BIOMASS AND PROTEIN YIELDS, N_2-FIXATION AND N TRANSFER IN ANNUAL FORAGE LEGUME-BARLEY (*Hordeum vulgare* L.) CROPPING SYSTEMS

by

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Short title:

ANNUAL LEGUME-BARLEY INTERCROPS FOR FORAGE PRODUCTION

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ABSTRACT

M.Sc. Helen G. Sampson Plant Science

BIOMASS AND PROTEIN YIELDS, N_2-FIXATION AND N TRANSFER IN ANNUAL FORAGE LEGUME-BARLEY (*Hordeum vulgare* L.) CROPPING SYSTEMS

In this study, six annual legumes and the perennial, red clover (*Trifolium pratense* L.) were monocropped (MC) and intercropped (IC) with barley in a field study with three N levels, 0, 30 and 60 kg N ha^{-1}. At 0 kg N ha^{-1}, N_2-fixation and N transfer were estimated by the ^{15}N isotope dilution (ID) method. At 60 kg N ha^{-1}, a direct ^{15}N labelling method was employed to study N transfer. The hypotheses were that the annual species would be more productive within one growing season than red clover, that increased N levels would increase herbage dry matter (DM) and crude protein (CP), that the proportion of N derived from N_2-fixation in IC-legumes would be higher than that of MC-legumes and that within intercrops there would be evidence of N transfer. In neither year was the total DM yield of red clover, MC or IC, less than the rest of the legumes. In 1991, the total DM yield of intercrops responded to 30 kg N ha^{-1}; in neither year did the estimated total CP yield of MC-legumes or intercrops respond to N levels. Only in 1992 was there evidence of N_2-fixation and the proportion of N derived from fixation by IC-legumes was 145 % higher than that of MC-legumes. Only the ^{15}N direct labelling method gave evidence of N transfer, to associated legume and barley plants in 1991, and to associated legume plants in 1992.

Résumé

M.Sc. Hélène G. Sampson Plant Science

La Biomasse et le Rendement en Protéine, La Fixation et Le Transfert de L'Azote dans une Culture d'Orge et de Légumineuses Fourragères Annuelles

La culture, avec apport de 0, 30, et 60 kg N ha^{-1}, de six espèces annuelles de légumineuses et d'une espèce péréenne de trèfle rouge en monoculture ou en culture intercalaire avec l'orge, est evaluée en plein champ. La méthode de dilution d'^{15}N est utilisée pour estimer la fixation et le transfert d'azote dans le traitement avec 0 kg N ha^{-1}, alors que la méthode employée pour étudier le transfert d'azote dans le traitement où 60 kg N ha^{-1} est incorporé, pourvoie directement de l'azote enrichi ^{15}N aux légumineuses . Les hypothèses de depart étaient que les rendements des espèces annuelles seraient plus élevés que le rendement du trèfle rouge pendant la periode d'éssai, qu'une teneur superieure en matière sèche et en protéine du fourrage correspondrait à un apport maximal d'azote, que le taux d'azote fixé serait meilleur pour les légumineuses en culture intercalaire et que le transfert d'azote en culture intercalaire serait évident. Pendant les deux années consécutives, la biomasse, en monoculture ou culture intercalaire, a été plus élevée pour le trèfle rouge. En 1991, un meilleur rendement en matière sèche fut obtenu à l'apport de 30 kg N ha^{-1} pour les légumineuses en culture intercalaire. Par contre, l'apport de différentes quantités d'azote ne changea pas la teneur en protéine des légumineuses, en monoculture ou en culture intercalaire. La fixation de l'azote était évidente seulement en 1992 et le pourcentage d'azote fixé par les légumineuses en culture intercalaire, était superieur de 145% à celui des légumineuses en monoculture. Seule la méthode de dilution d'^{15}N indiqua un transfert d'azote parmi les légumineuses et l'orge en 1991, et parmi les espèces légumineuses en 1992.

FORWARD

This thesis is submitted in the form of original papers suitable for journal publications. The first section is a literature review on this topic. The next two sections, each a complete manuscript, form the body of the thesis. The last section consists of an overall summary and conclusions of the research. This thesis format has been approved by the Faculty of Graduate Studies and Research, McGill University, and follows the conditions outlined in the Guidelines Concerning Thesis Preparation, section B.2 "Manuscripts and Authorship" which are as follows:

"The candidate has the option, subject to the approval of the deparment, including as part of the thesis the text, or duplicated published text, of an original paper, or papers.
-Manuscript-style theses must still conform to all other requirements explained in Guidelines Concerning Thesis Preparation.
-Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made on the importance and originality of the research reported.
-The thesis should be more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review and final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interests of cohesion.

It is acceptable for the thesis to include, as chapters, authentic copies of papers already published, provided these are duplicated clearly on regulation thesis stationary and bound as an integral part of the thesis. In such instances, connecting texts are mandatory and supplementary explanatory material is always necessary.
-Photographs or other materials which do not duplicate well must be included in their original form.

While the inclusion of manuscripts co-authored by the candidate and others is acceptable, the candidate is required to make an explicit statement on who contributed to such work an to what extent, and supervisors must attest to the accuracy of the claims before the Oral Committee. Since the task of the Examiners is made more difficult in these cases, it is in the candidate's interest to make the responsibilities of authors perfectly clear."

ACKNOWLEDGEMENTS

It has been my pleasure to have worked with Dr. Ralph C. Martin on this project. I would like to express my thanks for his thorough and thoughtful review and editing of these manuscripts. I have also appreciated the suggestions made by Dr. Don L. Smith and his time spent reviewing the manuscripts. I thank Dr. Bruce Coulman for his participation on my graduate committee.

Thanks are also extended to Dr. Suzan Altinok, who brought her beloved annual medic seeds from Turkey to Nova Scotia, and with whom it was enjoyable working.

The technical assistance of Stewart Liebovitch was also greatly appreciated. I would like to thank the following people, whose efforts during seeding and harvesting helped things go smoothly, Beverley (Vetch-Hater) Hattie, Rupert (Lupin-Lignin) Jannasch, Teklay Messele and Ignatius Augustin.

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CONTRIBUTIONS OF CO-AUTHORS TO MANUSCRIPTS FOR PUBLICATION

Section 3 comprises the manuscript of Sampson, Altinok, Smith, Papadopoulos and Martin (1993), to be submitted to Journal of Production Agriculture. Section 4 comprises the manuscript of Sampson, Smith and Martin (1993) to be submitted to Biology and Fertility of Soils. Dr. Martin, Dr. Altinok and Dr. Papadopoulos were each involved with the project's initial proposal. Dr. Smith provided use of laboratory equipment and supplies for ^{15}N analysis. Dr. Martin and Dr. Smith have each reviewed and edited these manuscripts; and Dr. Altinok and Dr. Papadopoulos will review manuscripts prior to submission for publication.

All laboratory procedures involving ^{15}N were performed by myself, while N analysis of samples for section 3 were performed by Beverley Hattie, Dr. Altinok and the Nova Scotia Department of Agriculture and Marketing Plant Industry Branch.

During field operations, I received technical assistance. I conducted the statistical analysis and wrote the manuscripts.

Section 2

GENERAL INTRODUCTION

2.0. Forage production in Nova Scotia:

Sixty-six percent of Nova Scotia's improved land is in forage crop production (Anon. 1991a.) and consists mainly of perennial legumes and grasses. Forage crops are frequently undersown with a cereal cover crop and red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.) are, generally, recommended over the use of alfalfa (*Medicago sativa* L.) and most other grasses (Anon. 1991b.). Currently, the only recommended annual forage legume in Nova Scotia is persian clover (*T. resupinatum* L.), monocropped or intercropped with annual ryegrass (*Lolium multiflorum* Lam.) for pasture or silage. In Prince Edward Island, Kunelius and Narasimhalu (1983) examined the potential of several forage legumes as summer annuals when monocropped or intercropped with either Italian or westerwolds ryegrass (*Lolium multiflorum* Lam.). Dry matter (DM) yields of mixtures were increased over monocropped legumes by 15 to 22 % when legumes were intercropped with Italian ryegrass and by 15 to 52 % when the grass component was westerwolds. Of the legumes, persian clover proved to be the most productive. Binary mixtures of persian clover, red clover, alfalfa and birdsfoot trefoil (*Lotus corniculatus* L.), each with Italian ryegrass, produced total DM yields which were, respectively, 84, 72, 63 and 50 % that of the N-fertilized monocropped grass. These same mixtures produced N yields which were, respectively, 91, 81, 73 and 49 % that of the fertilized monocropped grass. With the exception of the mixture containing birdsfoot trefoil, DM yield of mixtures with westerwolds were higher than those of the non-N-fertilized monocrop grass. Annual legumes intercropped with barley (*Hordeum vulgare* L.) for forage production, have potential for use in short term cropping rotations. As annual legumes complete their life cycle in one season, their growth, compared with that of a perennial, such as red clover, would be expected to be more vigorous during the establishment year. As such, annual legumes may compete more effectively in cereal intercrops. Investigations into the use of alternative annual legumes are necessary to expand their limited use and may provide growers with more flexibility and greater choice in their rotations.

2.1. Annual legume-cereal intercropping:

Compared with studies involving legume-grass intercrops, reports on legume-cereal intercrops are limited. Such studies have examined the cereal component grown for grain and forage production. Kunelius *et al.* (1992) evaluated barley production in Prince Edward Island by undersowing the cereal with either westerwolds ryegrass, Italian ryegrass or red clover. When intercropped in small plots (2 m x 5 m) in alternate rows, grain yields were reduced. Grain DM yield in mixture was most severely depressed by westerwolds ryegrass 'Aubade' and produced only 71 % of monocropped grain. Intercropped red clover had the least effect on grain yield, allowing for 90 % of the monocropped yield. Barley height was not affected by undersowing. Although the N concentration of red clover (26.9-36.1 g kg^{-1}) was higher than that of ryegrass (21.6-31.9 g kg^{-1}), red clover N yield (390-1545 kg ha^{-1}) was lower than that of ryegrass (775-2890 kg ha^{-1}) since ryegrass herbage yields, either monocropped or intercropped, were greater than those of red clover. The authors also studied large plots to simulate commercial conditions. In these plots, grain yields were less affected by ryegrass. Italian and 'Marshal' westerwolds ryegrass had no effect on grain yield. Red clover had the same effect on grain yields as in the smaller plots.

With the aim of examining biomass yield and weed control on two sites in Southern Ontario, Samson *et al.* (1989) interseeded red clover at different times into existing winter wheat (*Triticum aestivum* L.) fields and included treatments of Nitro alfalfa, crimson clover (*Trifolium incarnatum* L), and hairy vetch (*Vicia villosa* Roth.) drilled in wheat in early May. On a sandy loam site the authors found no differences in wheat yields between treatments, with the exception of the early frost seeding of red clover (3.35 t ha^{-1}) being significantly lower than those of the late frost seeded red clover (4.36 t ha^{-1}) and drilled crimson clover (*T. incarnatum* L.) (4.38 t ha^{-1}). There were no significant differences in wheat yields between treatments on a silt loam site. Of the legumes, vetch produced the highest forage biomass on each site, yielding 3367 and 2554 kg ha^{-1} on the silt loam and sandy loam, respectively. On the silt loam, drilled red clover (678 kg ha^{-1}), Nitro alfalfa (890 kg ha^{-1}) and crimson clover (844 kg ha^{-1}) yielded significantly lower than the red clover frost seeded treatments. On the sandy loam, Nitro alfalfa (1440 kg ha^{-1}) and crimson clover (1426 kg ha^{-1}) yielded significantly higher than red clover treatments. The authors stated that in general, weed suppression was greatest where forage production was greatest.

In a pot study, installed in a winter wheat field in Vienna, Hartl (1989) monocropped and intercropped winter wheat with a specific group of weeds, black medic (*Medicago lupulina* L.) and persian clover, and found a trend of more grain when winter wheat was intercropped with black medic (approximately 22.5 g pot^{-1}) than when monocropped (21.5 g pot^{-1}). Straw yield when intercropped with medic was significantly higher than weedy wheat, and weed DM, of weedy wheat intercropped with medic, was reduced by 55 % compared to weedy wheat. In wheat intercropped with persian clover, there was a trend for decreased grain yield (20 g pot^{-1}), compared to monocropped wheat. Persian clover significantly decreased straw yield compared to weedy wheat and decreased weed DM over 70 % when intercropped with weedy wheat, compared to weedy wheat. In a field study, the authors broadcast the clover and medic each into weedy winter wheat plots in the spring and found them to both significantly decrease grain yield compared to monocropped weedy wheat, and persian clover to significantly decrease straw yield. In a separate field study, under-sown white clover (*T. repens* L.) in weedy winter wheat, decreased weed DM by 36 %, in comparison with monocropped wheat. In addition, the author reported a trend of higher grain and straw yields when undersown with white clover.

Stewart *et al.* (1980) grew binary mixtures of four red clover cultivars and two white clover cultivars in association with one of three barley cultivars of varying straw length. Although there were higher proportions of clover in intercrops with red clover than white clover, and lower proportions of clover in intercrops with the long stemmed barley cultivar than the medium or short stemmed cultivars, the grain yield of monocropped barley did not differ from that of barley intercropped with any clover.

In a forage quality evaluation in Illinois, Roberts *et al.* (1989) examined wheat-hairy vetch intercrops fall seeded at four vetch seedling rates (0, 54, 108, and 162 (PLS) pure live seeds m^{-2}) which were harvested at three different wheat stages (boot, anthesis and milk). Intercropped shoot DM yields were found to increase with later harvests and to decrease with increasing vetch seeding rates; monocropped wheat and the intercrop with vetch seeding rate of 162 PLS m^{-2} yielded 7.3 and 6.5 Mg ha^{-1}, respectively. By increasing vetch seeding rates, the crude protein (CP) concentration of the intercrop increased: monocropped wheat was 12[illegible] g kg^{-1} DM while the CP concentration of the mixture at the highest vetch seeding rate was 161 g kg^{-1} DM. As well, the CP concentration of the wheat in intercrop increased with increasing vetch seeding rate; at the highest vetch seeding rate, wheat CP of 129 g kg^{-1} DM was higher than that intercropped with vetch at 54 PLS m^{-1} (117 g kg^{-1} DM).

Tidwell *et al.* (1985) investigated the quality of winter wheat-hairy vetch intercrops for spring harvested forages in Indiana. The study included two winter wheat cultivars monocropped or intercropped with vetch at two locations, harvested at five wheat maturity stages ranging from boot to soft dough. Although, the monocropped DM yield of each cultivar, averaged over each harvest, was not different from the average intercrop yield, the CP content of the intercrops harvested at milk and soft dough stage were 1.81 and 3.06 percentage units higher than monocropped wheat. Lunnan (1988) examined barley for forage production in Norway through intercrops with yellow lupin (*Lupinus luteus* L.), field bean (*Vicia faba* L.), three field pea cultivars (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.) at 60 and 120 kg N ha^{-1}. Plots were harvested at the barley dough stage. Nitrogen yields of intercrops with forage pea and vetch, averaged over six sites and three years, 146 kg ha^{-1} and 143 kg ha^{-1}, respectively, were higher than those of all other intercrops. The N yield of monocropped barley (85 kg ha^{-1}) was lower than the N yield of the intercrops, with the exception of the lupin intercrop (91 kg ha^{-1}). As well, the protein concentration (%) of mixtures including forage pea and vetch (13.2 and 12.9 %, respectively) were significantly higher than that of monocropped barley (7.4 %), or the other legumes. Of the legumes, forage pea and vetch were most competitive with barley, producing 43.7 and 39.1 % of the intercrop DM yield, and as such were significantly lower in percent weed DM than monocropped barley and the other mixtures. Barley intercropped with vetch or forage pea were disadvantageous as they resulted in the highest percent lodging.

Joost (1989) examined winter annual clovers intercropped with annual ryegrass during two field seasons in Baton Rouge. The following clovers were included in the study: two ball clover cultivars (cvs.) (*T. nigrescens*), two berseem clover cvs. (*T. alexandrinum*), three crimson clover cvs. and persian clover. Their productivity was compared with monocropped ryegrass fertilized with different N rates. While monocropped ryegrass at N rates of 168-224 kg N ha^{-1} yielded the highest DM, the 0 kg N ha^{-1} monocropped ryegrass yielded lower than all of the mixtures, with the exception of ryegrass with ball clover. All fertilized monocropped ryegrass treatments and all intercrops, with the exception of one ball clover cv., yielded higher in total CP than monocropped ryegrass at 0 kg N ha^{-1}. Of the intercrops, only multicut berseem clover yielded significantly higher in total N (812 kg ha^{-1}) than at least one of the fertilized monocropped ryegrass treatments (N fertilizer rate of 112 kg ha^{-1}, yielded 533 kg ha^{-1}). There was no evidence of N transfer from the legume to the associated grass during the growing season as, in none of the mixtures was the grass N yield higher than that of the 0 kg N ha^{-1} monocropped ryegrass treatment.

2.2. Grass response to legumes in mixture:

Legumes as components of forage swards contribute symbiotically fixed atmospheric N and they often increase grass yields (Ta and Faris 1987a). As grass herbage production is limited mainly by N availability (Burity *et al.* 1989), this increase in yield has been attributed to the transfer of symbiotically fixed N to the associated grass. Nitrogen transfer may be the result of its direct excretion from the legume, and its release by decomposition of the legume root system (Burity *et al.* 1989; Boller and Nosberger 1987). Such transfers may partly substitute N fertilizers (Brophy *et al.* 1987).

From 1973 to 1979, LLoyd and Hilder (1985) examined the benefits of barrel medic (*Medicago truncatula* Gaertn.) to a summer growing perennial grass, *Panicum coloratum*, through the application of 5 levels of N fertilizer (0, 50, 100, 200 and 400 kg ha^{-1}) by comparing the monocropped grass and grass/medic intercrops. In addition to N fertilizer increasing grass DM production, at the low N levels the medic increased grass DM production over monocropped grass during the latter part of the experiment. Examining the means of DM production and N uptake over the six years, it was found that the medics increased DM production and N uptake of grass in treatments of 0, 50, and 100 kg N ha^{-1} $year^{-1}$. At, 0 kg N ha^{-1}, medic increased grass DM from 2900 kg ha^{-1} $year^{-1}$ (monocropped) to 5500 kg ha^{-1} $year^{-1}$. Grass N concentration, at all N levels, was higher when intercropped. Medic DM yields were lower at the higher N levels, as illustrated in 1974-75, monocropped medic biomass yields at 0, 50, 100, 200 and 400 kg N ha^{-1} were 4.3, 4.0, 4.1, 2.8, and 1.8 t ha^{-1}, respectively.

Clarkson *et al.* (1987) examined the production of the winter annual legumes *Medicago scutellata* and *Medicago truncatula* monocropped and intercropped with summer growing grasses. Yields of the monocropped medics were higher than medic intercropped at two sites, and medic yields at the site which experienced low summer rainfall were not affected by grass competition. At sites receiving irrigation, grass yields in association with medic were twice that of monocropped grass, an effect equivalent to the application of 100 kg N ha^{-1} $year^{-1}$. On the most responsive dryland site, grass yields were also doubled when intercropped. Here, the medic effect was equivalent to N applications between 50 and 100 kg ha^{-1} $year^{-1}$. On the high fertility soils, grass yields when intercropped were increased by 25 %, equivalent to an N application of less than 50 kg ha^{-1} $year^{-1}$. In general, during the summer the authors found grass N concentration to increase when intercropped with medics or when N was applied.

Evans *et al.* (1990) evaluated eight white clover varieties with perennial ryegrass (*Lolium perenne* L.) under rotational sheep grazing over three years and found clover based swards to consistently outyield grass monocultures each year and to be, on average, 50 % more productive. While the authors attributed most of this effect to a direct clover contribution, the clover biomass yield, there was evidence of an indirect clover contribution to grass yield as an increase in grass yield grown in mixture over monocropped grass. This indirect increase was greatest during the spring of the second and third year. At these times, the clover variety Nesta indirectly contributed approximately 0.5 t ha^{-1} of extra grass biomass. Over the third year, Nesta's indirect contribution was equivalent to 54 kg ha^{-1} of nitrogen fertilizer. Lutwick and Smith (1977) observed the CP concentration of crested wheatgrass (*Agropyron cristatum* (L.)Gaertn.) to be greater when intercropped with alfalfa than when monocropped, as exemplified in the second year where non-N-fertilized monocropped grass was 7.9 % CP and intercropped grass was 12.2 %. Monocropped alfalfa yielded the highest in CP production and monocropped grass the lowest while the CP yield of the mixture was between these values. This ranking of protein yield is illustrated in the following 0 kg N ha^{-1} treatments of the first harvest year: monocropped alfalfa, 0.75 t ha^{-1}; monocropped grass, 0.17 t ha^{-1}; and intercrop, 0.50 t ha^{-1}. In an evaluation of crested wheatgrass (*Agropyron desertorum*), Russian wildrye (*Elymus junceus*) and pubescent wheatgrass (*A. trichophorum*) intercropped with sicklepod milkvetch (*Astragalus falcatus*), alfalfa, sainfoin (*Onobrychis viciifolia*) and crownvetch (*Coronilla varia*), McGinnies and Townsend (1983) found no differences in the CP content of the grasses when monocropped or intercropped.

Sollenberger *et al.* (1984) reported monocropped legume stands of alfalfa, red clover and birdsfoot trefoil contained markedly more weed DM than did stands intercropped with orchardgrass (*Dactylis glomerata* L.) or perennial ryegrass. To illustrate the extent to which the inclusion of grass decreased weed yield, the authors reported monocropped alfalfa averaged 1.0 t ha^{-1} of weed DM, while alfalfa-orchardgrass averaged only 0.05 t ha^{-1}. As well, higher legume seeding rates of monocrops also reduced weed yields. Jung *et al.* (1991) observed that in intercrops of perennial ryegrass and alfalfa, weed content was inversely related to the ryegrass seeding rate, but was not affected by alfalfa seeding rate.

2.3. Evaluation of intercrop efficiency:

To determine the effectiveness of an intercrop, the land equivalent ratio (LER) is the measure most frequently employed (Vandermeer 1989). The LER is based upon relative land requirements for intercrops versus monocrops. The LER, as described by Mead and Willey (1980): "represents the increased biological efficiency achieved by growing two crops together in the particular environment used". If one desired to produce two separate monocrops, the amount of monocropped land necessary to produce that which would be produced on one intercropped hectare is the LER (Vandermeer 1989). The LER is the summation of the relative yields, relative yield being a components yield in intercrop divided by its yield in monocrop. An LER which is higher than 1, indicates that monocrops use more land than an intercrop to provide an equal amount of crop yield (Hiebsch and McCollum 1987).

There can be problems in assessing the yield advantage obtained by intercropping, although if component crops are equally acceptable this is not complicated (Mead and Willey 1980). Simply evaluating the value of one intercrop over another by comparing LERs may not reflect their actual values as higher proportions of one component crop may be preferred even though resulting LERs are low (Mead and Willey 1980). Hiebsch and McCollum (1987) argue that as an intercrop often occupies land for greater duration than monocrops, LER is often inadequate as time is not included in its calculation. To address this inadequacy they developed and area-x-time equivalency ratio (ATER). Ofori and Stern (1987) claim that this ATER has not been widely adopted.

Ofori and Stern (1986) intercropped cowpea (*Vigna unguiculata* L.) and two maize (*Zea mays* L.) cultivars at four N levels (0, 25, 50 and

100 kg N ha^{-1}) and observed a tendency for the N applications to decrease LER values. As well, maize was competitive for N as the N content of intercropped cowpea was lower than when monocropped and with increasing N levels the maize grain yields, either intercropped or monocropped, were increased while cowpea yields were decreased. The authors speculated that the reductions of seed yield of intercropped cowpea under heavy N applications were probably due to direct effects of shading by maize on cowpea DM production. As maize growth under heavy N applications was enhanced, LER values were lower due to reduced cowpea yields. In a separate study, Ofori *et al.* (1987) found the intercrop advantage of maize-cowpea DM, in terms of LER, to be lower (33-55 %) when plots received N fertilizer (25 kg N ha^{-1}) than when no N was applied (56-69 %).

Hiebsch and McCollum (1987) evaluated 182 legume/maize intercrop studies by comparison of reported LERs with calculated area x time equivalency ratios (ATER). The intercrop advantage, as determined by ATER, was found to be greatest when the maize was grown without adequate N. In ten of the surveyed experiments, which were under low N status conditions, the average ATER was approimately 1.3; 69 of the experiments were on soils of fairly high N supplying capacity and gave an average ATER of approximately 1.1; 103 of the studies received 42-270 kg N ha^{-1} and produced an average ATER of approximately 1.04.

The most common way that an intercrop will produce LER values greater than one is through the components complementary use of resources (Trenbath 1976). In intercrops of legume-nonlegumes the nonlegume will exploit available soil N while the legume is capable of obtaining N through N_2-fixation. It is on soils which are deficient in available N that legume components can be most useful and that high LERs may be obtained (Trenbath 1976).

2.4. Nitrogen and its fixation in general:

The gas N_2 constitutes 78 % of the earth's atmosphere, and in this form is unavailable to most higher plants. Nitrogen fixation by *Rhizobium* or *Bradyrhizobium*, sybmiotically associated with legumes, is one of the modes by which it is converted to plant usable forms of N (Tisdale *et al.* 1985). Nitrogen fixing organisms contain nitrogenase, an oxygen sensitive metalloenzyme. Nitrogenase can reduce N_2 to ammonia according to the equation: $N_2 + 8H^+ + 8e^- = 2NH_3 + H_2$ (Richards 1990).

Most of the N requirements of forage legumes can be supplied by biologically fixed-N_2 (Danso *et al.* 1988; Heichel and Henjum 1991). Based on the results of a ^{15}N dilution study, Cadish *et al.* (1989) concluded that the total amount of N derived from symbiotic N_2-fixation is based on herbage yield, N concentration in plant tissue, and the percentage of N derived from fixation. They reported herbage yield of several tropical legumes to be the most variable parameter and that it was adequate for ranking legumes in terms of N_2-fixation. Nitrogen concentration was the next most affected parameter, followed by the percentage of N derived from symbiotic fixation. In an intercrop of white clover, alfalfa and fescue, Danso *et al.* (1991) found the proportion of N derived from the atmosphere to vary little over harvests. They did find large variations in the amounts of N fixed between the legumes and harvests, the result of herbage yield differences. As pointed out by La Rue and Patterson (1981), symbiotic N_2-fixation cannot be considered free fertilizer, plants must provide photosynthate energy. As the nitrogenase enzyme requires energy, N_2-fixers prefer to use combined N if it is available (Sprent 1990).

The nitrogen in nature is a mixture of two stable isotopes, ^{14}N and ^{15}N. The natural abundance of ^{15}N in the atmosphere is 0.366 atom % $\pm$ 1.5 % relative (Fiedler and Proksch 1975). The chemical properties of ^{15}N and ^{14}N are almost identical, but $^{15}N_2$ will diffuse slightly more slowly than $^{14}N_2$ (Bergersen 1980). The half lives of the radioactive isotopes of N are too short for tracer studies dealing with plant systems and the stable isotope ^{15}N must be employed in N tracer experiments (Fielder and Proksch 1975).

In an analysis of atmospheric N_2, Bergersen (1980) found the natural abundance of ^{15}N to range between 0.362 and 0.368 atoms %. Delwiche and Steyn (1970) found the average ^{15}N content of clover (0.0008 atom % excess ^{15}N - compared to atmospheric N_2) to be significantly less than that of grass or soil which suggested that the grass N came primarily from the soil and that much of the clover N was from the atmosphere. The authors concluded that N_2-fixing legumes have an N isotope composition similar to the atmosphere while non-N_2 fixers have a composition similar to their growth medium. Studying various soils, Ledgard *et al.* (1984) found the ^{15}N natural abundance of the total soil N to increase with depth, while there was no such change in plant extractable N.

As defined by Fiedler and Proksch (1975), the number of ^{15}N atoms in the total amount of N atoms is referred to to as atom % or % abundance. When the ^{15}N abundance of natural N is subtracted from the ^{15}N % abundance of the sample, the ^{15}N atom % excess is obtained. The $^{14}N/^{15}N$ ratio of a system can be determined by mass spectrometric or emission spectrometric techniques on N_2 gas generated from the sample.

2.5. Effect of mineral N on legume nodulation and N_2-fixation:

Shomberg and Weaver (1992) studied the time required by arrowleaf clover (*T. vesiculosum* L.) roots to nodulate when exposed to three different temperatures in a growth chamber: 18, 32 and 25°C; two strains of effective *R. trifolii* and three rates of starter N fertilizer: 0, 0.5 and 1.0 mg N plant^{-1}. At 25°C the rate of 0.5 mg N had no effect on the time to nodulate while 1.0 mg N delayed nodulation by 1.5 days over the 0 mg N $plant^{-1}$ treatment. Under 18 and 32°C with application of 1.0 mg N, nodules were visible 10.5 to 11.5 days after inoculation while they were visible after approximately 8.5 days when no N was applied.

The N difference method was employed by Shomberg and Weaver (1990) to examine the N_2-fixation activity of arrowleaf clover when uninoculated or inoculated with two *R. trifolii* strains and grown in sand culture in a growth chamber at the following five N rates applied at seeding: 0, 14, 28, 42 and 56 mg N pot^{-1}. Plants were harvested 40 days after planting and it was determined that low rates of N fertilizer aided in N_2-fixation. The amount of N_2 fixed at the 14 N level was twice that of the N_2 fixed at the 0 N level. Although the amounts of N_2 fixed at the 28 N and 42 N levels were higher than that at the 0 N level, the amount of N_2 fixed declined at levels over 14 N. The N contents of clover either inoculated with the strain RP114-2, or uninoculated, receiving 56 mg N were similar and therefore showed no evidence of N_2-fixation.

Through the application of ground rhodesgrass (*Chloris gayana* Kunth.) plant material, which was labelled with ^{15}N, to pot cultures of sirato (*Phaseolus atropurpureus* D.C. var. Sirato), Henzell *et al.* (1968) were able to determine that it was at the time of early nodulation that sirato relied most highly upon soil N. By determining the enrichment of sirato harvested over 15 weeks and that of rhodesgrass to indicate the enrichment of the soil N, they were able to separate sirato's symbiotically fixed-N_2 from soil derived N. The authors observed the time of the beginning of effective nodule formation, at three weeks, to correspond with the time that soil N contributed most to sirato N. In monocropped sirato and sirato intercropped with rhodesgrass, 50 and 43 % of sirato's N, respectively, was of soil origin while after 15 weeks, only 4 and 2 % of sirato's N was of soil N origin.

The effect of two concentrations of N (5 and 10 Mm) as either urea, nitrate, ammonium, and nitrate + ammonium on the nodulation and N_2-fixation, as determined by acetylene reduction assay (ARA), was reported by Rongqing *et al.* (1992) on the following legumes: faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.), and medic (*Medicago rugosa* Desr. cv. Paraponto). All forms of N were found to negatively affect the number of active nodules of all crops, with the medic most severely affected and the highest N concentration having the greatest effect. Nodule initiation was also delayed by most N treatments. At the first sampling date, day 15, medic which had received either N concentration had no active nodules while the control medic had 5. Nitrogenase activity was also depressed at all N treatments, with NH_4Cl being most depressive and urea, the least.

In an ARA study of barrel medic (*Medicago truncatula* Gaertn.) grown in pots, N_2-fixation was found to be negatively correlated with mineral N and to not be related to the total N content of the soils (Alston and Graham 1982).

In examining N_2-fixation, by the ^{15}N isotope dilution (ID) method, of different clover cultivars in ryegrass-clover swards, Ledgard *et al.* (1990) found a large decrease in the proportion of N_2-fixed due to an increase of inorganic soil N resulting from dry soil conditions. They concluded the clover substituted N from fixation with the uptake of soil N. Heichel and Henjum (1991) found the percent N derived from the atmosphere of legumes in mixture with reed canarygrass (*Phalaris arundinacea* L.) over four years to increase with stand age and believed this occurrence to be related to the depletion of soil N. Studying N_2-fixation, by the ID method, of swards of white clover, red clover and birdsfoot trefoil, each combined with tall fescue (*Festuca arundinacea* Schreb.), Mallarino and Wedin (1990) found N fertilizer to temporarily increase herbage yield. With 100 kg N ha^{-1} there was a lengthy period during which the proportion of legume decreased. The percentage of nitrogen derived from the atmosphere was decreased for a number of weeks after the fertilization application while there was a lengthy decrease in fixed N yields resulting from the decrease in legume yield. When the N treatment was applied to established swards annual fixed-N yields were reduced from 178 to 148 kg ha^{1} while N applications at seeding reduced annual fixed-N yields from 65 to 29 kg ha^{-1}.

Using the ^{15}N natural abundance technique, Ofori *et al.* (1987) found that, whether monocropped or intercropped with maize, two thirds of the N of field cropped cowpea was derived from N_2-fixation. In the greenhouse portion of the study, the authors found monocropped cowpea to have derived less N from the atmosphere (42-50 %) than that of the field study, and speculated that this may have been in response to higher rates of availability of mineralized N. In the greenhouse, % N_2-fixed values as determined through the application of ^{15}N-urea (25 kg N ha^{-1}), were lower than those calculated by the natural abundance technique. The authors had expected such a response due to the negative effects of combined N on N_2-fixation. In the field, this response only occurred in monocropped cowpea, and not in the cowpea-maize intercrop. McAuliffe *et al.* (1958) grew ladino clover (*T. repens* var. *latum*) and tall fescue together in a pot study and observed the application of N, up to 224 kg ha^{-1}, to established plants to not increase the total (of three cuts) N yield. The authors explained this lack of response by the occurrence of N-uptake by clover accompanied by a decrease in N_2-fixation. On a Cecil clay, clover, which had received 28 kg N ha^{-1}, derived 65 % of its N from N_2-fixation in cut 1, while clover which had received 224 kg N ha^{-1} (as a solution of $(N^{15}H_4)_2SO_4$) derived only 10 % of its N from N_2 fixation. Over time, the clover increased its reliance on fixed N_2 in response to an apparent reduction of soil N. By the time of the second cut, at the N rate of 224 kg ha^{-1}, the clover derived 43 % of its N from N_2-fixation and at cut 3, 75 %, while clover at the 28 kg N level derived 91 and 93% of its N from N_2-fixation at cuts 2 and 3, respectively.

2.6. Principles of the ^{15}N isotope dilution (ID) method:

In field studies, the ^{15}N ID method can give an integrated value of N^2-fixation, and it is the only method that can determine N contributions to the plant from soil, fertilizer and atmosphere (Danso 1986). The ^{15}N ID method is based upon the comparison of an N_2-fixing and non-N_2-fixing system. The possible N sources of these systems are: fertilizer, soil, and in the case of the fixing system, the atmosphere. The N in the plants derived from the soil or fertilizer will be enriched in ^{15}N and in the legume, fixed atmospheric $^{14}N_2$ will dilute it. The non-fixer is required to determine the amount of N derived from the soil and fertilizer (Rennie and Rennie 1983).

In a pot study, McAuliffe *et al.* (1958) used ^{15}N labelled fertilizer to determine the ratio of absorption of fixed to applied N. Ladino clover and alfalfa were each grown with tall fescue as a reference plant. They used the following formula to determine the % N in the legume from non-fixing sources: unfixed N = (atom % excess ^{15}N in the legume / atom % excess ^{15}N in the grass) x 100. The % N fixed by the legume equals the above value subtracted from 100. Their work was based on the assumption that the legume and grass absorbed the same proportion of soil and fertilizer N and, that the legume did not release fixed N to the grass.

2.7. The ^{15}N isotope dilution (ID) method compared to other methods:

By exposing a legume to $^{15}N_2$ in a contained system one can directly measure the amount of N_2-fixed. McNeill and Wood (1990) exposed white clover, intercropped with perennial ryegrass, to $^{15}N_2$, and were able to detect N_2-fixation as the clover had a higher ^{15}N content than the grass. Studies involving such systems are not practical in field studies. The

^{15}N ID method provides a direct measurement of N_2-fixation which lends itself to field studies. With this technique, the soil N is enriched with ^{15}N and the rate at which the plant ^{15}N is diluted gives an estimate of N_2-fixation (Bergersen 1980).

In a pot experiment involving alfalfa and meadow fescue, Martensson and Ljunggren (1984) concluded that when estimating the total amount of N fixed by a legume, the ^{15}N ID method may be preferable to the acetylene reduction assy (ARA) as it integrates diurnal and plant phenological variations. They found the difference method to be comparable to ^{15}N ID. Rennie (1979) compared the ^{15}N ID method and the classical total N difference method (N fixed = total legume N - total non-legume N) for calculating N_2-fixation of navy bean (*Phaseolus vulgaris*) and reported the ^{15}N ID method yielded the most logical estimation. Contrary to Martensson and Ljunggren's (1984) findings, the difference method was not in agreement. Talbot *et al.* (1982) also concluded the ^{15}N ID method to be more accurate than the difference method. They found the total N accumulation in soybean to be more variable than ^{15}N concentrations.

A two year field study on the N_2-fixation of bean (*Phaseolus vulgaris* L.) by Rennie and Kemp (1984) found the ARA to severely underestimate N_2-fixation compared to that of the ^{15}N ID method. They concluded the ARA to be at a disadvantage as it is a measure of nitrogenase activity only at the time of sampling while the ^{15}N ID method estimate is integrated over time.

In an irrigation and fertilizer study of N_2-fixation by white bean (*Phaseolus vulgaris* L.) and soybean, estimated by ARA, Smith and Hume (1985) explained their results due to the underestimation of N_2-fixation by ARA. In a comparison of N_2-fixing estimates for white bean and soybean, Smith and Hume (1987) reported no difference between the difference method and the ^{15}N ID method, while estimates by the ARA were lower for both crops. The authors reported ARA N_2-fixation estimates of field grown soybeans, soybeans grown in a growth room and white bean grown in a growth room to be half those of the difference method while ARA values of white bean grown in the field were only 10 % that of difference method and ID method. Legg and Sloqger (1975) compared the estimates of soybean N_2-fixation by the ARA, the difference method and the ID method and also found the ARA estimates to be much lower than those of the other methods.

In a ^{15}N ID study on soybean N_2-fixation, Rennie (1982) concluded that there is potential in using naturally occurring ^{15}N as it has the advantage of a greater degree of soil N homogeneity, although it is easier to detect isotopically labelled N. Ofori *et al.* (1987) found good agreement, in a field and greenhouse study, between the ^{15}N natural abundance and ^{15}N ID method in the estimation of N_2-fixation of mono-cropped cowpea and cowpea intercropped with maize. The authors do state that a highly sensitive mass spectrometer is required when employing the natural abundance method. In a greenhouse study on N fertilizer uptake, Meints *et al.* (1975) also compared the two techniques and reported that unenriched fertilizer N underestimated the amount of fertilizer N taken up by sudangrass and that the standard deviations of these estimates were larger. In conclusion they stated that methods based on natural abundance could at best be termed roughly quantitative.

2.8. Nitrogen transfer:

The excretion of N compounds by plants, as stated by Wilson and Wyss (1937), is known to occur. In an enclosed root chamber study Brophy and Heichel (1989) found the sum of N released to the root zone of sand cultured soybean roots (harvested after 65-80 days) to be 10.4 % of total plant N while alfalfa, over 173 days, released 4.5 %. Under water deficit conditions and shoot harvest, the authors noted an increase in released N.

Lory *et al.* (1992) quantified the deposition of symbiotically fixed-N_2 into the soil surrounding monocropped alfalfa roots and nodules resulting from excretion or leakage from roots and nodules, or from the sloughing off of root cells. The authors employed the ^{15}N ID method in a field and greenhouse study to compare the ^{15}N concentration of the rhizosphere of effectively nodulated alfalfa with that of an ineffectively nodulated alfalfa. In the field study, plants were harvested three times over 135 days. There was no evidence of deposition of symbiotically fixed N_2 to the rhizosphere (soil adhering to roots), atom % ^{15}N and soil N concentration was similar for the effective and ineffective alfalfa. In the greenhouse study, there was evidence that over two harvests (112 days) 1 kg of fixed N_2 was deposited into the rhizosphere at the 0-15 cm depth. In both the field and greenhouse, there was evidence of deposition of symbiotically fixed N_2 into the nodusphere soil (soil adhering to nodules). In the field and greenhouse these values were 0.1 kg ha^{-1} and 0.4 kg ha^{-1}, respectively. The authors questioned the validity of these values due to the possibility of leakage resulting from the use of the sonicator in soil removal.

Vallis *et al.* (1967) suggested that when a legume transfers N, which originated from the soil, to an intercropped grass, the grass receives no practical benefit. Ta *et al.* (1986), examined the root excretions, defined as substances lost through root epidermis, of hydroponically grown alfalfa. Once the plants reached the 10 % bloom stage, they were exposed to an environment enriched in $^{15}N_2$. It was determined that the nodule-root system excreted 15 ug N $plant^{-1}$ day^{-1}, approximately 3 % of the total daily fixed N. As the hydroponic solution was enriched in ^{15}N only one day after the plants were exposed to $^{15}N_2$, it was determined that recently fixed N_2 contributed to the excreted N. As stated by Wahua and Miller (1978), the fact that legumes may fix N_2 which could become available to associated non-legumes, is one reason for intercropping.

In ^{15}N ID methods of calculating N transfer, the enrichment of the non-N_2 fixer intercropped with a legume and that of a monocrop of the non-N_2 fixer are compared. In intercrop, the extra N resulting from transfer will dilute added ^{15}N of the non-N_2-fixer in proportion to the amount of N transferred (Vallis *et al.* 1977). Vallis *et al.* (1967) first used ^{15}N labelled fertilizer to study N transfer from legumes to grass while studying the N economy of a rhodes-grass/Townsville lucerne (*Stylosanthes humilis* H.B.K.) mixture. Although their pot study was unable to detect significant transfer of unlabelled N, the authors found evidence of N sparing as two grass plants grown with two alfalfa plants each received almost half of the available N while four grass plants grown together each received 1/4 of the available soil N. It was speculated that the legumes were unable to compete for available soil N.

As pointed out by Brophy *et al.* (1987) the ^{15}N ID method can only provide information on the N transferred from unlabelled sources. Any uptake by the non-fixer of unlabelled N other than that originating from the atmosphere can result in errors. When the ^{15}N ID method is used to measure the % N of a non-legume obtained from transfer, one assumes that the dilution of ^{15}N in the non-fixer intercroped with an N_2-fixing crop, compared to that of the non-fixer in monocrop, is a result of the transfer of fixed N_2 from the legume (Boller and Nosberger 1988).

Boller and Nosberger (1988) found that intercropped grasses appeared to have deeper root systems than those monocropped. When ^{15}N fertilizers are spread on the soil surface they suggested this difference would result in an underestimation of N transferred from white clover. In 1 year of a four year study, Heichel and Henjum (1991) found white clover to transfer more N (53 kg N ha^{-1}) to associated reed canary grass during one season than the actual amount of fixed N in white clover plants in two combined years (approximately 10 kg N ha^{-1}). This suggested to the authors that the root systems may have stimulated the release of soil organic N.

Hardarson *et al.* (1988) studied monocropped and intercropped perennial ryegrass to measure the N_2-fixation of alfalfa in a ^{15}N ID study and found the atom % ^{15}N excess of the intercropped ryegrass to be slightly, but not significantly, lower than that of the monocropped grass in the second harvest. They suggested this may have been due to an insignificant uptake of N derived from the atmosphere by the intercropped grass. They used the following formula to estimate % N transferred: 1-(atom % ^{15}N excess of intercropped ryegrass / atom % ^{15}N excess in monocropped ryegrass) X 100. The amount of N transferred (kg ha^{-1}) was then calculated as: (% N transferred / 100) x total N in intercropped ryegrass. In the second harvest, only 4 kg N ha^{-1} was estimated to have been transferred.

Results of a ^{15}N dilution field study by Eaglesham *et al.* (1981) showed that in zero and low N fertilizer treatments (25 kg N ha^{-1}), maize intercropped with cowpea was significantly higher in N, in terms of mg N $plant^{-1}$ and % N content, than monocroped maize. At the 25 kg N ha^{-1} treatment the ^{15}N concentration of intercroped maize was significantly diluted compared with intercropped maize, this suggested N excretion by cowpea. The fact that the components of the intercrop absorbed a similar amount of N fertilizer ruled out any possible N sparing by the cowpea. The ^{15}N concentration of intercroped maize was not diluted in the 100 kg N ha^{-1} treatment, and as ARA activity was reduced and nodulation reduced at the flowering stage, the high rate of N was believed to have inhibited nodulation. The authors concluded that N excretion by a legume will be of significant benefit to an associated crop, only under conditions of low mineral soil N. Martin *et al.* (1991a) also observed that N transfer, as determined by ^{15}N ID, was best demonstrated under conditions of low mineral N. Only on an N depleted soil, with one exception, were the authors able to detect the transfer of N from nodulating soybean to non-nodulating soybean and maize. In the 1988 growing season, the nodulated soybean, seeded at 33 % of the monocrop rate, on N-depleted soil transferred 3.68 kg N ha^{1} to the associated tall maize cultivar, seeded at 67 % of the monocrop rate. Also on N-depleted soil, N transfer was evident from nodulated soybean, seeded at 67 % of the monocrop rate, to associated non-nodulated soybean, seeded at 33 % of the monocrop rate, and was determined to be 1.62 kg N ha^{-1}. On non-N-depleted soil the nodulated soybean, seeded at 67% of the monocrop rate, transferred 3.29 kg N ha^{-1} to associated non-nodulated soybean, seeded at 33% of the monocrop rate.

Boller and Nosberger (1987) also found isotopic evidence of the field transfer of fixed N_2 from white clover to perennial ryegrass and of red clover to Italian ryegrass. Plants were fertilized with 0 kg N ha^{-1} or 30 kg N ha^{-1} at each cut. While N fertilizer had little effect on the % N derived from fixation, it resulted in a decrease in the % N transferred from clover to grass. In the seeding year of 1983, white clover, at 0 kg N ha^{-1}, transferred 11 kg N ha^{-1} to the associated grass, in N fertilized plots there was no evidence of transfer. During this season, red clover transferred 42 kg N ha^{-1} to associated grass receiving no N fertilizer, and 31 kg N ha^{-1} to grass in N fertilized plots.

A field study by Patra *et al.* (1986), employing the ^{15}N ID method, indicated that 28 % of the total N uptake of maize (21.2 kg N ha^{-1}) intercroped with cowpea was of atmospheric origin, the result of N transfer from cowpea.

Using the ^{15}N ID method, Mallarino *et al.* (1990) concluded that white clover, red clover, and birdsfoot trefoil each intercropped with tall fescue, transferred small amounts of N to the tall fescue shortly after seeding and that with time the amount transferred increased significantly. In year one of the experiment established in 1983, the total N values estimated to have been transferred to tall fescue from white clover, red clover and birdsfoot trefoil were 14.7, 12.8 and 9.9 kg ha^{-1}, respectively, and in the second year were 31.1, 23.3 and 20.6 kg ha^{-1}, respectively. With an increase in the legume component of mixtures, the authors reported that although the concentration of legume derived N in the tall fescue increased, the actual amount transferred was not affected.

In a ^{15}N dilution field study, Burity *et al.* (1989) observed that when either timothy or bromegrass (*Bromus intermis* Leyss.) was grown with alfalfa, the % of alfalfa N in the grasses which was derived from N_2-fixation increased throughout the growing season. They concluded that the amount of N transferred before the first cut was due to the direct excretion of N compounds, while transfer in later cuts was a result of decomposing nodules and roots. In a ^{15}N dilution study carried out by Ta and Faris (1987a), it was noted that in intercrops of alfalfa and timothy N transfer increased with increased proportions of alfalfa as well as increasing with progressive cuts. This led them to suggest that transfer may have been the result of direct excretion of N as well as the decomposition of alfalfa roots and nodules.

Brophy *et al.* (1987), employing the ^{15}N ID method, observed an increase in the % of reed canary grass N derived from transfer from intercroped alfalfa and birdsfoot trefoil as the season progressed. They suggested this increase was due to greater root mingling, greater fixation of the legume, and an increase in the depletion of available soil N.

In a greenhouse study, Ledgard *et al.* (1985) labelled subterranean clover with ^{15}N through a foliar absorption technique and found 2.2 % of the labelled N had transferred to the associated annual ryegrass over 29 days. In a similar study in the field they observed no such transfer from either subterranean clover or alfalfa. They concluded the greater contact of the two roots in pots, would make it easier for any N released by the legume to be absorbed by the grass.

Van Kessel *et al.* (1985) devised a soybean split root technique to detect N transfer to intercroped maize. ^{15}N labelled ammonium sulfate was applied to a pot containing only half of the soybean root. The other half of the root was grown with maize, and half of the pots including the maize were inoculated with VAM fungi. As the atom % ^{15}N excess of VAM-infected maize plants was higher than that of the non-VAM-infected maize, it was concluded that the VAM fungi enhanced N transfer from the soybean. Using the ^{15}N dilution method in a field study of intercropped maize and soybean, Hamel *et al.* (1990) found no indication of N transfer from the legume to cereal when plots were inoculated with an endomycorrhizal fungus, nor was there any transfer when plots were uninoculated. The authors did observe a 55 % increase in the N_2-fixation rate of soybean when plots were inoculated with the fungus, and that in a number of cases the % N derived from the atmosphere in the maize was a negative value. The authors suggested that these negative values could be due to the effects of N sparing. Due to the competition of the intercropped grass for N, the nodulating soybean could have relied more on their N_2-fixing ability. This reliance on N_2-fixation would have made a larger pool of ^{15}N available for the associated corn, thus resulting in a larger uptake of ^{15}N by the maize intercroped with nodulating soybean than that of the maize intercropped with non-nodulating soybean.

In a separate intercrop field study, Hamel and Smith (1991) employed a direct ^{15}N labelling method which demonstrated N transfer from nodulating soybean to maize. Also, this transfer was observed to be greater when plots were inoculated with mycorrhiza as opposed to non-inoculated plots.

As pointed out by Hamel *et al.* (1990), the N sparing effect will result in a higher ^{15}N concentration of the maize while N transfer from the legume to maize will dilute the ^{15}N concentration of the maize and result in a lower ^{15}N concentration. These two effects will cancel each other out if they are approximately equal. This is a potentially serious drawback of the ^{15}N ID method as the N transfer effect may be hidden. Direct labelling methods do not have this potential problem.

By applying ^{15}N fertilizer in solution directly to the petiole of nodulating soybean in a field study, Martin *et al.* (1991b) were able to detect significantly higher ^{15}N levels in adjacent maize than when unenriched N was similarly applied. They also directly labelled soybean roots but found no evidence of N transfer to associated maize plants. In a greenhouse study employing the split root technique, they were able to detect transfer to intercropped maize and non-nodulated soybean. The authors suggest that although the ^{15}N transferred was not derived from N_2-fixation they felt it could be assumed that a similar transfer could take place with fixed N.

2.9. Hypotheses:

The overall hypothesis of this research was that the intercropping of annual legumes with barley would produce herbage of higher biomass and of higher protein yields than that of the component crops monocropped on the same land area.

The specific hypotheses tested, were that:

1. the annual species included in the study: the annual medics, Persian clover, hairy vetch and cv. Nitro alfalfa, would be more productive than the perennial red clover.
2. with increasing N-fertilizer levels, the biomass and protein yields of monocrops and intercrops would increase.
3. within intercrops, the ^{15}N dilution method would give evidence of N-transfer from the legumes to barley during the growing season.
4. the labelling of intercropped legume petioles with ^{15}N would result in the transfer of labelled N to barley and associated legumes.
5. the proportion of N derived from the atmosphere in intercropped legumes would be higher than that of monocropped legumes.

2.10. Objectives:

The objectives were:

- to compare herbage biomass yields of barley and annual legumes under monocropped and intercropped systems.

- to evaluate the effectiveness of intercropped systems, at three N-fertilizer levels, through comparison of LERs.

- to compare herbage CP yields and concentrations of barley and annual legumes under monocropped and intercropped systems.

- to evaluate the effects of three N-fertilizer levels on herbage biomass yields and protein concentrations and yields of barley and annual legumes under monocropped and intercropped systems.

- to determine the agronomic potential of annual legumes, especially annual medics, under growing conditions in Truro, N.S.

- to determine if N-transfer from legume to barley occurs during the growing season with the ^{15}N dilution method, and if it does, to quantify the amounts transferred.

- to assess legume-N-transfer to barley by directly labelling legume petioles with N enriched in ^{15}N.

- to evaluate the N_2-fixation capabilities of annual legumes by the ^{15}N isotope dilution method, within monocropped and intercropped systems.

Preface to Section 3

Section 3 consists of a manuscript by Sampson *et al.* (1993a) to be submitted to Journal of Production Agriculture. The manuscript will undergo further editing prior to submission. Tables are presented on the page, or pages, immediately following the page in which they are first referred to. References cited in this section are listed within the thesis reference section.

The experiment presented in this section compares the herbage and crude protein production of barley, six annual forage legumes and the perennial, red clover (*Trifolium repens* L.) under monocropped and legume-barley intercropping systems and evaluates the effect of three levels of N-fertilizer on the cropping systems.

Section 3

THE BIOMASS AND PROTEIN PRODUCTION OF ANNUAL FORAGE LEGUMES MONOCROPPED AND INTERCROPPED WITH BARLEY (*Hordeum vulgare* L.)

3.0. Abstract:

In this study, the annual legumes persian clover (*Trifolium resupinatum* L.), hairy vetch (*Vicia villosa* Roth.), Nitro alfalfa (*Medicago sativa* L.), *M. ciliaris* (L.) Mill., *M. scutellata* (L.) Mill., *M. polymorpha* L. and the perennial, red clover (*Trifolium pratense* L.) were monocropped (MC) and intercropped (IC) with barley. The experiment, seeded in 1991 and 1992 at Truro, N.S., was a randomized complete block design in a split plot arrangement with four replicates. Three main plot units were comprised of three N levels: 0, 30 and 60 kg N ha^{-1}. Subplots within main plot units were in a 2 x 7 factorial with two levels of cropping systems: MC-legumes and legumes IC with barley, and the seven legume species. One MC plot of barley was included in each main plot unit. The hypotheses were that the annual species would be more productive within one growing season than red clover and, that increasing N fertilizer levels would increase herbage dry matter (DM) and crude protein (CP). Three legume harvests were taken each season but barley was harvested only in the first cut. In neither year were total DM (TDM) and estimated total CP (ETCP) yields of red clover, monocrops or intercrops, lower than the rest of the MC legumes or intercrops, with an exception in 1992, where ETCP yield of red clover intercrops was lower than intercrops of vetch. In 1991, the TDM of intercrops responded positively to 30 kg N ha^{-1} while 60 kg N ha^{-1} appeared to have a negative effect on TDM. In neither year did the ETCP of MC-legumes or intercrops respond to N levels.

3.1. Introduction:

Legumes, as components of forage swards, contribute symbiotically fixed atmospheric N and they often increase grass yields (Ta and Faris 1987a). On irrigated sites, Clarkson *et al.* (1987) found the inclusion of *Medicago scutellata* (L.) Mill. and *Medicago truncatulata* Gaertn., in mixture with summer growing grasses, to double grass yields, an effect equivalent to the application of 100 kg N ha^{-1}. Joost (1989) examined winter annual clovers intercropped (IC) with annual ryegrass (*Lolium multiflorum* Lam.) and reported that while monocropped (MC) ryegrass at N fertilizer rates of 168 and 224 kg ha^{-1} yielded highest in total seasonal dry matter (DM) (5958 and 6496 kg ha^{-1}, respectively), MC-ryegrass with 0 kg N ha^{-1} (0 N) yielded lower (3013 kg ha^{-1}) than all intercrops, with the exception the intercrop of ball clover (*Trifolium nigrescens* Viv.). Although in none of the intercrops was grass N yield higher than that of 0 N MC-grass (252 kg N ha^{-1}), all N fertilized MC-grass treatments and all intercrops (812 to 450 kg N ha^{-1}), with the exception of the ball clover intercrop, yielded higher in total N yield than 0 N MC-grass.

In Prince Edward Island, Kunelius and Narasimhalu (1983) found 0 N binary mixtures of persian clover (*T. resupinatum* L.), red clover (*T. pratense* L.), alfalfa (*M. sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.), each with Italian ryegrass (*Lolium multiflorum* Lam.), produced DM yields which were, respectively, 84, 72, 63 and 50 % that of MC-grass fertilized with 225 kg N ha^{-1} over the season. These same mixtures resulted in N yields which were, respectively, 91, 81, 73, and 49 % that of N fertilized MC-grass.

Compared with studies involving legume-grass mixtures, reports on legume-cereal intercropping are limited. Such studies have examined the cereal component grown for grain and forage production. In a pot study, installed in a winter wheat (*Triticum aestivum* L.) field in Vienna, Hartl (1989) monocropped and intercropped winter wheat with a specic group of weeds, black medic (*M. lupulina* L.) and persian clover and found a trend of more grain when winter wheat was intercropped with black medic (approximately 22.5 g pot^{-1}) than when monocropped (21.5 g pot^{-1}). Wheat straw yield, when intercropped with medic was significantly higher than that of weedy wheat and weed DM of weedy wheat intercropped with medic was reduced by 55 % compared to weedy wheat. Grain yield when intercropped with persian clover (20 g pot^{-1}) was slightly decreased, although not significantly, compared to MC-wheat and weed DM decreased by over 70 % compared to weedy wheat. Kunelius *et al.* (1992) found barley grain yields to be reduced when sown in small plots (2 m x 5 m) in alternating rows with either westerwolds ryegrass or Italian ryegrass (*L. multiflorum* Lam.). Grain DM was most severely reduced by westerwolds ryegrass 'Aubade', producing only 71 % of monocropped grain yield. Red clover had the least effect on grain yield, allowing for 90 % of the monocropped yield.

As annual legumes complete their life cycle in one season, their growth, compared with that of a perennial, such as red clover, would be expected to be more vigorous during the establishment year. As such, annual legumes may compete more effectively in intercrops with cereals such as barley. Samson et *al.* (1989) interseeded red clover, at different times, into existing winter wheat fields and drilled several annual legumes into winter wheat in early May at two sites in Ontario. There were no significant differences in wheat yields between treatments on the silt loam site. On the sandy loam site, wheat interseeded with the early frost seeding of red clover (3.35 t ha^{-1}) was significantly lower than that interseeded with the late frost seeding of red clover (4.36 t ha^{-1}) and the drilled annual, crimson clover (*T. incarnatum* L.) (4.38 t ha^{-1}). Wheat yield when interseeded with drilled red clover (3.78 t ha^{-1}), Nitro alfalfa (3.98 t ha^{-1}) and hairy vetch (*Vicia villosa* Roth.) (3.82 t ha^{-1}) did not differ from any treatments. Of the legumes, hairy vetch produced the highest forage biomass on each site, yielding 3367 and 2554 kg ha^{-1} on the silt and sandy loam, respectively. On the silt loam, drilled red clover (678 kg ha^{-1}), Nitro alfalfa (890 kg ha^{-1}) and crimson clover (844 kg ha^{-1}) yielded significantly lower than the red clover frost seeded treatments. On the sandy loam, Nitro alfalfa (1440 kg ha^{-1}) and crimson clover (1426 kg ha^{-1}) yielded significantly higher than the red clover treatments.

Lunnan (1988) examined barley for forage production in Norway through monocrops and intercrops with yellow lupin (*Lupinus luteus* L.), field bean (*Vicia faba* L.), three pea cultivars (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.). Nitrogen yields of intercrops of forage pea and vetch (146 and 143 kg ha^{-1}, respectively), averaged over 6 sites and 3 years, were significantly higher than those of all other intercrops and MC-barley yielded significantly lower (85 kg ha^{-1}) than the intercrops, with the exception of the lupin intercrop (91 kg ha^{-1}). In addition, the protein concentration of intercrops including forage pea and vetch (13.2 and 12.9 %, respectively) were significantly higher than those of MC-barley (7.4 %) and the other legume-barley intercrops.

Grasses, as stated by Chestnutt and Lowe (1970), are adapted to succeed over clover with their earlier growth, deeper roots and more upright growth. As noted by Rhodes (1981), white clover (*T. repens* L.) will often appear drought tolerant when monocropped, but in mixtures, lacks this tolerance. Under conditions of limiting moisture, legumes adapted to low rainfall environments would be expected to be more effective as components of intercrops since they should be better able to withstand moisture competition. Annual medics are native to arid sites in North Africa and the Middle East (Brahim and Smith 1993) and in Australia are the main pasture legumes over approximately 50 million ha (Crawford *et al.* 1989). Annual medic pastures were developed mainly in Southern Australia's semiarid agricultural zones, Mediterranean type environments with annual rainfalls between 250 and 500 mm (Crawford *et al.* 1989).

In North America, the distribution of annual medics "is generally restricted to the southern states and the Pacific Coast west of the Cascade and Sierra Nevada mountain ranges" (Rumbaugh and Johnson 1986). In 1990, six annual medic species were brought from Turkey, their area of origin, with the objective of evaluating their productivity in monocrops under Truro, Nova Scotia conditions. Their forage yields and regrowth were higher than those observed in Turkey (Altinok, unpublished). Forage crops in Nova Scotia are frequently undersown with a cereal cover crop. Red clover and timothy (*Phleum pratense* L.) are, generally, recommended over the use of alfalfa and most other grasses (Anon. 1991b). Currently, the only recommended annual forage legume in Nova Scotia is persian clover, grown monocropped or intercropped with annual ryegrass for pasture or silage. Annual legumes, such as annual medics, intercropped with barley for forage production, may have potential for use in short term cropping systems.

The objectives in this study were to compare herbage and crude protein production of barley, three annual medic species, the annual, persian clover, the semi-dormant alfalfa cultivar 'Nitro' and hairy vetch, which grow as annuals in N.S. and the commonly grown perennial red clover, under monocrop and legume-barley intercrop systems; to evaluate the effect of three levels of N fertilizer cn cropping systems and to evaluate the effectiveness of these intercrop systems.

3.2. Materials and Methods:

This study was conducted in 1991 and 1992 at the Nova Scotia Agricultural College, Bible Hill, Nova Scotia, on a Truro sandy loam. Soil samples were collected on November 21, 1990 and on November 6, 1991 for analysis, by the Nova Scotia Department of Agriculture and Marketing Plant Industry Branch. Soil organic matter was 1.6% in 1990 and 2.1% in 1991. Soil pH was 6.2 in 1990 and 6.1 in 1991. Limestone was applied, as required, to raise soil pH to 6.4. On May 14, 1991, 1 t ha^{-1} of granular dolomitic limestone was applied and hand raked into the soil. On May 26, 1992, 2 t ha^{-1} of pelletized dolomitic limestone was applied and worked into the soil with an s-tyne cultivator.

Although monthly rainfall totals in 1991 did not vary greatly from their 30 year normals, the rainfall was not evenly distributed and parts of the growing season were relatively dry. Weekly totals from several weeks in June were well below the 30 year normals and 49 % of the total monthly rainfall fell in a single day (June 13) (Table 3.1.). Total monthly rainfalls in May, June, July, August, September, October and November were: 74.2, 64.0, 71.2, 146.1, 202.8, 128.0 and 165.5 mm, respectively (Anon. 1991c). Rainfalls in the same months of 1992 were: 44.2, 58.0, 68.8, 57.0, 84.7, 96.4 and 84.0, respectively (Anon. 1992).

The experiment was of split plots arranged in a randomized complete block design with four replicates. The three main plot units were comprised of three N levels (N) of 0, 30 and 60 kg N ha^{-1} (0 N, 30 N and 60 N, respectively). Subplots within main plot units were of a 2 x 7 factorial of two levels of cropping systems (C): monocropped (MC) legumes and legumes intercropped (IC) with barley (cv. Chapais) and seven legume species (Sp) (Table 3.2.). Each main plot unit contained one MC-barley plot. Plots were hand seeded on May 27 (replicates 1 and 2) and May 28 (replicates 3 and 4) in 1991; and on May 28 (replicates 1,2 and 3) and May 29 (replicate 4) in 1992. Pathways were seeded with annual ryegrass and trimmed throughout the season. Seed of several legume species was limited and therefore restricted the plot area to 0.288 m^{-2} (60 cm x 48 cm). Monocropped legume plots consisted of six rows, spaced 8 cm apart and were seeded to standard seeding rate recommendations (Table 3.2.). Intercropped plots were 3 rows of legume alternating with 3 rows of barley. Seeding rate of the IC-legumes was half that of the MC-legume plots as three legume rows were replaced with barley. Barley, monocropped and intercropped, was seeded in three rows at a rate of 400 seeds m^{-2}. Prior to seeding, vetch and the medic seeds were scarified and all legume seeds were inoculated with the appropriate strain of *Rhizobium* (Table 3.2.).

Table 3.1. 1991 and 1992 rainfall data (mm) for the last week of May and month of June.

Dates	Fri.	Sat.	Sun.	Mon.	Tues.	Wed.	Thurs.	Week Tctal	30 Year Normal
1991									
May 24-May 30	5.8	0.2	0.0	11.2	0.3	0.0	0.0	17.5	19.6
May 31-June 6	0.0	1.4	2.2	5.0	0.0	1.0	0.0	9.6	14.8
June 7-June 13	0.0	0.8	0.0	0.0	0.5	5.0	31.2	37.5	14.0
June 14-June 20	1.8	0.3	0.0	0.0	0.0	0.0	0.0	2.1	14.0
June 21-June 27	0.0	0.0	3.0	0.0	0.0	2.6	2.0	7.6	14.0
June 28-July 4	5.8	1.4	0.0	0.0	0.0	0.0	0.0	7.2	18.0
1992									
May 22-May 28	0.0	0.0	0.2	0.0	0.0	3.2	0.0	3.4	
May 28-June 4	0.0	0.0	0.0	4.4	6.6	0.0	0.4	11.4	
June 5-June 11	0.0	1.9	0.2	1.0	1.4	0.8	0.0	5.3	
June 12-June 18	1.4	0.3	1.8	15.6	0.0	0.0	0.0	19.1	
June 19-June 25	0.0	0.0	0.0	3.8	0.0	0.0	14.0	17.8	
June 26-July 2	1.0	0.0	0.0	0.0	3.4	1.0	1.0	6.4	

Table 3.2. Agronomic practices in field plots of annual legumes and barley.

Legume Species	Legume cultivar and common name	Seeding Rate ($kg\ ha^{-1}$)	Inoculum
Trifolium pratense L.	cv. Marino double cut red clover	10	"B" culture *Rhizobium trifolii*
T. resupinatum L.	cv. Felix persian clover	10	"R" culture *Rhizobium trifolii*
Medicago sativa L.	cv. Nitro alfalfa	10	"A" culture *Rhizobium meliloti*
Vicia villosa Roth.	cv. Dr. Baumanns hairy vetch	40	"C" culture *Rhizobium leguminosarum*
M. ciliaris (L.) Mill.	Sea hedgehog medic	14.5	"A" culture *Rhizobium meliloti*
M. scutellata (L.) Mill.	Snail medic	18.3	"A" culture *Rhizobium meliloti*
M. polymorpha L.	Toothed medic	14	"A" culture *Rhizobium meliloti*

In 1991, barley germination was poor, (approximately 65 percent) due to low rainfall and plants were counted in the first and second barley rows on June 23 and additional barley sown to make up the desired population. Weeds were controlled throughout the experiment by hand cultivation.

N levels were applied as ammonium nitrate (34-0-0) on June 25, 1991 and on June 16, 1992. Each application was mixed with approximately 1.5 g of sand, to facilitate application, and applied in three shallow trenches per plot such that each row had access to the fertilizer on one side only. Soil was then sprinkled over the fertilizer to minimize volatilization..

Barley stem lengths were taken on July 18th and 19th, 1991 and on July 30th 1992, from the middle row, as an average of the height of five plants per row.

Plots were harvested, by hand, as for silage to a height of 5 cm and the first cut was taken when barley reached the early dough stage (Table 3.3.). The harvest area of IC-plots was 0.12 m^2 (24 cm x 50 cm), including the middle barley row and the two legume rows on either side of the barley row. A 50 cm length of each row was harvested, leaving a five cm border at either end. In MC-legume and barley plots, the harvest area was 0.08 m^{-2} (16 cm x 50 cm) with two middle rows harvested from MC-legume plots and the one middle row harvested from MC-barley plots.

Table 3.3. Harvest dates in 1991 and 1992.

	1991			1992		
Species	Cut 1	Cut 2	Cut 3	Cut 1	Cut 2	Cut 3
T. pratense	July 22-3	Aug.28	Oct.22	July 31	Sept.4	Oct.21
T. resupinatum	"	Aug.20	Sept.18	"	Aug.31	Sept.24
M. sativa	"	Aug.29	Oct.22	"	Sept.4	Oct.21
V. villosa	"	Sept.4	-	"	Sept.4	Oct.21
M. ciliaris	"	Aug.20	Oct.22	"	Aug.31	Oct.21
M. scutellata	"	Aug.28	Oct.22	"	Sept.9	Oct.21
M. polymorpha	"	Aug.20	Oct.22	"	Aug.31	Oct.21
Barley	"	-	-	"	-	-

Leaf area index (LAI) was estimated in 1992 using a LAI-2000 Plant Canopy Analyzer (LI-COR, Inc., Lincoln, NE, USA) which was not calibrated due to the small plot size. LAI was calculated from one above-canopy reading and four below-canopy readings replicated five times, per plot. The first set of measurements was recorded on August 22 and 23rd, the second set of measurements was taken on November 4th and 5th.

Barley was harvested once. Following the barley harvest, legumes were again harvested when they reached approximately 20-30 percent flowering. In both years, three harvests of legumes were taken, with the exception of vetch in 1991 which only produced two cuts. After each sampling, all plots were completely clipped and clippings removed. Fresh weights were taken in the field on a triple beam balance. Samples

00were then placed in a solar dryer and later dried in a 75° C hot air dryer for at least 48 hours and dry weights (DM) taken. Total DM production was calculated in IC-plots as the sum of barley DM in the first cut and the total legume DM production of the three cuts. In MC-legume plots, total DM production was equivalent to the total legume DM production of the three cuts.

Dry matter Land Equivalent Ratio (LER) indices were determined using formula 1 (Mead and Willey 1980).

Formula 1. $LER = La + Lb = \frac{Ya}{Sa} + \frac{Yb}{Sb}$

La and Lb are ratios for the individual crops.
Ya and Yb are the IC-yields of the individual crops.
Sa and Sb are the MC-yields of the individual crops.

Dried barley and legume plants were ground separately in a Wiley mill (A.H. Thomas Co., Philadelphia, PA)(20 mesh with 1 mm openings). The concentration of total N was determined on sub-samples from the ground tissue from each species in the first and second harvest only. Nitrogen values of material from the first harvest in the main plot units receiving 30 and 60 kg N ha^{-1} were obtained using a Leco analyzer (Leco FP220 Nitrogen Analyzer) while values for main plot units of 0 kg N ha^{-1} were determined by Kjeldahl analysis in conjunction with the ^{15}N analysis of a separate study. Nitrogen values of all material from the second harvest were determined using the Leco analyzer. Crude protein (CP) was calculated as 6.25 times percent N.

Since CP analysis was not carried out on the third legume cut, due to budgetary constraints, comparisons of total CP yield with MC-barley do not accurately depict the full potential of the legume total CP yield. An estimate of legume CP yield in the third cut was determined based upon the CP concentration of the second cut and the actual legume DM yield of the third cut. Fraser *et al.* (1993) reported the CP concentration of two white clover cultivars (*T. repens* L.), when grown in binary mixture with four grass species, to remain fairly stable over four cuts, and the CP concentration of each cultivar in cut 3 to be

Oeither similar to that of cut 2, or to rank higher. In addition, MacLeod *et al.* (1972) reported CP concentration values of two monocropped alfalfa cultivars which were similar between cuts 2 and 3. Therefore, in this study, the lower herbage from the third cut was expected to have CP concentration values the same or higher than in the higher yielding second cut, and the third cut CP yield was more likely underestimated than overestimated. In MC-legume plots, these values were then compiled with CP yields of the first and second cuts to give an estimated total CP (ETCP) yield. In IC-plots, the ETCP yield was calculated as the sum of barley CP production in the first cut and the total legume CP production of the three cuts.

Yields and observations were analyzed by analysis of variance (Statistical Analysis System Institute Inc. 1985) at the 5 % level of significance. A protected LSD test (Steel and Torrie, 1980), unless otherwise noted, was utilized for means comparison where significant differences were indicated.

3.3 Results and Discussion:

TOTAL DRY MATTER (DM) PRODUCTION

MONOCROPPED LEGUMES AND INTERCROPS IN 1991

In 1991, there was no cropping system main effect on total DM production (Table 3.4.). There was a C*N interaction and within the IC-plots the 30 kg N ha^{-1} level, averaged over each legume species, produced a total DM yield of 8916 kg ha^{-1} which was significantly higher than those at 0 N (7313 kg ha^{-1}) and 60 N (7174 kg ha^{-1}). Of the three N levels, averaged over all legume species, only in the 30 N level were there cropping system effects on total DM yields and IC-plots yielded 8916 kg ha^{-1}, which was significantly higher than the 6991 kg ha^{-1} yield of the MC-legume plots. This positive response of intercrops to 30 N suggests that soil N was limiting total DM yield, while the 60 N appears to have had a negative effect on total DM yields. There were no N level responses within MC-legumes, indicating that N was not limiting their DM production. It would be expected that of the cropping systems, the intercrop would be the most responsive to N as grasses are able to exploit N to a high degree (Walker *et al.* 1954). Within a barley-field pea (*Pisum sativum* L.) intercropping study, Izaurralde et *al.* (1990) reported that although barley grain yields were not increased with an N fertilizer application of 80 kg ha^{-1}, barley straw responded with a yield increase of 880 kg ha^{-1} over 0 N fertilized IC-barley; neither pea grain or pea straw responded to the N fertilizer. That moisture was a limiting factor may explain why there was no continued response of the intercrops to 60 N as this was a modest application and grasses are capable of responding to a wide range of N fertility (Walker *et al.* 1954). In southern inland Queensland, Clarkson *et al.* (1987) intercropped tropical grass pastures of either sorghum (*Sorghum* spp.), buffel grass (*Cenchrus ciliaris*) or green panic (*Panicum maximum*) with either *M. scutellata* or *M. truncatula* and fertilized MC-grass with

Table 3.4. Total dry matter yields (TDM kg ha^{-1}) and estimated total crude protein yields (ETCP kg ha^{-1}) in 1991.

		TDM			ETCP		
N	Sp	MC-L	IC(B+L)	MC-B	MC-L	IC(B+L)	MC-B
0	Pol	6577	5128	9189	1066	639	1046
	Scu	5433	5793		864	649	
	Cil	5646	6206		837	719	
	Per	8473	6302		1686	872	
	Nit	11625	8429		2265	1265	
	Vet	6121	8792		1645	1356	
	Red	8967	10542		1937	1648	
30	Pol	4411	6795	8883	731	829	896
	Scu	4094	7263		724	860	
	Cil	7977	7592		1261	932	
	Per	7307	10789		1368	1707	
	Nit	10024	9959		1797	1391	
	Vet	5215	9702		1338	1534	
	Red	9911	10310		2114	1654	
60	Pol	5847	5115	10328	989	554	1101
	Scu	3358	6573		577	921	
	Cil	7074	6168		1104	718	
	Per	11153	7370		2079	1086	
	Nit	7818	8419		1465	1227	
	Vet	8049	7072		2083	1161	
	Red	8639	9501		1873	1502	
	LSD	4249	2288	-	878	369	-
	Sig. Effects[a]	Sp, C*N			C, Sp		
	LSD	-	2468		-	365	
	Sig. Effects[b]	-	Sp		-	Sp, Sp*N	

See footnotes on following page.

Footnotes to table 3.4.

N = N fertilizer levels; Sp = species; Pol = *M. polymorpha*; Scu = *M. scutellata*; Cil = *M. ciliaris*; Per = persian clover; Nit = Nitro alfalfa; Vet = vetch; Red = red clover; MC-B = monocropped barley; C = cropping system; MC-L = monocropped legume; IC(B+L) = intercrop(barley + legume); LSD = least significant difference; [a] = monocrop legumes and intercrops, excluding MC-barley; [b] = intercrops, including MC-barley; Sig. Effects = significant effects ($p < 0.05$).

either 0, 50 or 100 kg N ha^{-1} $year^{-1}$. Under two of the irrigated sites, the grass responded to inclusion of the medics by doubling in yield, a response similar to the application of 100 kg N ha^{-1} $year^{-1}$. Grass on the non-irrigated site at Roma, did not respond to either N fertilizer or medics due to low summer rainfalls which were on average, 297 mm over five seasons. In this study, there was a significant legume species main effect in 1991 on total DM yields. Red clover, Nitro alfalfa and persian clover produced similar total DM yields of 9645, 9379 and 8566 kg ha^{-1}, respectively. Total DM yields of red clover and Nitro 0alfalfa were higher than those of the other legumes. Red clover was the only perennial included in the study, and may be viewed as the check species. The three annual medics ranked lowest among the legumes in terms of total DM production, with the exception of *M. ciliaris* which did not differ from vetch.

MONOCROPPED LEGUMES AND INTERCROPS IN 1992

In 1992, there was a main effect of cropping system on total DM production, with IC-plots producing 51 % more DM than MC-legumes (Table 3.5.). This response was similar to a study by Kunelius *et al.* (1983) in which TDM yields of intercrops of legume-annual ryegrass were higher than MC-legumes. The authors reported intercropping legumes with Italian ryegrass to increase DM yields from 15 % (over MC-persian clover) to 22 % (over MC-birdsfoot trefoil); while intercropping with

Table 3.5. Total dry matter yields (TDM kg ha^{-1}) and estimated total crude protein yields (ETCP kg ha^{-1}) in 1992.

		TDM			ETCP		
N	Sp	MC-L	IC(B+L)	MC-B	MC-L	IC(B+L)	MC-B
0	Pol	2828	5254	8091	362	447	606
	Scu	4806	6565		759	658	
	Cil	3003	4925		539	370	
	Per	6878	8039		1409	1078	
	Nit	8522	7530		1597	956	
	Vet	3550	9699		997	2010	
	Red	7633	12027		1596	1969	
30	Pol	3059	6850	8203	376	562	914
	Scu	4884	6323		803	708	
	Cil	3069	4992		334	407	
	Per	5778	7460		1123	864	
	Nit	8705	8912		1590	1138	
	Vet	5251	11555		1435	2148	
	Red	6219	10203		1230	1699	
60	Pol	4119	6433	10319	446	599	893
	Scu	3800	7498		691	731	
	Cil	2888	6856		461	642	
	Per	5104	6539		935	798	
	Nit	8028	8045		1397	967	
	Vet	5403	11825		1557	2122	
	Red	7196	9401		1508	1400	
	LSD	2617	2301	-	500	404	-
	Sig. Effects[a]	C, Sp, C*Sp			Sp, C*Sp		
	LSD	-	2420		-	431	
	Sig. Effects[b]	-	Sp		-	Sp	

See footnotes to table 3.4.

westerwolds ryegrass increased DM yields from 15 % (over MC-persian clover) to 52 % (over MC-birdsfoot trefoil). In this study, a C*SP interaction existed and within the two cropping systems, total DM yields of the medics, averaged over each N level, did not differ. The MC-medics ranked lowest of the MC-legumes in total DM production, although *M. scutellata* was not significantly different from persian clover and vetch, while *M. polymorpha* did not differ from vetch. Within the IC-system the total DM yield of the medics were lower than all other IC-legumes, with the exception of *M. scutellata* and *M. polymorpha* which did not differ from persian clover. Within the IC-system, the total DM yields of intercrops of vetch (11026 kg ha^{-1}) and red clover (10543 kg ha^{-1}), averaged over each N level, were higher than those of the other intercrops. In the MC-legume system, the total DM yield of Nitro alfalfa (8418 kg ha^{-1}), averaged over each N level, outyielded all other MC-legumes, with the exception of red clover (7016 kg ha^{-1}). There were no significant N level effects on total DM production under either cropping system.

INTERCROPS AND MONOCROPPED BARLEY

In 1991, the DM yield of MC-barley (9467 kg ha^{-1}), averaged over each N level, did not differ from the total DM yields of intercrops with red clover, Nitro alfalfa and vetch (Table 3.4.). Similarly, Tidwell (1985) reported the MC-forage yields of two wheat cultivars, averaged over five maturity stages, to not differ from yields of wheat-hairy vetch intercrops. Roberts *et al.* (1989) examined the forage quality of wheat-vetch intercrops of four vetch seeding rates and reported DM yields of MC-wheat to be higher than IC yields, with the exception of vetch at the lowest seeding rate (54 pure live seeds (PLS) m^{-2}). Intercrops with vetch at the highest seeding rate (162 PLS m^{-2}) produced 89 % of MC-wheat yields. In this study, the DM yield of MC-barley was higher than the total DM yields of intercrops of persian clover and the annual medics. Kunelius *et al.* (1983) reported MC-annual ryegrass fertilized with 225 kg N ha^{-1} over the season, to have produced higher DM yields than those of 0 N fertilized intercrops. Intercrops containing Italian ryegrass produced DM yields ranging from only 50 % of MC-Italian ryegrass yield (when IC with birdsfoot trefoil) to 84 % of MC-grass yield (when IC with persian clover); intercrops containing westerwolds ryegrass produced DM yields ranging from 52 % of MC-westerwolds ryegrass yield (when IC with birdsfoot trefoil) to 70 % of MC-grass yield (when IC with persian clover).

In 1992, the DM yield of MC-barley (8871 kg ha^{-1}), averaged over each N level, was lower than the total DM yields of intercrops of vetch (11026 kg ha^{-1}) and red clover (10543 kg ha^{-1}). Kunelius et al. (1983) found 0 N MC-westerwolds ryegrass to yield lower than all intercrops, with the exception of intercrops of birdsfoot trefoil, and intercrops of red clover produced 29 % more DM than MC-grass. In this study, similar to 1991, the DM yield of MC-barley did not differ from Nitro alfalfa intercrops and was higher than intercrops of persian clover and the medics (Table 3.5.).

ESTIMATED TOTAL CRUDE PROTEIN PRODUCTION

CROPPING SYSTEMS OF MONOCROPPED LEGUMES AND INTERCROPS

In 1991, although the total DM yields of MC-legumes and IC-plots were similar, there was a cropping system main effect on estimated total CP (ETCP) yields with MC-legumes (1419 kg ha^{-1}) outyielding intercrops (1106 kg ha^{-1}) (Table 3.4.). The species main effect of red clover (1788 kg ha^{-1}) was higher than persian clover (1466 kg ha^{-1}) and the annual medics, yet similar to those of Nitro alfalfa and vetch. The annual medics were lowest in ETCP.

In 1992, although the total DM yield of IC-plots was higher than MC-legumes, there was no cropping system main effect on ETCP yields (Table 3.5.). There was a C*Sp interaction, and in monocrops the species main effect of Nitro alfalfa (1528 kg ha^{-1}) was similar to red clover and vetch but higher than persian clover and the medics with MC-medics yielding lowest in ETCP. Under the intercropping system the species main effect of vetch (2093 kg ha^{-1}) was highest of all intercrops in ETCP production and red clover (1689 kg ha^{-1}) was higher than the other intercrops. The medics were lowest in IC-ETCP with the exception of *M. scutellata* which yielded similarly to persian clover.

INTERCROPS AND MONOCROPPED BARLEY

Within the IC-plots and MC-barley of 1991 there was a Sp*N interaction (Table 3.4.). In 1991, at 0 N the CP yield of MC-barley was lower than the ETCP yield of intercrops of red clover and was higher than those of *M. scutellata* and *M. polymorpha*. At the 30 N level, intercrops of persian clover, red clover, vetch and Nitro alfalfa did not differ and were higher in ETCP than the annual medic intercrops and MC-barley. At 60 N, the ETCP of red clover intercrops was highest, while MC-barley was higher than intercrops of *M. ciliaris* and *M. polymorpha*.

In 1992, the CP yield of MC-barley (804 kg ha^{-1}), averaged over all N levels, ranked lower than the ETCP yields of intercrops of vetch, and red clover (2093 and 1689 kg ha^{-1}, respectively) (Table 3.5.). Similarly, Lunnan (1988) reported MC-barley (whole plant silage) to be significantly lower in N yield than barley-legume intercrops, with the exception of yellow lupin and MC-barley produced only 58 and 59 % of the N yields of intercrops of forage pea and vetch, respectively. Roberts *et al.* (1989) found the CP concentration of MC-wheat to be significantly lower than intercrops with vetch, and as the seeding rate of the IC-vetch increased, the CP yield of the intercrop increased. Intercrops of the highest vetch seeding rate produced 20 % more CP than MC-wheat. In this study, the CP yield of MC-barley was only higher than the ETCP yields of intercrops of *M. polymorpha* and *M. ciliaris*.

BARLEY OBSERVATIONS

In both 1991 and 1992, there were no treatment effects on barley height (data not shown). Kunelius *et al.* (1992) also reported barley height was not affected when intercropped with either westerwolds ryegrass, Italian ryegrass or red clover. Hartl (1989) reported that wheat stem lengths were not affected when intercropped with either black medic (*M. lupulina*) or persian clover in either a pot or field study. But, in the pot study, intercropping with a specific group of weeds decreased wheat stem length by 6.7 cm compared to MC-wheat and stem length was decreased by 4.1 cm when black medic was included in the wheat-weed intercrop.

In both 1991 and 1992, there were no treatment effects on barley CP concentration (data not shown). Ta and Faris (1987b) reported the CP concentration of the following grasses: timothy (*Phleum pratense*), bromegrass (*Bromus inermis*), red fescue (*Festuca ruba*), tall fescue (*Festuca arundinacea*) and orchardgrass, to be higher when intercropped with each of the following legumes: alfalfa, red clover and birdsfoot trefoil. This trend was illustrated by the CP concentration of MC-tall fescue, 181 g kg^{-1}, at cut four, which was significantly lower than those of tall fescue intercropped with either of the legumes: the CP of tall fescue intercropped with alfalfa, red clover, and birdsfoot trefoil was 209, 206 and 212 g kg^{-1}, respectively. Lunnan (1988) found the protein content of MC-barley (74 g kg^{-1}) to not differ from that of barley intercropped with the following legumes: yellow lupin (*Lupinus luteus*), field bean (*Vicia faba*) and two pea cultivars (*Pisum sativum*) but, barley intercropped with forage pea (91 g kg^{-1}) and vetch (88 g kg^{-1}) was higher in protein content than MC-barley. In our study in 1991, barley DM, averaged over all cropping treatments, at 30 N (7034 kg ha^{-1}) tended to yield higher, although not significantly, than at 60 N (6021), or 0 N (5955 kg ha^{-1}) (Table 3.6.). There was a significant main effect of species on barley DM yields and barley CP yields.

BARLEY DRY MATTER PRODUCTION

In 1991 and 1992, IC-legumes were apparently competing with barley for resources as MC-barley DM yields (9467 and 8871 kg ha^{-1},

Table 3.6. Barley dry matter yield (kg ha^{-1}) (DM) and crude protein yield (kg ha^{-1}) (CP) in 1991 and 1992.

	1991			1992	
N	Barley CT	DM	CP	DM	CP
0	Pol	4701	536	4925	400
	Scu	5514	640	5825	532
	Cil	5959	672	4825	357
	Per	4851	558	5779	598
	Nit	5421	601	4565	376
	Vet	5032	563	3887	386
	Red	6973	767	4727	335
	MC	9189	1046	8091	606
30	Pol	6372	739	6715	546
	Scu	7011	810	6054	658
	Cil	7081	831	4931	401
	Per	6848	817	5323	437
	Nit	6585	731	5677	479
	Vet	6818	793	5710	491
	Red	6669	790	5160	502
	MC	8883	896	8203	914
60	Pol	4869	511	6127	557
	Scu	6383	876	7033	653
	Cil	5755	647	6712	627
	Per	5024	576	4840	452
	Nit	5283	593	5479	492
	Vet	4425	470	6479	628
	Red	6102	722	5117	439
	MC	10328	1101	10319	893
	LSD	2324	302	2069	327
	Sig. Effects	Sp	Sp	Sp	Sp

CT = cropping treatment; MC = monocrop; see footnotes to table 3.4.

respectively), were higher than barley in any intercrop (Table 3.6.). Roberts *et al.* (1985) also found wheat shoot yield when intercropped with hairy vetch to be lower than MC-wheat, and as vetch seeding rate increased, the IC-wheat shoot yield also decreased. Wheat intercropped with vetch at 162 pure live seeds m^{-2} produced only 67 % of MC-wheat shoot yield. Although Stewart *et al.* (1980) reported that grain yields of MC-barley did not differ from that of barley intercropped with each of four red clover cultivars and two white clover cultivars, the authors did observe that towards the time of harvest, the broad red clover cultivars appeared to dominate shorter strawed barley cultivars. In a pot study, Hartl (1985) also reported a trend for less grain when winter wheat was intercropped with persian clover compared to MC-wheat, and wheat straw yielded lower in the intercrop, compared to weedy wheat. Also, the authors noted a trend for more grain when the IC-legume was black medic and that straw yields were higher in the intercrop compared to weedy wheat. On a silt loam site, Sampson *et al.* (1989) found the wheat yields of several wheat-legume intercrops to not differ and on a sandy loam, the only difference in wheat yields between intercrops was with the early frost seeding of red clover (3.35 t ha^{-1}) being lower in wheat yield than that of the late frost seeding of red clover (4.36 t ha^{-1}) and that of the drilled annual, crimson clover (4.38 t ha^{-1}). In our study in 1991, the DM yield of barley intercropped with *M. polymorpha* (5314 kg ha^{-1}) was lower than barley intercropped with red clover (6581 kg ha^{-1}) while barley intercropped with all other legumes did not differ.

In 1992, barley DM yields when intercropped with Nitro alfalfa and red clover (5240 and 5001 kg ha^{-1}, respectively) were lower than barley intercropped with *M. scutellata* (6304 kg ha^{-1}) while barley intercropped with all other legumes did not differ.

In neither year did barley DM respond to N fertilizer applications. Plant available N was not limiting plant growth.

BARLEY CRUDE PROTEIN PRODUCTION

In both years, the CP yield of MC-barley exceeded those of IC-barley (Table 3.6.); this was related to DM yields as the CP concentration of barley was similar under the two cropping systems (data not shown). In 1991, CP yields of barley intercropped with vetch and *M. polymorpha* (609 and 596 kg ha^{-1}) were lower than those of barley intercropped with *M. scutellata* and red clover (776 and 760 kg ha^{-1}, respectively). Barley CP yield when intercropped with all other legumes did not differ.

In 1992, the CP yield of barley intercropped with *M. scutellata* (614 kg ha^{-1}) was higher than barley intercropped with red clover (425 kg ha^{-1}), while barley intercropped with all other legumes did not differ.

In neither year did barley CP yield respond to N fertilizer applications.

PERCENT LEGUME DRY MATTER YIELD OF INTERCROPS IN CUT 1

In 1991, the percent DM yield of vetch in intercrops (13.6 %), averaged over each N-level, was higher than those of the other legume species (Table 3.7.). Lunnan (1988) also reported a high vetch content in legume-barley intercrops, when intercrops of vetch and forage pea had the highest legume composition, producing 43.7 % and 39.1 %, respectively, of the intercrop DM yield. In this study, all other species did not differ, with the exception of *M. scutellata* (1.0 %) which was lower than persian clover (5.8 %).

In 1992, there was a SP*N interaction (Table 3.7.). At each N-level the percent legume yield of IC-vetch ranked highest of the legumes. Within the 0 N level the percentage legume yield of the other IC-legumes did not differ, with the exception of *M. ciliaris* which was also lower than red clover and persian clover. At 30 N, the percent legume yield of IC-persian clover ranked higher than that of Nitro alfalfa and the IC-medics ranked lowest. At 60 N, the percent legume yield of IC-persian clover was higher than that of *M. scutellata* and

Table 3.7. Land equivalent ratio (LER), intercrop crude protein yield (kg ha^{-1}) (IC-CP) and percent legume component of intercrop dry matter yield (L %) in cut 1 of 1991 and 1992.

		1991			1992		
N	Sp	LER	IC-CP	L %	LER	IC-CP	L %
0	Pol	0.58	569	4.3	0.68	423	4.4
	Scu	0.63	649	0.8	0.76	549	2.3
	Cil	0.75	691	2.5	0.68	366	1.7
	Per	0.65	594	5.6	0.87	680	12.2
	Nit	0.67	621	3.2	0.71	426	8.5
	Vet	0.93	831	17.9	1.64	800	31.6
	Red	0.89	806	2.8	0.90	466	12.2
30	Pol	0.94	764	2.6	0.98	559	1.7
	Scu	0.97	822	1.0	0.77	667	1.0
	Cil	0.98	868	3.9	0.65	404	0.8
	Per	1.08	894	5.8	0.88	515	8.0
	Nit	0.95	769	3.4	0.85	532	5.3
	Vet	1.16	974	11.4	1.25	778	17.0
	Red	0.93	842	3.6	0.85	567	6.6
60	Pol	0.51	539	3.4	0.66	583	3.5
	Scu	0.69	890	1.2	0.77	666	1.5
	Cil	0.61	671	3.1	0.73	635	1.4
	Per	0.62	641	6.0	0.66	503	5.6
	Nit	0.62	631	4.3	0.61	522	3.4
	Vet	1.88	613	11.4	1.12	837	12.4
	Red	0.71	767	3.6	0.60	475	3.9
	LSD	ns	ns	4.8	0.35	271	7.1
	Sig. Effects	-	-	Sp	Sp	Sp	Sp, Sp*N

ns = not significantly different ($p<0.05$); See footnotes to table 3.4.

N. ciliaris. Within intercrops of vetch, red clover, persian clover and Nitro alfalfa, the proportion of legume in the intercrop tended to increase as the rate of N fertilizer decreased. Similarly, Frame and Boyd (1987a) reported increasing spring N fertilizer applications, of 0, 25, 50 and 75 kg N ha^{-1}, to a mixed sward of two perennial ryegrass cultivars and two white clover cultivars, progressively decreased white clover content. In addition, Frame and Boyd (1987b) applied four annual rates of N fertilizer (0, 120, 240 and 360 kg N ha^{-1}) to mixtures of four white clover varieties with perennial ryegrass, and reported that although fertilizer N increased total herbage production, there was a steady decline in white clover content with increasing N rates.

LEGUME DRY MATTER YIELD

CROPPING SYSTEMS OF MONOCROPPED LEGUMES AND INTERCROPS

A C*SP interaction existed in all three cuts for both years including the total of these cuts in 1992 (Tables 3.8. and 3.9.). As well, in each cut of both years, with the exception of cut 3 in 1992, and including the total of these cuts, there was a main effect of cropping system on legume DM yield. Legume DM yields of MC-legumes were higher than those of IC-legumes. The IC-legumes were seeded at half the rate of the MC-legumes, but, at cut 1 in both years the IC-legumes yielded much less than half that of the MC-legumes as a result of barley competition. Sulc *et al.* (1993) monocropped and intercropped alfalfa, in all plots at 18 kg ha^{-1}, with five ryegrass cultivars and oats (*Avena sativa* L.) under different environments. In environments receiving adequate rainfall, alfalfa yields were suppressed by all intercrop components, and produced only 12 % to 28 % of MC-alfalfa yield. In an environment in which moisture was limiting during the time of establishment, ryegrass growth was reduced, allowing for higher IC-alfalfa yields. The intercrop component of oats resulted in the lowest alfalfa yields, producing only 24 % of MC-alfalfa in one year and 39 % of MC alfalfa in the next year. In our study in cut 1 of 1991,

Table 3.8. Legume dry matter yields (kg ha^{-1}) of the three cuts and their totals in 1991.

N	Sp	Cut 1 MC-L	Cut 1 IC-L	Cut 2 MC-L	Cut 2 IC-L	Cut 3 MC-L	Cut 3 IC-L	Total MC-L	Total IC-L
0	Pol	5147	218	909	76	521	133	6577	427
	Scu	1281	44	3001	108	1151	127	5433	279
	Cil	3029	140	1266	83	1352	24	5646	247
	Per	2533	261	4278	721	1661	469	8473	1451
	Nit	2627	171	5371	1466	3627	1370	11625	3008
	Vet	2671	1006	3450	2754	-	-	6121	3760
	Red	1691	181	3923	1182	3353	2207	8967	3569
30	Pol	2759	154	609	102	1042	166	4411	422
	Scu	1134	63	2124	50	836	138	4094	252
	Cil	4753	240	1569	178	1655	93	7977	511
	Per	2813	397	3538	1706	956	1838	7307	3941
	Nit	2574	214	4320	1814	3130	1345	10024	3373
	Vet	2294	708	2922	2176	-	-	5215	2884
	Red	2977	238	4348	1496	2586	1907	9911	3641
60	Pol	4047	171	1019	75	781	0	5847	246
	Scu	897	69	1895	112	366	8	3358	189
	Cil	4194	167	1350	150	1530	97	7074	414
	Per	3264	322	6022	1167	1867	857	11153	2346
	Nit	2249	224	3456	1619	2113	1293	7818	3136
	Vet	1593	603	6456	2044	-	-	8049	2647
	Red	2093	219	4052	1335	2494	1846	8639	3399
	LSD	2125	261	2799	739	948	476	4249	1045
Sig. Effects		C, Sp, C*Sp		C, Sp, C*Sp		C, Sp, N, C*Sp C*Sp*N		C, Sp	

MC-L = monocropped legume; IC-L = intercropped legume; See footnotes to table 3.4.

Table 3.9. Legume dry matter yields (kg ha^{-1}) of the three cuts and their totals in 1992.

N	Sp	Cut 1 MC-L	Cut 1 IC-L	Cut 2 MC-L	Cut 2 IC-L	Cut 3 MC-L	Cut 3 IC-L	Total MC-L	Total IC-L
0	Pol	3229	219	463	88	79	23	3771	329
	Scu	2609	123	1666	485	531	131	4806	740
	Cil	1500	71	563	17	941	13	3003	100
	Per	2781	544	2681	940	1415	776	6878	2260
	Nit	2556	336	2997	796	2969	1833	8522	2966
	Vet	1578	1646	1172	2691	800	1475	3550	5812
	Red	2378	650	2592	2521	2663	4129	7633	7300
30	Pol	2503	117	497	17	59	2	3059	135
	Scu	2016	54	1978	106	891	108	4887	269
	Cil	2075	38	684	21	309	2	3069	60
	Per	2303	435	2459	838	1016	864	5778	2137
	Nit	1956	298	3077	868	3672	2069	8705	3235
	Vet	2559	1179	1613	2203	1078	2462	5251	5845
	Red	1622	325	2023	1605	2575	3113	6219	5042
60	Pol	3522	221	447	73	150	13	4119	306
	Scu	1538	102	1556	156	706	206	3800	465
	Cil	1625	94	509	29	753	21	2888	144
	Per	1938	290	2191	769	976	641	5104	1700
	Nit	2672	188	2674	701	2681	1677	8028	2565
	Vet	2031	877	1457	1621	1916	2848	5403	5346
	Red	2122	206	2568	1011	2506	3067	7196	4284
	LSD	ns	310	1070	616	880	1015	2578	1440
Sig. Effects		C, Sp, C*Sp		C, Sp, C*Sp		Sp, C*Sp, Sp*N		C, Sp, C*Sp	

MC-L = monocropped legume; IC-L = intercropped legume; ns = not significantly different ($p < 0.05$); See footnotes to table 3.4.

IC-legumes yielded 10 % of MC-legume yield and in 1992, were 17 % of MC-legume yield. In 1991, at cut 2, IC-legumes produced 31 % of MC-legume yield and at the third cut IC-legumes produced 45 % of MC-legume yield, resulting in total legume DM yield of IC-legumes being 26 % that of the MC-legumes. In 1992, at cut 2, IC-legumes yielded 49 % of MC-legume yield and by the third cut yielded 88 % of MC-legume yield, resulting in the total legume DM yield of intercrops being 46 % of the MC-legume yield.

In 1991, IC-legumes produced 4.9 % (overall mean) of cut 1 intercrop DM yield while in 1992, IC-legumes produced 6.9 % of this yield. The IC-legumes of 1991 were less able to recover from barley competition, and as a result, IC-total DM yields were similar to MC-legume total DM yields. The apparently more vigorous IC-legumes of 1992 were better able to recover from barley competition, resulting in a continued high performance of the intercrops.

It is possible to theoretically compare equal legume seeding rates of the two cropping systems by dividing MC-legume yields in half. In cut 1 of 1992, MC-vetch would have only yielded 83 % of its intercrop yield, while in 1991 no MC-legumes would have been lower than their intercrop yield. In cut 2 of 1992, MC-vetch would only have yielded 33 % of its intercrop yield and red clover, 70 % of its intercrop yield, but in 1991, MC-vetch would have yielded 92 % of its IC-yield. In cut 3 of 1992, all MC-legumes yielded less in monocrop than intercrop, with the exception of the medics, while in 1991 only MC-red clover and MC-persian clover yielded less in monocrop. With the exception of the medics, DM yield of the IC-legumes appeared to have been stimulated in 1992. In 1992, the MC-legumes may have been competing for similar resources and the replacement of half of the legumes with barley lessened the degree of intraspecific competition. After barley had been harvested in cut 1, its growth in cuts 2 and 3 was negligible, and it was highly probable that intraspecific competition was higher at the greater plant densities of the monocrops than the low density intraspecific competition plus marginal interspecific competition of the intercrops.

In 1991 there was a species main effect on total legume DM yield with Nitro alfalfa and red clover (6498 and 6354 kg ha^{-1}) yelding higher than all other legumes, with the exception of persian clover. The total legume DM yield of the medics were lowest with the exception of *M. ciliaris* which yielded similarly to vetch. The only significant N level effect on legume DM yield, averaged over each legume species, occurred in the third cut of both 1991 and 1992. In the third cut of 1991 there was an N level main effect on legume DM with 0 N and 30 N (1333 and 1308 kg ha^{-1}, respectively) yielding higher than 60 N (1121 kg ha^{-1}). If the 60 N of cut 3 resulted in decreased N_2-fixation due to exposure to mineral N, as reported by McAuliffe *et al.* (1958) and Rongquing *et al.* (1992), this alone would not explain the negative response to the main effect of 60 N. This additional N would have been available in monocrops and in intercrops but the barley did not respond to this additional N (Table 3.6.).

LEGUME DRY MATTER YIELD IN MONOCROPS

In each cut of 1991 there was a significant species main effect on MC-legume DM production (Table 3.8.). In the first cut, MC-*M. ciliaris* and *M. polymorpha* produced respective DM yields of 3992 and 3985 kg ha^{-1} which were similar to persian clover but, higher than Nitro alfalfa, red clover, vetch and *M. scutellata*. As the annual medics are native to arid sites in North Africa and the Middle East (Brahim and Smith 1993), their success may have been related to drought tolerance in June and July 1991. By the time of the second cut, the MC-medics ranked lower than the other legumes which yielded similar DM values. In the third cut, Nitro alfalfa (2957 kg ha^{-1}) and red clover (2811 kg ha^{-1}) yielded higher than the other legumes, while *M. ciliaris* (1512 kg ha^{-1}) and persian clover (1495 kg ha^{-1}) did not differ and were higher yielding than *M. scutellata* and *M. polymorpha*. The only main effect of N level on legume DM yield, occurred in the third cut where the 0 N level legume DM yield of 1944 kg ha^{-1} was higher than either the 30 N or 60 N levels (1701 and 1559 kg ha^{-1}, respectively). McAuliffe *et al.* (1958) also observed a negative effect of N fertilizer (224 kg N ha^{-1}) on ladino clover grown in association with tall fescue in a pot study in which clover growth was decreased and chlorosis occurred at this N level. The authors could offer no explanation for this effect. Chestnutt and Lowe (1970) noted several reports in which small amounts of combined N aided in the establishment of legumes and their nodulation, but increasing N levels led to a reduction in the number of effective nodules. In this study, the applied 60 N may have inhibited the early nodulation of the legumes and by the time of the third cut, the compensatory effect of the early applied N may have been exhausted.

There was no main effect of species on MC-legume DM production in the first 1992 cut. This lack of response may have been due to the more evenly distributed rainfalls; the legumes were not moisture limited as in 1991. There were main effects of species on MC-legume DM production in the following cuts and of the total of these cuts (Table 3.9.). In the second cut, monocrops of Nitro alfalfa, persian clover and red clover (2916, 2444 and 2394 kg ha^{-1}, respectively) were not different and were higher in legume DM yield than the other MC-legumes, while *M. ciliaris* (585 kg ha^{-1}) and *M. polymorpha* (469 kg ha^{-1}) ranked lowest of the MC-legumes. At the time of the third cut, the Nitro alfalfa DM yield (3107 kg ha^{-1}) was highest of the MC-legumes followed by red clover (2581 kg ha^{-1}) which was higher than the other MC-legumes, while *M. polymorpha* (98 kg ha^{-1}) ranked lowest. The total MC-legume DM yields of Nitro alfalfa (8418 kg ha^{-1}) and red clover (7016 kg ha^{-1}) did not differ and were higher than those of vetch and the medics, and red clover did not differ from persian clover (5920 kg ha^{-1}). In monocrops, Kunelius and Narasimhalu (1983) found that in terms of total DM yield (of three cuts), persian clover was the most productive legume (7138 kg ha^{-1}), followed by red clover (5949 kg ha^{-1}), alfalfa (5157 kg ha^{-1}) and birdsfoot trefoil (4024 kg ha^{-1}). In this study, there were no significant N level effects in any of the cuts.

LEGUME DRY MATTER YIELD IN INTERCROPS

In each cut of 1991, there was a main effect of species on IC-legume DM yield (Table 3.8.). In each of these cuts, the three medics ranked lowest in DM yield, although in cut 1, *M. ciliaris* and *M. polymorpha* were similar to red clover and Nitro alfalfa. At the time of the first and second cuts, IC-vetch DM yields (773 and 2325 kg ha^{-1}, respectively) were higher than those of the other legumes. In the third cut a SP*N interaction existed. Within each N level of the third cut, IC-legume DM yields of red clover were highest of the IC-legumes but at 30 N red clover did not differ from persian clover and Nitro alfalfa. The yields of the IC-medics were lower than the other legumes at each N level with the exception of *M. polymorpha* and *M. scutellata* at 0 N, which yielded similarly to persian clover.

In 1992, a SP*N interaction existed for cuts 1 and 2; at the first cut, vetch had the highest IC-legume DM yields at each N level and at 60 N all other IC-legumes did not differ in DM yield (Table 3.9.). Also in cut 1, within the 0 N level, red clover DM yields were higher than those of *M. scutellata* and *M. ciliaris*, and at 30 N the medics were also lower than persian and red clovers. At the second cut under the 0 N and 30 N levels, vetch and red clover did not differ in legume DM yield and outyielded the other legume species. At 60 N, vetch outyielded all other IC-legume species. At cut 3 and for the total of the 3 cuts, the legume DM yields of the IC-medics did not differ from each other and were lower than those of the other legumes. At cut 3, red clover (3436 kg ha^{-1}) yielded highest of the legumes while vetch and Nitro alfalfa (2262 and 1860 kg ha^{-1}) were higher than persian clover (760 kg ha^{-1}). The total legume DM yields of IC-vetch and red clover

(5667 and 5542 kg ha^{-1}, respectively) were higher than the other legumes while Nitro alfalfa (2922 kg ha^{-1}) was higher than persian clover.

The competitive ability of vetch grown with barley was clearly demonstrated in cuts one and two, of both years. Although in both years, vetch legume DM was higher than the other legumes when intercropped with barley, its associated barley yielded DM values which did not differ from those of the other legumes. Therefore, vetch was not competing more with barley than the other legumes for growth factors, it was either relying more on its own resources, possibly through higher N_2-fixation, or it was exploiting soil volumes not explored by barley roots or received more light than other legumes. Samson *et al.* (1989) also found vetch to be highly productive when intercropped with winter wheat and to not depress grain yields. The authors found drilled vetch on a silt loam site to yield a fall biomass of 3367 kg ha^{-1}, which was highest of the legumes, early frost seeded red clover produced the second highest biomass (1569 kg ha^{-1}) while drilled red clover (678 kg ha^{-1}), Nitro alfalfa (890 kg ha^{-1}) and crimson clover (844 kg ha^{-1}) did not differ. On a sandy loam site, vetch biomass (2554 kg ha^{-1}) was also highest of the legumes, followed by Nitro alfalfa (1440 kg ha^{-1}) and crimson clover (1426 kg ha^{-1}), which did not differ, and drilled and frost seedings of red clover produced the least biomass. In our study, vetch DM yield ranked much lower under monocrop conditions. In the third cut of our study in 1991, regrowth of vetch was negligible and this material was not harvested.

LEGUME CRUDE PROTEIN CONCENTRATION

In 1991 a C*SP interaction existed in cuts one and two in terms of legume CP concentration, and there was a main effect of cropping systems on legume CP concentration (Table 3.10.). In cut one, the CP concentration of MC-legumes (202 g kg^{-1}) was higher than that of IC-legumes (191 g kg^{-1}), although *M. polymorpha* and *M. ciliaris* tended to be

Table 3.10. Legume crude protein concentration (g kg^{-1}) in cut 1 and 2 of 1991.

N	Sp	Cut 1 MC-L	Cut 1 IC-L	Cut 2 MC-L	Cut 2 IC-L
0	Pol	159	156	185	318
	Scu	235	192	135	-
	Cil	128	135	172	266
	Per	217	199	195	229
	Nit	180	167	197	225
	Vet	278	264	268	264
	Red	228	213	213	248
30	Pol	146	177	222	257
	Scu	225	193	155	207
	Cil	132	150	186	230
	Per	218	196	170	231
	Nit	176	177	181	198
	Vet	232	244	285	265
	Red	232	215	207	240
60	Pol	165	169	205	207
	Scu	242	195	143	238
	Cil	142	144	179	189
	Per	218	204	182	222
	Nit	189	164	186	204
	Vet	262	239	271	268
	Red	234	210	210	234
	LSD	29	37	31	41
	Sig. Effects	C, Sp, C*Sp		C, Sp, C*Sp, C*N, C*Sp*N	

MC-L = monocropped legume; IC-L = intercropped legume; See footnotes to table 3.4.

higher in intercrop. By the time of the second cut, the opposite was true and the CP concentration of IC-legumes (235 g kg^{-1}) was higher than that of MC-legumes (197 g kg^{-1}), although vetch tended to be higher in monocrop. Within MC-legumes and IC-legumes at cut 1 and cut 2 of 1991, the vetch CP concentration was highest of the legumes, although at cut 2 within IC-legumes the vetch CP concentration was not significantly higher than that of *M. polymorpha* (255 g kg^{-1}). Of several legumes intercropped with barley, Lunnan (1988) also found the protein content of vetch (190 g kg^{-1}) to be highest, although not significantly higher than forage pea. Under monocrops and intercrops, at cut 1, *M. ciliaris* (134 and 143 g kg^{-1}, respectively) yielded the lowest of the legumes for CP concentration.

In the 1992 cut one, the CP concentration of MC-legumes (172 g kg^{-1}), was higher than that of the IC-legumes (162 g kg^{-1}) (Table 3.11.). This is in contrast to results of Ofori and Stern (1986) who found intercropping did not affect N concentrations of maize and cowpea. The CP concentration of vetch (249 g kg^{-1}), averaged over each N-level and cropping system, was the highest of the legumes, followed by red clover (202 g kg^{-1}) which was the highest of the remaining legumes. Of the MC-legumes studied by Kunelius and Narasimhalu (1983), the CP content of red clover (209 g kg^{-1}) ranked highest, followed by alfalfa (207 g kg^{-1}), birdsfoot trefoil (192 g kg^{-1}) and persian clover (169 g kg^{-1}). In this study, the medics ranked lowest of the legumes, with the CP concentration of *M. scutellata* (161 g kg^{-1}) being similar to that of Nitro alfalfa. At the time of the second 1992 cut there was no significant difference between cropping systems for CP concentration and a C*Sp interaction existed and in MC-legumes there was a main effect of N-level on CP concentration with the 0 N (217 g kg^{-1}) being higher than those of the 60 N (198 g kg^{-1}) or 30 N (192 g kg^{-1}). Under both cropping systems of cut 2, the CP concentration of vetch (MC,303 and

Table 3.11. Legume crude protein concentration (g kg^{-1}) in cut 1 and 2 of 1992.

N	Sp	Cut 1 MC-L	Cut 1 IC-L	Cut 2 MC-L	Cut 2 IC-L
0	Pol	102	104	208	187
	Scu	162	134	165	186
	Cil	115	111	212	135
	Per	186	160	216	242
	Nit	163	160	198	197
	Vet	248	254	310	292
	Red	207	200	209	226
30	Pol	106	107	164	138
	Scu	164	175	164	212
	Cil	90	81	149	125
	Per	200	182	193	205
	Nit	158	179	188	206
	Vet	256	244	289	291
	Red	199	209	198	245
60	Pol	103	113	173	167
	Scu	194	139	182	179
	Cil	127	88	157	136
	Per	188	181	184	208
	Nit	163	160	182	185
	Vet	256	241	308	289
	Red	219	180	201	227
	LSD	42	46	33	37
Sig. Effects		C, Sp		Sp, N, C*Sp, Sp*N	

MC-L = monocropped legume; IC-L = intercropped legume; See footnotes to table 3.4.

IC, 290 g kg^{-1}) ranked highest of the legumes and the medics ranked lowest in CP concentration, although in MC-legumes *M. polymorpha* (179 g kg^{-1}) was similar to Nitro alfalfa and persian clover while in IC-legumes, *M. scutellata* (192 g kg^{-1}) was similar to Nitro alfalfa.

LEGUME CRUDE PROTEIN PRODUCTION

As expected, in cuts 1 and 2 of 1991 and 1992, there was a main effect of cropping system on legume CP yield, whereby MC-legumes were higher than IC-legumes (data not shown).

In cut 1 of 1991, MC-legumes yielded 508 kg ha^{-1} of CP while IC-legumes yielded 59 kg ha^{-1}, the CP yield of IC-legumes being only 12 % that of MC-legumes (data not shown). A C*SP interaction existed for legume yield at cut one, whereby vetch yielded higher than other lagumes under intercrop but not under monocrop. In the second 1991 cut, there were no interactions and the legume CP yield of monocrops was 629 kg ha^{1} while intercrops produced 39 % of the MC yield (243 kg ha^{-1}). The CP yield of vetch (875 kg ha^{-1}), averaged over each cropping system and N-level, was higher than the other legumes and the medics yielded the lowest CP.

Within cut one of 1992, there were no interactions for legume CP yield and the MC-legumes produced 380 kg ha^{-1} CP, while IC-legumes (77 kg ha^{-1}) yielded only 20 % of the MC-legume CP yield (data not shown). In 1992, cut one, vetch CP yield (412 kg ha^{-1}) averaged over all cropping systems and N-levels, was higher than those of the other legumes. By the second cut in 1992, a C*SP interaction existed but MC-legumes yielded 350 kg ha^{-1} CP, averaged over each legume species and N level, while IC-legumes yielded 202 kg ha^{-1} having increased to 58 % that of MC-legumes. Within MC-legume plots of cut 2, Nitro alfalfa, red clover, persian clover and vetch (554, 488, 484 and 425 kg ha^{-1}, respectively) had similar CP yields and outyielded the medics. A SP*N interaction existed for IC-legumes. Within each N level, vetch yielded highest in

CP production. The next highest CP yielding legume was red clover which ranked higher than the remaining legumes with an exception at 60 N where red clover and persian clover yields were not different.

INTERCROP OBSERVATIONS: CUT 1

INTERCROP (Legume + Barley) DRY MATTER PRODUCTION

In 1991 and 1992, there were no treatment effects on intercrop DM yield at cut 1 (data not shown). Differences between component legume DM yields were apparently evened out by equivalent gains or losses of IC-barley yields and vise versa for differences between component barley DM yields.

LERs

In 1991, there was a trend for all species to have higher LERs at 30 N, with the exception of vetch which was highest under 60 N. In 1991, at 0 N there were no intercrops with LERs greater than 1 (Table 3.7.). At the 30 and 60 N levels IC-vetch LERs were 1.16 and 1.88 respectively, and at the 30 N level IC-persian clover LER was 1.08. In 1992, there was no overall trend for higher LERs at any fertilizer level but only IC-vetch produced LERs greater than one and in decreasing order as N input increased: 0 N of 1.64, 30 N of 1.25 and 60 N of 1.12 (Table 3.7.). That vetch would be an efficient species when intercropped with barley is probably associated to its growth habit; the tendrils of vetch allowed it to attached to the barley thus providing support, and allowing it to receive more light. The lack of a consistent trend in LERs is contrary to results of a maize-cowpea intercropping study by Ofori and Stern (1986) who observed a tendency for N applications (25, 50 and 100 kg N ha^{-1}) to decrease LER values. As well, Hiebsch and McCollum (1987), through the evaluation of 182 legume-maize intercrop studies, found the intercrop advantage, as determined by area-x-time equivalency ratio, to be greatest when the maize was grown without adequate N.

INTERCROP CRUDE PROTEIN PRODUCTION

In 1991, there were no treatment effects on intercrop CP yield at the first cut (Table 3.7.). In 1992, there was a species effect on intercrop CP yield as IC-vetch CP yield (805 kg ha^{-1}) was higher than those of other intercrops and IC-*M. scutellata* CP yield (627 kg ha^{-1}) was also higher than that of IC-*M. ciliaris* (468 kg ha^{-1}) (Table 3.7.). All other intercrops did not differ in CP.

LEAF AREA INDEX OBSERVATIONS

August LAIs

A significant linear correlation was obtained between LAI, taken at least eight days before the second harvest, and the legume DM yield of the second cut (r = 0.782). There was a significant main effect of cropping system on LAI with MC-legumes (1.40) being higher than IC-legumes (0.82) (Table 3.12.). Also there was a significant species main effect, a C*Sp interaction and Sp*N interaction. Within monocrops, the red clover LAI of 2.71, averaged over all N-levels, was the highest of the legumes, although not significantly higher than MC-Nitro alfalfa. MC-vetch and MC-*M.scutellata* (1.21 and 0.85) were also lower than Nitro alfalfa (2.29) and persian clover (2.06) while *M. ciliaris* and *M. polymorpha* (0.31 and 0.27) were lowest of the MC-legumes.

Within intercrops, LAIs at the 0 N and 30 N levels (0.95 and 0.91) were higher than those at 60 N (0.61) and a Sp*N interaction existed. At 30 N and 60 N, the LAIs of IC-vetch (1.93 and 1.16) were the highest of the legumes, but at 30 N were not different from red and persian clovers and at 60 N were not different from red clover. Within the 0 N level red clover (2.40) was higher than all other legumes. Within each N level the LAI of the IC medics were lowest although at 0 N, *M. scutellata* (0.42) was not different from Nitro alfalfa and at 30 N *M. polymorpha* (0.36) was not different from persian clover and Nitro alfalfa, and *M. scutellata* (0.18) and *M. ciliaris* (0.16) were not

Table 3.12. Leaf area index (LAI) readings taken in August and November of 1992.

N	Sp	August MC-L	August IC-L	November MC-L	November IC-L
0	Pol	0.43	0.20	0.03	0.08
	Scu	1.02	0.42	0.06	0.17
	Cil	0.18	0.30	0.04	0.07
	Per	2.35	1.35	0.34	0.51
	Nit	2.85	0.64	0.53	0.30
	Vet	1.07	1.35	0.02	0.12
	Red	3.43	2.40	0.03	0.06
30	Pol	0.19	0.36	0.03	0.03
	Scu	0.89	0.18	0.03	0.07
	Cil	0.42	0.16	0.04	0.03
	Per	1.85	1.21	0.62	0.64
	Nit	1.83	0.75	0.36	0.13
	Vet	1.74	1.93	0.03	0.26
	Red	2.07	1.82	0.07	0.14
60	Pol	0.23	0.15	0.03	0.07
	Scu	0.67	0.21	0.04	0.09
	Cil	0.32	0.35	0.04	0.06
	Per	1.99	0.82	0.42	0.65
	Nit	2.20	0.51	0.32	0.07
	Vet	0.81	1.16	0.04	0.04
	Red	2.62	1.08	0.05	0.03
	LSD	0.81	0.51	0.24	0.24
	Sig. Effects	C, Sp, C*Sp, Sp*N		Sp, C*Sp	

MC-L = monocropped legume; IC-L = intercropped legume; See footnotes to table 3.4.

different from Nitro alfalfa, and at 60 N *M. ciliaris* (0.35) and *M. scutellata* (0.21) were similar to Nitro alfalfa.

<u>November LAIs</u>

As the November LAI readings were taken after the third cut, no correlation between these LAI readings and the legume DM yield of the third cut was attempted. LAI readings taken at this time were expected to predict the potential of these annuals as winter cover crops. There was a significant main effect of species on LAI, and a C*Sp interaction existed (Table 3.12.). Within monocrops, persian clover and Nitro alfalfa (0.46 and 0.40) ranked highest, while the LAIs of all other legumes were not significantly different. Within intercrops persian clover (0.60) had the highest average LAI of all legumes. As the third cut of persian clover was taken nearly one month earlier than the other species, it had a longer regrowth period before LAIs were taken, and this probably accounted for its higher LER value.

3.4. Conclusion:

In terms of TDM and ETCP production, red clover was highly productive under both monocrop and intercrop systems in both years, while the annual medics were least productive.

In 1991, averaged over both cropping systmes, Nitro alfalfa and persian clover produced as much TDM as red clover while vetch yielded similarly to persian clover and *M. ciliaris*. In 1992, TDM yields of MC-Nitro alfalfa and MC-persian clover did not differ from that of MC-red clover, but intercrops of Nitro alfalfa and persian clover were less productive than intercrops of vetch and red clover.

In 1991, while Nitro alfalfa and vetch, averaged over both cropping systems, were as productive in ETCP as red clover, persian clover was not. In 1992, although monocrops of Nitro alfalfa, vetch and persian clover did not differ in terms of ETCP, from red clover, intercrops of vetch were higher than intercrops of red clover while intercrops of Nitro alfalfa and persian clover were lower than red clover ETCP.

The IC-legumes competed with barley for resources, resulting in lower barley DM yield in intercrops at cut one, compared to MC-barley. Although the CP concentration of MC-barley did not differ from that of barley intercropped with any legume, in both years the CP yield of MC-barley was higher than that of barley intercropped with any legume. In addition, in 1991, the CP yields of barley intercropped with *M. scutellata* and red clover were higher than barley intercropped with vetch and *M. polymorpha* and in 1992 the CP yield of barley intercropped with *M. scutellata* was higher than that of barley intercropped with red clover.

In 1991, intercrops of red clover, Nitro alfalfa and vetch did not differ, in terms of TDM production, from MC-barley, and in 1992, intercrops of vetch and red clover outyielded MC-barley.

In 1991, intercrops of red clover outyielded MC-barley, in ETCP production, at each N level. In addition, at 30 N, intercrops of persian clover, vetch and Nitro alfalfa also outyielded MC-barley. In 1992, only ETCP yields of vetch and red clover intercrops were higher than MC-barley.

In 1991, the TDM production of intercrops responded positively to 30 N while 60 N appeared to have a negative effect on TDM. In neither year did the ETCP production of MC-legumes or intercrops respond to N fertilizer applications. Similarly, barley did not respond to N levels.

In 1991, the intercrops at cut 1 did not differ in CP or DM production. Also in 1992, intercrops at cut 1 did not differ in DM production, but intercrops of vetch produced higher CP yields than other intercrops.

In 1991 there was a trend for greater intercrop effectiveness at 30 N. Only monocrops of the components of vetch and persian clover intercrops at 30 N and vetch at 60 N would require more land to produce similar intercrop DM yeilds at cut 1. In 1992, only intercrops of vetch had an intercrop advantage.

Preface to Section 4

Section 4 consists of a manuscript by Sampson et *al.* (1993b) to be submitted to Biology and Fertility of Soils. The manuscript will undergo further editing prior to submission. Tables are presented on the page, or pages, immediately following the page in which they are first referred to. References cited in this section are listed within the thesis reference section.

In section 3, several of the intercrops in 1991 had total N yields which were at least 1.4 times higher in shoot N than that of monocropped barley and in 1992, intercrops of vetch and red clover were 2.6 and 2.1 times higher in N yields than monocropped barley. In the following section the N_2-fixation capabilities of the legumes are examined in the 0 kg N ha^{-1} level of section 3 through use of the ^{15}N isotope dilution method, and the occurrence of N transfer determined. In addition, within the 60 kg N ha^{-1} level of section 3, the occurrence of N transfer was determined by directly labelling legume petioles with ^{15}N enriched fertilizer.

Section 4

NITROGEN FIXATION AND TRANSFER IN ANNUAL FORAGE LEGUME-BARLEY (*Hordeum vulgare* L.) CROPPING SYSTEMS

4.0. Abstract:

In this study, the N_2-fixation abilities of six annual legumes, and the perennial, red clover (*Trifolium pratense* L.), monocropped (MC) and intercropped (IC) with barley, were investigated in a 2 x 7 factorial plus one MC-barley plot. Nitrogen transfer was also examined using the ^{15}N isotope dilution (ID) method. Microplots were enriched with 1.5 kg ^{15}N ha^{-1} as ammonium sulphate (99 atom % ^{15}N). The hypotheses were that the proportion of N derived from N_2-fixation in IC-legumes would be higher than that of MC-legumes and that within intercrops there would be evidence of N-transfer from the legumes to associated barley during the growing season. In separate plots, having received 60 kg N ha^{-1}, a 2 x 3 factorial was established with two levels of N fertilizer, enriched in ^{15}N (99 atom % ^{15}N) or unenriched, and three legume species, red clover, persian clover (*T. resupinatum* L.) and *Medicago ciliaris* (L.)Willd., were directly labelled with the fertilizer. Associated plants were harvested one week later with the hypothesis that labelled N would be detected in these plants. Only in 1992, was there evidence of N_2-fixation, and the proportion of N derived from N_2-fixation by IC-legumes was 145 % higher than that of MC-legumes. The N_2-fixing MC-legumes yielded similar values of fixed N while IC-vetch yielded higher (64 kg N ha^{-1}) than the rest of the IC-legumes. While the ^{15}N ID method did not give evidence of N transfer during the growing season, the direct labelling method provided evidence of N transfer from legumes to associated legume and barley plants in 1991, and to associated legume plants in 1992.

4.1 Introduction:

Nitrogen advantages have been reported in legume-non legume intercrops, compared to monocropped (MC) non-legumes. In Alberta, Izaurralde *et al.* (1990) reported barley-field pea (*Pisum sativum* L.) intercrops produced grain N yields 1.2 to 1.7 times higher than that of monocropped (MC) barley. Tidwell *et al.* (1985) monocropped and intercropped (IC) two winter wheat cultivars (*Triticum aestivum* L.) with hairy vetch (*Vicia villosa* Roth.) and harvested plots at five wheat maturity stages ranging from boot to soft dough, and reported the crude protein content of intercrops harvested at milk and soft dough stage to be 1.81 and 3.06 percentage units, respectively, higher than MC-wheat while MC-dry matter yields of each cultivar, averaged over each harvest, did not differ from the intercrop yield.

This N advantage is often attributed to the ability of *Rhizobium* or *Bradyrhizobium*, symbiotically associated with legumes, to convert the gas N_2, which is unavailable to most higher plants, to plant usable forms of N (Tisdale *et al.* 1985). Most of the N requirements of forage legumes can be supplied by biologically fixed N_2 (Danso *et al.* 1988; Heichel and Henjum 1991) but as legumes may also utilize mineral N, one cannot assume 100 % of legume N is derived from the atmosphere (Ledgard and Steele 1992). In addition, symbiotic N_2-fixation cannot be considered a free fertilizer since plants must provide photosynthate energy (LaRue and Patterson 1981) and for this reason, N_2-fixers prefer to use combined N if it is available (Sprent 1990).

One reason for intercropping legumes with non-legumes is that this fixed N may become available to associated non-legumes (Wahua and Miller 1978). The excretion of N compounds by plants, as stated by Wilson (1937) is known to occur. Lory *et al.* (1992) quantified the deposition of symbiotically fixed N_2 into the soil surrounding MC-alfalfa (*Medicago sativa* L.) roots and nodules resulting from excretion or leakage from roots and nodules, or from the sloughing off of root cells. The authors employed the ^{15}N ID method in a field and greenhouse study to compare the ^{15}N concentration of the rhizoshpere of effectively nodulated alfalfa with that of ineffectively nodulated alfalfa. In the field study, there was no evidence of deposition of symbiotically fixed N_2 to the rhizosphere (soil adhering to roots) and in the greenhouse study, there was evidence that 1 kg of fixed N_2 was deposited into the rhizosphere. In both the field and greenhouse, there was evidence of deposition of symbiotically fixed N_2 into the nodusphere soil (soil adhering to nodules). In the field and greenhouse these values were less than 0.1 kg ha^{-1} and approximately 0.4 kg ha^{-1}, respectively. The authors questioned the validity of these values due to the possibility of leakage resulting from the use of the sonicator in soil removal. Ta *et al.* (1986) examined the root excretions, defined as substances lost through root epidermis, of hydroponically grown alfalfa. Once the plants reached the 10 % bloom stage, they were exposed to an environment enriched in $^{15}N_2$. It was determined that the nodule-root system excreted 15 *ug* N $plant^{-1}$ day^{-1}, approximately 3 % of the total daily fixed N. As the hydryponic solution was enriched in ^{15}N only one day after the plants were exposed to $^{15}N_2$, it was determined that recently fixed N contributed to the excreted N.

In a greenhouse study, Ledgard *et al.* (1985) labelled subterranean clover (*T. subterraneum* L.) with ^{15}N through a foliar absorption technique and found 2.2 % of the clover N had transferred to the associated annual ryegrass (*Lolium rigidum* Gaud). In a similar study in the field they observed no such transfer from either the clover or alfalfa and concluded the greater contact of the two roots in pots, compared to field conditions, would make it easier for any N released by the legume to be asborbed by the grass. Martin *et al.* (1991b) also employed the ^{15}N foliar absorption technique in a field study and reported evidence of N transfer. By applying urea enriched with ^{15}N

directly to the petiole of nodulating soybeans (*Glycine max* L.) they were able to detect higher ^{15}N levels in adjacent maize (*Zea mays* L.) than when unenriched urea was similarly applied.

Through the use of the ^{15}N ID method, Brophy *et al.* (1987) observed an increase in the percentage of reed canarygrass (*Phalaris arundinacea* L.) N derived from transfer from associated alfalfa and birdsfoot trefoil (*Lotus corniculatus* L.) to increase as the season progressed. The percentage of grass N derived from associated alfalfa increased from 64 % at cut 1 to 68 % at cut 3, and similarly, the percentage of grass N derived from associated birdsfoot trefoil increased from 68 % to 79 %. In a four year study of alfalfa, birdsfoot trefoil, red clover and white clover (*T. repens* L.), in binary mixture with reed canarygrass, Heichel and Henjum (1991) measured N transfer using the ^{15}N ID method. Although negligible N was transferred from the legumes to the grass in year 1, the amount of N transferred in years 2, 3 and 4, averaged over each legume, were 28 kg N ha^{-1}, 10 kg N ha^{-1} and 6 kg N ha^{-1}, respectively.

Although Izaurralde *et al.* (1992) were unable to detect N transfer, using the ^{15}N ID method, during the growing season from field pea to IC-barley, grown either in the same rows or alternating rows, the authors concluded that such intercrops may provide benefits such as greater N_2-fixation efficiency. The authors reported the percent N of the IC-peas derived from fixation (84 %) to be higher than that of MC-peas (60 %) and that it was N competition between the IC-components which resulted in this increase. Brophy *et al.* (1987) studied N_2-fixation and transfer in alfalfa and birdsfoot trefoil with reed canarygrass in plots of alternating rows of grass and legume which formed a gradient from grass to legume. The authors found the highest estimates of the percent legume N derived from fixation, for four of the six species-harvest combinations, to be in legume rows surrounded by the highest grass population (legume-grass ration 2:3), while the lowest estimates occurred in the first harvest in legume rows near the plot edge. The maximum effect of grass on alfalfa N_2-fixation at cut 3 resulted in the legume fixing 95 % of its N, while MC-alfalfa fixed 86 % of its N. The maximun effect of grass on birdsfoot trefoil N_2-fixation at cut 3 resulted in the legume fixing 92 % of its N, while the MC-legume fixed 80 % of its N. The authors reasoned that the observed increase in legume reliance on fixation of legumes associated with grass resulted from the uptake of soil N by the grass, and that this depletion of mineral N also lessened the negative effect of mineral N on N_2-fixation.

In an associated study of seven annual legumes monocropped and intercropped with barley (Sampson et *al.* 1993a), we found the total N yield of several intercrops in 1991, to be at least 1.4 times higher in shoot N than that of MC-barley and in 1992, intercrops of vetch and red clover were 2.6 and 2.1 times higher in N yields than MC-barley. The objectives in this study were to determine, through use of the ^{15}N ID method, whether N transfer from these annual legumes to barley occurred during the growing season and to quantify any transfer; to assess legume N transfer to barley by directly labelling legume petioles with N enriched in ^{15}N; and to evaluate the N_2-fixation capabilities of annual legumes by the ^{15}N ID method, within monocrop and intercrop systems.

4.2. Materials and Methods:

This experiment was conducted in conjunction with the study in section three, and all materials and methods are the same unless otherwise stated.

<u>^{15}N Dilution Study</u>

The experiment was a randomized complete block design with four replicates and was established within the 0 N main plot units of a separate agronomic evaluation in a split plot arrangement. The experiment was a 2 x 7 + 1 factorial with two levels of cropping system:

monocropped (MC) legumes and legumes intercropped (IC) with barley (cv. Chapais), seven levels of legume species and one MC-barley plot (as described in chapter one). On July 9, 1991 and June 16, 1992, 1.5 kg ^{15}N ha^{-1} was applied to all plots as ammonium sulphate (99 atom % ^{15}N). The area of application was within 0.04 m^2 (16 cm x 25 cm) microplots, delineated by a plastic insert, to a depth of 2.5 cm in 1991 and to a depth of 6 cm in 1992. The plastic insert served to prevent the possible lateral movement of the applied nitrogen (Haystead and Lowe 1977; Heichel *et al.* 1981). Each microplot was placed in the middle of each plot and contained two legume rows in the MC-legume plots, one legume row and one barley row in IC-plots and one barley row (middle) in MC-barley plots. N was applied in solution to the area between the two middle rows and was applied directly to the left of the middle row of MC-barley plots. The N application container was rinsed twice with water and soil was sprinkled on the area of application to prevent N volatilization.

Plots were harvested as described in section three with the area of harvest consisting of the entire microplot. The concentration of total N was determined on subsamples from ground tissue from each species. Percent ^{15}N abundance of all plant material was determined by an adaptation of the Dumas method (Preston *et al.* 1981; Fiedler and Proksch, 1975). Kjeldahl distillate solution was converted to nitrogen gas as described by Martin *et al.* (1991b) for analysis on a JASCO N-150 Emission Spectrometer.

The percentage of nitrogen derived from the atmosphere (% Ndfa) by the legumes was calculated using formula 1 (Morris and Weaver 1987; Izaurralde *et al.* 1992) with MC-barley as the non-N_2-fixing control. The assumption of this formula was that the N_2-fixer and non-fixer absorb soil and applied N in the same proportion (McAuliffe *et al.* 1958).

Formula 1. $$\%Ndfa = \left(1 - \frac{\text{atom \% }^{15}\text{N excess in legume}}{\text{atom \% }^{15}\text{N excess in MC-barley}}\right) \times 100$$

where atom % ^{15}N excess = atom % ^{15}N abundance - 0.3663

Nitrogen fixation was only assumed to have occured when the atom % ^{15}N excess of the legume was significantly lower than that of the MC-barley.

The percentage of nitrogen transferred (%N T) from the legume to the IC-barley was calculated using formula 2 (Izaurralde *et al.* 1992). Monocropped barley was the control for barley intercropped with legumes for measuring N transfer by the ^{15}N ID method.

Formula 2. $$\%N\ T = \left(1 - \frac{\text{atom \% }^{15}\text{N excess in IC-barley}}{\text{atom \% }^{15}\text{N excess in MC-barley}}\right) \times 100$$

Nitrogen transfer was only assumed to occur when the atom % ^{15}N excess of the IC-barley was significantly lower than that of the MC-barley (Martin *et al.* 1991a; Okereke and Anyama 1992).

To determine if N_2-fixation and N transfer occurred, atom % ^{15}N excess data were analyzed by analysis of variance (Statistical Analysis System Institute Inc. 1985) at the 5 % level of significance after a log transformation of the data, and where significant differences were indicated, Dunnet's Multiple Comparison Procedure was used (Dunnet 1955) to compare treatments with a control (MC-barley). In determining the occurrence of N_2-fixation, the control, atom % ^{15}N excess MC-barley was compared with the atom % ^{15}N excess of the legumes while in determining the occurrence of transfer, the control, atom % ^{15}N excess of MC-barley was compared with the atom % ^{15}N excess of IC-barley. Yields and observations were also analyzed by analysis of variance at the 5 % level of significance and a protected LSD test (Steel and Torrie, 1980), unless otherwise noted, was utilized for means comparison where significant differences were indicated.

Direct Labelling Study

The experiment was a randomized complete block design with four replicates and was established within the 60 kg N ha^{-1} main plot units of a separate agronomic evaluation in a split plot arrangement. The main plot of 60 kg N ha^{-1} was chosen for the study as N transfer from directly labelled nodulating soybean to adjacent corn plants had previously been observed on N depleted soil (Martin *et al.* 1991b), and it was of interest to determine if such transfer could be detected against high background levels of soil N. The experiment was a 2 x 3 factorial with two levels of N fertilizer, enriched in ^{15}N or unenriched, and three plant species red clover, persian clover and *M. ciliaris*. The legumes were intercropped with barley and established as described in the ^{15}N ID study, with the addition of 60 kg ha^{-1} as ammonium nitrate (34-0-0) on June 25, 1991 and on June 16, 1992. Microplots were not employed as in the ^{15}N ID study.

On July 12, 1991 and on July 16, 1992, one legume plant (donor legume) in each ^{15}N enriched plot (E) was labelled with 30 mg N as a 2 % (w/v) solution of double labelled urea (99 atom % ^{15}N). Similarly, one legume plant (donor legume) in each unenriched plot (UE) was labelled with 30 mg ^{14}N urea. In a preliminary greenhouse study, this concentration of urea was found to be an acceptable level for petiole uptake. The trifoliate at the first or second node was cut off and the petiole immersed in the urea solution. The vial of urea was securely fastened to a support and the vial and petiole covered with parafilm. Petioles were checked daily for damage, and damaged petioles were replaced with petioles from the next node. In 1991, petioles were removed from solution after seven days (July 19) and in 1992, were removed after eight days (July 24).

Plants were harvested by hand on July 22, 1991 and on July 24, 1992. Harvested plants in each plot included the donor legume and the closest plants, a neighbouring legume (receiver legume) and two neighbouring barley plants (receiver barley). Plants were dried for at least 48 hours in a 75°C dryer. Dried plant material was ground and analyzed for % ^{15}N abundance as described in the ^{15}N ID study. Percent ^{15}N abundance values of the two receiver barleys were averaged together.

To determine if transfer occurred, atom % ^{15}N excess data were analyzed by analysis of variance (Statistical Analysis System Institute Inc. 1985) at the 5 % level of significance after a log transformation of the data and a protected LSD test (Steele and Torrie, 1980) was utilized for means comparison where significant differences were indicated. Where significant differences were indicated, the proportion of N transferred (T) was determined by formula 3 (Ledgard *et al.* 1985) which included the proportion of transferred ^{15}N in receiver plants.

Formula 3. $T = N_r(R_E - R_{UE}) \ / \ [N_l(L_E - L_{UE}) + N_r(R_E - R_{UE})]$

where N_r and N_l are the amounts of nitrogen assimilated by the receiver plant and donor legume, respectively.
R_E and R_{UE} are the atoms % ^{15}N of the enriched and unenriched receiver
L_E and L_{UE} are the atoms % ^{15}N of the enriched and unenriched donor legume

The amount of N transferred was determined by multiplying T by the amount of nitrogen assimilated by the legume.

4.3. Results and Discussion:

NITROGEN FIXATION: ^{15}N ISOTOPE DILUTION (ID) METHOD

The ^{15}N ID method is based upon the comparison of an N_2-fixing and a non-N_2-fixing system. The possible N sources of these systems are: fertilizer, soil, and in the case of the N_2-fixing system, the atmosphere. The N in plants derived from fertilizer in the soil will be enriched in ^{15}N and in the N_2-fixer, fixed atmospheric $^{14}N_2$ will dilute it, compared to the non-N_2-fixer. In 1991, there were no legumes with atom % ^{15}N excess lower than that of the MC-barley (Table 4.1.), and as such, there was no evidence of N_2-fixation by any of the legumes.

Actually, the atom % ^{15}N excess of IC-*M. polymorpha* and IC-*M. scutellata* were higher than that of MC-barley. Compared to these medics, the ^{15}N of the barley was diluted. When ^{15}N labelled fertilizers are applied to soils, a highly enriched layer is created near the surface (Peoples and Herridge 1990). As the ^{15}N fertilizer was applied 42 days after seeding in our study, due to dry soil conditions, the barley may have explored soil volumes of different enrichment compared to these medics. If IC-*M. polymorpha* and IC- *M. scutellata* had access only to N near the soil surface, they would have been more enriched in ^{15}N, as is the case.

Results from soil tests taken in the fall of 1990 and 1991 recommended no N fertilizer be applied to the site. Plant available N was not limiting plant growth, as seen in an associated study on the site in which barley, monocropped or intercropped with annual legumes, did not respond to N applications of 30 or 60 kg N ha^{-1}. In addition, although monthly rainfall totals in 1991 did not vary greatly from their 30 year normals, the rainfall was not evenly distributed and parts of the growing season were relatively dry, as reported in chapter one. Ledgard *et al.* (1990) reported the proportion of N fixed by several white clover cultivars (*T. repens* L.) and red clover, as estimated by the ^{15}N ID method, declined during the summer in response to an increase in inorganic soil N which resulted from dry soil conditions.

That the legumes in our study were exposed to a high level of soil N may partially explain the lack of evidence of N_2-fixation. The proportion of legume N derived from soil N may have been so high that any dilution of ^{15}N by fixed atmospheric N was insignificant. The onset of N_2-fixation by these legumes may also have been slow, and in fact the legumes were harvested only 55 days after seeding. Perhaps N_2-fixation might have been detected in subsequent cuts. Indeed, Ledgard *et al.* (1990) observed that clovers fixed over 50 % of their N four months after sowing.

Table 4.1. Log transformation of atom % ^{15}N excess in monocropped (MC) legumes, intercropped (IC) legumes and monocropped barley in 1991 and 1992. Untransformed values in brackets.

	Sp	1991		1992	
MC	Pol	-0.8882	(0.1361)	-0.1573	(0.8952)
	Scu	-0.7593	(0.1899)	-0.3472	(0.4600)
	Cil	-0.7124	(0.2011)	0.0214	(1.1255)
	Per	-0.9541	(0.1175)	-0.3559 *	(0.4625)
	Nit	-0.9681	(0.1085)	-0.2766	(0.5425)
	Vet	-1.0098	(0.0983)	-0.6060 *	(0.2530)
	Red	-0.8112	(0.1548)	-0.3700 *	(0 4311)
IC	Pol	-0.3737 *	(0.5133)	-0.2008	(0.6402)
	Scu	-0.4930 *	(0.3501)	-1.0477 *	(0 0953)
	Cil	-0.7188	(0.2119)	-0.4265 *	(0.5047)
	Per	-0.5252	(0.3415)	-0.1055 *	(0.0952)
	Nit	-0.7737	(0.1839)	-0.7111 *	(0.2028)
	Vet	-0.8672	(0.1390)	-1.3410 *	(0.0465)
	Red	-0.8534	(0.1449)	-0.9267 *	(0.1267)
M	Barley	-0.8879	(0.1461)	0.0479	(1.1260)
Dunnett's critical T value		2.955		2.955	

* significantly different than MC barley ($p < 0.05$); Sp = species; pol = *M. polymorpha*; scu = *M. scutellata*; cil = *M. ciliaris*; per = persian clover; nit = Nitro alfalfa; vet = vetch; red = red clover; MC = monocropped; IC = intercropped.

The ^{15}N ID method is based on the assumption that the N_2-fixer and non-N_2-fixer absorb the same proportion of soil and fertilizer N and, that the legume does not release fixed N to the non-N_2-fixer (McAuliffe *et al.* 1958). Therefore, one assumes the enrichment of the assimilated N of the non-N_2-fixer equals that of the N_2-fixer (Knowles 1980). Peoples and Herridge (1990) stated that it is when the proportion of N derived from N_2-fixation is small, that errors resulting from incompatible non-N_2-fixer and N_2-fixer are most important. As the ^{15}N enrichment of the soil N pool may decline over time and with depth, it is under ideal conditions that roots of the non-N_2-fixer and N_2-fixer explore a similar volume of soil of similar enrichment. Papastylianou and Danso (1990), in a study of vetch (*Vicia sativa* L.) monocropped and intercropped with oats (*Avena sativa* L.), reported the use of organic material enriched in ^{15}N to label the soil, resulted in oat herbage atom % ^{15}N excess which was higher than corresponding vetch and therefore indicated N_2-fixation. The use of inorganic ^{15}N fertilizer at 20 and 60 kg N ha^{-1} resulted in several values of atom % ^{15}N excess which were similar for the oats and vetch, and did not support the occurrence of N_2-fixation. The authors reported the decline of ^{15}N enrichment decline of soil labelled with mineral N was over twice that of soil labelled with organic matter; and as the time course of total N uptake by the two species were not paralled, the authors concluded estimates using labelled organic matter were most accurate. In our study, barley and the annual legumes may have had different patterns of N-uptake and have assimilated N of unequal enrichment which may account for the lack of evidence of N_2-fixation, especially since the proportion of legume N derived from N_2-fixation was probably low due to high levels of inorganic N.

Since rainfall was more evenly distributed in 1992, ^{15}N fertilizer was applied earlier (by 22 days) than in 1991. Also, barley was slower to develop in 1992, resulting in delayed harvest compared with 1991. Therefore, in 1992, N_2-fixation was integrated over 44 days, the time between ^{15}N fertilizer application and harvest, while in 1991, the time of integration, only 13 days, was much shorter. In 1992, there was evidence of N_2-fixation as the atom % ^{15}N excess of several legumes was lower than that of the MC-barley (Table 4.1.). There was no evidence of N_2-fixation by MC-Nitro alfalfa, the MC-medics and IC-polymorpha.

1992 PERCENT NITROGEN DERIVED FROM N_2-FIXATION (% NFIX)

There was a main effect of cropping system on the % NFIX by the legumes; and IC-legumes (71 %) were higher than MC-legumes (29 %) (Table 4.2.). Similar response of intercropped legumes has also been reported by Izaurralde *et al.* (1992) for field pea intercropped with barley and by Brophy *et al.* (1987) for alfalfa and birdsfoot trefoil, each intercropped with reed canarygrass. That the replacement of half of the monocropped legumes by barley in our study resulted in an increased reliance of the legumes on N_2-fixation was probably due to the barley being more competetive for soil N. Through the employment of ^{15}N enriched fertilizer, Henzell *et al.* (1968) observed that in intercrops of rhodesgrass (*Chloris gayana* Kunth.) and sirato (*Phaseolus atropurpureus* D.C.), the grass took up two units of N for each unit of N taken up by the legume, while in monocrops, the authors found the component plants to be equally efficient in the uptake of added ^{15}N enriched fertilizer. As well, Butler and Ladd (1985) reported that on soils which released low amounts of inorganic N, *Medicago littoralis* outyielded annual ryegrass (*Lolium multiflorum*) while ryegrass outyielded the medic when they were grown on soils of higher N availability.

Table 4.2. Percent nitrogen derived from fixation (Nfix) and total atmospheric nitrogen (FN) (kg ha^{-1}) fixed by legumes in 1992.

	% Nfix		FN (kg ha^{-1})	
Sp	MC	IC	MC	IC
Pol	0	0	0	0
Scu	0	91.2	0	2.5
Cil	0	52.0	0	1.0
Per	58.6	91.7	50.7	16.0
Nit	0	82.3	0	6.6
Vet	77.0	95.7	47.5	63.6
Red	61.0	88.3	48.8	18.7
LSD	10.6	25.0	21.3	14.2
Sig. Effects	C, Sp, C*Sp		Sp, C*Sp	

LSD = least significant difference; Sig. Effects = significant effects ($p < 0.05$);
C = cropping system; see footnotes to table 4.1.

The reduction of mineral N in intercropped plots may also have lessened negative effects of high mineral N on the N_2-fixation process and have allowed intercropped legumes to fix a greater proportion of their N than monocropped legumes. In a study of alfalfa monocropped and intercropped with timothy (*Phleum pratense* L.), Ta and Faris (1987a) reported that in year 1, the soil N, compared to year 2, was higher and led to a greater proportion of legume N fixed by intercropped alfalfa compared to monocropped alfalfa. As high soil N levels inhibit nitrogenase activity, the authors reasoned that the intercropped timothy may have taken up more N during establishment and lessened the amount of soil N in the soil volume explored by the intercropped alfalfa. Rongquing *et al.* (1992) found the application of 5 and 10 Mm of N, per pot, as either urea, nitrate, ammonium, nitrate + ammonium, on faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.) and medic (*M. rugosa* Desr.) to negatively affect the number of active nodules, with the highest N concentration having the greatest effect. In addition, nodule initiation was also delayed by most N treatments and nitrogenase activity, determined by acetylene reduction assay, was also depressed by all N treatments. Alston and Graham (1982) reported the N_2-fixation of barrel medic (*M. truncatulata* Gaertn.) to be negatively correlated with mineral N and to not be related to the total N content of the soils.

In our study, there also existed a cropping system (C) * species (Sp) interaction . Within MC-legumes the % NFIX of vetch was highest of the legumes while the values for red clover and persian clover were similar. Within IC-legumes, % NFIX of the fixing legumes were similar, with the exception of *M. ciliaris*, which was lower than the rest.

1992 FIXED ATMOSPHERIC NITROGEN (FN)(kg ha^{-1})

There was no difference between cropping systems in terms of FN yield. This resulted from the monocropped legumes having produced higher dry matter (2344 kg ha^{-1}), averaged over each legume species, than intercropped legumes (513 kg ha^{-1}) which evened out the higher proportion of intercropped legume N derived from N_2-fixation. A C*Sp interaction existed (Table 4.2.) and within the monocropped legumes, FN values of persian clover, red clover and vetch were similar. Within the intercropped legumes, FN yield of vetch was highest of the legumes while values for red clover, persian clover and Nitro alfalfa were similar. Fixed N_2 yields of *M. ciliaris* were also lower than those of red clover and persian clover. Species ranking for FN mostly reflected dry matter yield and N yield differences (Table 4.3.).

NITROGEN TRANSFER: ^{15}N ID METHOD

In both 1991 and 1992, there were no intercropped barley treatments with atom % ^{15}N excess significantly lower than that of MC-barley (Table 4.4.), suggesting fixed N_2 did not dilute the ^{15}N enrichment of intercropped barley. Therefore, there was not sufficient

Table 4.3. Legume dry matter yield (DM) (kg ha^{-1}) and legume N yield (kg ha^{-1}) in 1991 and 1992.

		DM		N	
1991	Sp	MC	IC	MC	IC
	Pol	5147	218	128.5	5.2
	Scu	1281	44	47.8	1.3
	Cil	3029	140	63.6	3.1
	Per	2533	261	87.5	7.7
	Nit	2627	171	76.9	4.3
	Vet	2671	1006	118.8	42.8
	Red	1691	181	62.1	6.2
LSD		1955	149	51.5	7.5
Sig. Effects		C, Sp, C*Sp		C, Sp	
1992					
	Pol	3229	219	58.4	3.6
	Scu	2609	123	63.9	2.7
	Cil	1500	71	31.0	1.4
	Per	2781	544	82.7	17.4
	Nit	2556	336	66.6	8.1
	Vet	1578	1646	62.3	66.2
	Red	2378	650	78.6	21
LSD		ns	439	ns	14.0
Sig. Effects		C, C*Sp		C, Sp, C*Sp	

LSD = least significant difference; ns = not significantly different ($p < 0.05$);
Sig. Effects = significant effects ($p < 0.05$); see footnotes to table 4.1.

evidence for current-season N transfer of fixed N_2 from any legume species to IC-barley. In addition, only intercrops of vetch yielded higher in N yield (128 kg N ha^{-1}), although not significantly higher, than MC-barley (97 kg N ha^{-1})(data not shown). This was probably related to the fact that of the intercropped legumes, the estimated value of N fixed by vetch was highest. The N yield of MC-barley (data not shown) was similar to that of all intercrops, with the exception of those of *M. ciliaris* (59 kg ha^{-1}). Also, the dry matter yield of MC-barley (8091 kg ha^{-1}) was higher than all intercrops, with the exception of persian clover intercrops (6323 kg N ha^{-1}). That there was no intercropping advantage over MC-barley supports the lack of evidence of N transfer.

Table 4.4. Atom % ^{15}N excess in barley intercropped (IC) with legumes and monocropped (MC) in 1991 and 1992.

	Sp	1991		1992	
IC	Pol	-0.8578	(0.1483)	-0.0262	(0.9484)
	Scu	-0.9679	(0.1088)	-0.1950	(0.9123)
	Cil	-0.9089	(0.1267)	-0.0455	(0.9392)
	Per	-0.8591	(0.1418)	-0.0298	(1.3835)
	Nit	-0.8996	(0.1357)	-0.0458	(1.1969)
	Vet	-0.9147	(0.1260)	-0.0094	(1.1143)
	Red	-0.8774	(0.1351)	0.0481	(1.1276)
MC	Barley	-0.8879	(0.1461)	0.0479	(1.1260)
Dunnett's critical T value		ns			ns

ns = not significantly different than MC-barley ($p < 0.05$); see footnotes to table 4.1.

The fact that the soil was high in mineral N may explain the lack of evidence of transfer. On a highly fertile soil, with a high N mineralization capacity, Izaurralde *et al.* (1992) found no evidence of N transfer, during the growing season, from field pea to associated barley. Eaglesham *et al.* (1981) intercropped maize with cowpea (*Vigna unguiculata* (L.) Walp.) under different levels of N fertilizer and concluded that only under conditions of low soil mineral N may N transfer fr. the legume to associated non-legumes be detected using the ^{15}N ID meth... Similarly, Martin *et al.* (1991a) observed that N transf... ...ermined by the ^{15}N ID method, was best demonstrated under conditio... ... mineral N. Only on an N depleted soil, with one exception, were ... authors able to detect the transfer of N from nodulating soybean to associated non-nodulating soybean and maize.

Nitr... Transfer: ^{15}N Direct Labelling

In 1991 ... evidence of transfer of ^{15}N from donor legumes to the associ... ...ume and to two associated receiver barley plants ar... ...ess of receiver plants associated with enriched ... higher than those associated with unenriched donor legumes ...able 4...). The amount of N transferred to individual receiver plants from *M. ciliaris* ranged from 0.027 mg to 0.212 mg, and from persian clover ranged from 0.061 mg to 0.687 mg and from red clover ranged from 0.057 mg to 0.220 mg (data not shown).

In 1992, there was evidence of transfer of ^{15}N from the donor legume only to the associated receiver legume as only the atom % ^{15}N excess of the receiver legumes associated with enriched donor legumes were higher than those associated with unenriched donor legumes. No transfer of ^{15}N was observed to the associated receiver barley plants (Table 4.6.). The amount of N transferred from *M. ciliaris* ranged from 0.002 mg to 0.006 mg, and from persian clover ranged from 0.004 mg to 0.153 mg, and from red clover ranged from 0.009 mg and 0.034 mg (data not shown).

Table 4.5. Log transformation of atom % ^{15}N excess of receiver legume and receiver barley plants associated with donor legumes fertilized with N either enriched in ^{15}N (E) or unenriched in ^{15}N (UE) in 1991. Untransformed values in brackets.

	Receiver Legume				Receiver Barley			
Donor Legume	UE		E		UE		E	
Cil	-1.2171	(0.0927)	-0.5957	(0.2885)	-1.3900	(0.0432)	-0.8554	(0.1405)
Per	-1.2312	(0.0683)	-0.3078	(0.8302)	-1.3428	(0.0462)	-0.8024	(0.1759)
Red	-1.4634	(0.0350)	-0.1594	(0.6930)	-1.3355	(0.0463)	-0.9954	(0.1071)
Mean	-1.2950	(0.0680)	-0.3930	(0.5860)	-1.3562	(0.0453)	-0.8844	(0.1412)
Fertilizer LSD		0.3790				0.1468		

LSD = least significant difference; See footnotes to table 4.1.

Table 4.6. Log transformation of atom % ^{15}N excess of receiver legume and receiver barley plants associated with donor legumes fertilized with N either enriched in ^{15}N (E) or unenriched in ^{15}N (UE) in 1992. Untransformed values in brackets.

	Receiver Legume				Receiver Barley			
Donor Legume	UE		E		UE		E	
Cil	-1.4353	(0.0384)	-0.6840	(0.6022)	-1.5530	(0.0284)	-1.3414	(0.0588)
Per	-1.5361	(0.0294)	-0.7344	(0.2973)	-1.5320	(0.0303)	-1.4470	(0.0358)
Red	-1.3659	(0.0465)	-0.6664	(0.2418)	-1.5279	(0.0317)	-1.5001	(0.0372)
Mean	-1.4380	(0.0390)	-0.6950	(0.3800)	-1.5377	(0.0302)	-1.4295	(0.0440)
Fertilizer LSD	0.3300				ns			

LSD = least significant difference; ns = not significant ($p < 0.05$); See footnotes to table 4.5.

Martin *et al.* (1991b) reported evidence of N transfer on an N depleted field from soybean, enriched in ^{15}N by labelling petioles, to associated maize plants. Ledgard *et al.* (1985) had previously reported no evidence of N transfer in field grown microplots of subterranean clover intercropped with annual ryegrass (*Lolium rigidum* Guad.) or of alfalfa intercropped with the grass, when clover trifoliate leaves and alfalfa stems were labelled with ^{15}N fertilizer. Although the N which was transferred in this study was not derived from fixation, it may be assumed that or this soil of a high mineral N level, fixed N_2 could similarly be transferred from the legume to an associated plant (Martin *et al.* 1991b).

4.4. Conclusion:

In 1991, there was no evidence of N_2-fixation by any legume species. In 1992, there was evidence of N_2-fixation by the legumes, with the exceptions of MC-Nitro alfalfa, MC-medics and IC-*M. polymorpha*.

In 1992, the percentage of N derived from N_2-fixation of the intercropped legumes (71 %) was higher than that of the monocropped legumes (29 %). Of the monocropped legumes, vetch fixed a higher proportion of N than MC-red clover and MC-persian clover. The intercropped legumes fixed similar proportions of N with the exception of *M. ciliaris* which fixed a lower proportion of N than the rest of the N_2-fixing legumes.

The amount of N fixed by the legumes in 1992, reflected dry matter yield and nitrogen yield differences as the N_2-fixing monocropped legumes yielded similar fixation values while IC-vetch yielded higher in fixed-N than the rest of the intercropped legumes. Intercropped red clover, persian clover and Nitro alfalfa fixed similar estimates of N, while red clover yielded higher in fixed N than the annual medics and persian clover also yielded higher than IC-*M. ciliaris*.

While there was no evidence of N transfer by the ^{15}N ID method, the direct labelling method did provide evidence of N transfer in both years under conditions of high soil mineral N.

Section 5

GENERAL DISCUSSION

The total dry matter yields and estimated total crude protein yields of red clover monocrops and intercrops were highly productive in both years. In neither year were total dry matter and estimated total crude protein yields of red clover monocrops or intercrops lower than the rest of the monocropped (MC) legumes or intercrops, with an exception in 1992, where the estimated total crude protein yield of red clover intercrops was lower than intercrops of vetch, but was higher than that of the other intercrops. Monocrops and intercrops of the annual medics were least productive.

Although intercropped (IC) legumes competed with barley for resources, resulting in decreased barley dry matter yield and crude protein yield compared to MC-barley, many intercrops were as productive as MC-barley in terms of total dry matter production and estimated total crude protein yield. In 1991, the total dry matter yield of intercrops of red clover, Nitro alfalfa and vetch did not differ from MC-barley but the rest of the intercrops yielded lower than MC-barley. In 1992, intercrops of vetch and red clover outyielded MC-barley, which did not differ from intercrops of Nitro alfalfa and outyielded the rest of the intercrops. In 1991, the estimated total crude protein yields of red clover intercrops outyielded MC-barley at each N level. In addition, at 30 kg N ha^{-1}, intercrops of persian clover, vetch and Nitro alfalfa also outyielded MC-barley. Only the intercrops of *M. scutellata* and *M. polymorpha* at 0 kg N ha^{-1} and of *M. ciliaris* and *M. polymorpha* at 60 kg N ha^{-1} yielded less estimated total crude protein than MC-barley. In 1992, the estimated total crude protein yield of intercrops of vetch and red clover outyielded MC-barley while MC-barley outyieded intercrops of *M. polymorpha* and *M. ciliaris*.

In neither year did the dry matter production of intercrops differ at cut 1. In 1991, the intercrops at cut 1 did not differ in crude protein yield. In 1992, the crude protein yield of intercrops of vetch at cut 1 outyielded the rest of the intercrops and intercrops of *M. ciliaris* yielded lower in crude protein than intercrops of *M. scutellata*.

In neither year did the estimated total crude protein production of monocropped legumes or intercrops respond to N fertilizer applications, nor did barley. In 1991, the total dry matter production of intercrops responded positively to 30 kg N ha^{-1} while 60 kg N ha^{-1} appeared to have a negative effect on total dry matter yield. Indeed, in 1991, there was a trend for greater intercrop effectiveness at 30 kg N ha^{-1}. Only monocrops of the components of vetch and persian clover intercrops at 30 kg N ha^{-1} and vetch at 60 kg N ha^{-1} would require more land to produce similar intercrop dry matter yields at cut 1. In 1992, only intercrops of vetch exhibited an intercrop advantage.

The ^{15}N ID method did not provide evidence of legume N_2-fixation in 1991. This lack of evidence was probably related to the high mineral N level of the soil and any dilution of legume ^{15}N by fixed atmospheric N may have been insignificant. In addition, the ^{15}N ID method assumption, that the N_2-fixer and non-N_2-fixer absorbed the same proportion of soil and fertilizer N (McAuliffe *et al.* 1958), may not have been met. In 1992, there was evidence of N_2-fixation, with the exceptions of MC-Nitro alfalfa, MC-annual medics and IC-*M. polymorpha*, and the proportion of N derived from N_2-fixation by intercropped legumes was 145 % higher than that of monocropped legumes.

In 1992, while monocrops of persian clover, red clover and vetch did not differ in terms of fixed N yield, the fixed N yield of IC-vetch was highest of the intercropped legumes while those of persian clover and Nitro alfalfa did not differ from that of IC-red clover.

The ^{15}N ID method gave no evidence of current season transfer of symbiotically fixed N. As with the lack of evidence of N_2-fixation in 1991, this too probably related to the high level of soil mineral N (Eaglesham *et al.* 1981; Martin *et al.* 1991a).

Although Ledgard *et al.* (1985) were unable to detect N transfer in the field using the ^{15}N direct labelling method, and Martin *et al.* (1991b) reported evidence of N transfer, on an N depleted soil, also using this method, we were able to obtain evidence of N transfer from *M. ciliaris*, red clover and persian clover, by directly labelling legume petioles with ^{15}N, to associated barley and legume plants in 1991, and to associated legume plants in 1992, on a soil high in mineral N.

Section 6

SUGGESTIONS FOR FUTURE RESEARCH

1. There should be further evaluation of the legumes intercropped with an annual grass, which would regrow after each cut.

2. Nitrogen fixation of these legumes and their transfer of N to associated grasses, as determined by the ^{15}N isotope dilution method, should be examined further on N depleted soils, or on soils known to be low in mineral N.

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