[C_2H_2]$ fixing activity, on a per plant basis for sainfoin than lucerne. Although nodulation of sainfoin appears to be good (Karpov, 1957; Stergeeva, 1957; Burton & Curley, 1968; Major *et al.* 1979) and acetylene reducing activity appears to be adequate (Copley, 1972; Major *et al.* 1979),there is very strong evidence (Koter, 1965a; Burton & Curley, 1968; Sims *et al.* 1968; Roath & Graham, 1968; Meyer, 1975) that sainfoin is less able to provide for its own nitrogen requirements via symbiotic fixation than other forage legumes such as lucerne and red clover.

There is an apparent contradiction between the seemingly low quantity of N fixed, and relatively high $N_2[C_2H_2]$ fixing activity per weight of plant compared with, for example, lucerne. It could be that the acetylene reduction technique over-estimates N_2 fixing activity in sainfoin relative to lucerne, and that greater inefficiencies, or energy losses, perhaps in the form of H_2 evolution (section 2.2.2), are present in the nitrogen fixing system of sainfoin.

There are, however, contrasting reports which indicate that sainfoin can fix adequate amounts of N. Badoux (1965) reports that yields of sainfoin in pure stands were slightly reduced by the application of 45 and 90 kg N per hectare, and that, in the glasshouse, added nitrate decreased top and root weights, and the number and size of nodules. There are two possible explanations for this result. It could indicate that symbiotic fixation of N was adequate to meet the requirements of the plant, or that the amount of N added was sufficient to inhibit N_2 fixing activity and partially replace N_2 fixation, but not sufficient to give a growth response. Stergeeva (1957) reported that sainfoin was found to have a greater soil enriching effect than lucerne. Crops were found to have greater yields and higher %N content following sainfoin. The greater nodule weights, and more highly developed root systems of sainfoin (section 1.3), and their subsequent decay on cultivation may contribute to this effect. An alternative explanation could be that there is leakage of N from living sainfoin plants into the soil.

The information on N_2 fixation in sainfoin can be summarised as follows:

1. Many reports of N deficiency symptoms in sainfoin stands.
2. Many reports of responses to added N.
3. A lesser number of reports comparing the response of sainfoin to added N with the response of other forage legumes, in which sainfoin is usually found to give a greater response than the better performing plants, such as lucerne or red clover.
4. Nodulation generally appears to be adequate in terms of number and weight of pink nodules. Nodule weight on a plant weight basis appears to be greater than that of lucerne.
5. From the little work that has been done, the $N_2[C_2H_2]$ fixing activity of sainfoin appears to be lower than that of lucerne on a weight of nodule basis, but higher on a per plant or weight of plant basis.

Overall, the evidence appears to suggest that sainfoin, although it produces a proportionally greater weight of nodule tissue than for example lucerne, and may have a higher $N_2[C_2H_2]$ fixing activity per weight of plant (thus using more energy), actually fixes, or utilises, less N_2 than lucerne.

Burton & Curley (1968) speculate that tannins contained in sainfoin would have an adverse effect on the symbiotic relationship between plant and bacteria. This could perhaps explain the relatively low $N_2[C_2H_2]$ fixing activity per weight of sainfoin nodule tissue.

Because of the possibly higher energy demand of the symbiotic N_2 fixing system of sainfoin and the apparently lesser ability of sainfoin to utilise photosynthetically active radiation (section 1.4) relative to lucerne, it could be that the energy relationships of sainfoin are particularly critical, and that relationships between such parameters as leaf area and nodule weight or $N_2[C_2H_2]$ fixing activity are more critical than in other legumes such as lucerne.