FORAGE LEGUME INOCULATION AND GRASS-LEGUME ASSOCIATION STUDIES

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CERTIFICATE

This is to certify that the thesis entitled "FORAGE LEGUME INOCULATION AND GRASS-LEGUME ASSOCIATION STUDIES" submitted by Mr. H.S.Gopala Gowda, for the degree of MASTER OF SCIENCE in AGRICULTURAL MICROBIOLOGY of the University of Agricultural Sciences, Bangalore, is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis of the award of degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

I. INTRODUCTION

The outstanding value of grass-legume association rests on the property of the legume to fix nitrogen when nodulated by appropriate strains of rhizobia. The nitrogen so fixed becomes available to the associated grass through decay of dead leaves, stems, roots and nodules of the legume. The more effective the rhizobial strain the more dry weight is produced. Usually land with low fertility is grown to grasses. The use of nitrogenous fertilizers is generally not advocated, because of low economic returns and comparatively high cost of nitrogen fertilizers.

Whyte et al. (1953) listed India (undivided, including Burma) as a centre, next to China in importance for origin of legumes. Norris (1966) contended that legumes were tropical in origin and he pictured their spread to temperate and arid regions as an outward migration from the tropics. This indicated that India has the right environmental conditions for growing a wide variety of legumes. Yet very little effort is made in India to exploit this natural advantage to augment grass yields through mixed pastures of grass and legume.

Grass-legume association was studied by various workers for the increased production of fodder using different combinations of grass and legumes, depending upon the agroclimatic conditions.

Roberts and Olson (1942) reported that higher yields could be obtained per unit area of grass in grass-legume mixtures than in pure stand of either grass or legume. Henzell and Norris (1962) have cited instances of transfer of N fixed by tropical forage legumes to grass in the pasture consisting of Stylosanthes gracilis/ Heteropogon contortus, Pueraria thunbergiana/Melinis minutiflora and Panicum maximum. Fernando (1961) reported that the yield of pasture was considerably increased by legume association consisting of Brachiaria brizantha/ Centrosema pubescens as compared to pure grass stand. In India, Singh and Chatterjee (1968a and b) have conducted experiments during 1962-65 to compare herbage production under frequent and infrequent defoliation with 12 grasses and one legume (Centrosema pubescens). They have reported that under both defoliation treatments, total forage yield in grass/Centrosema mixture was more than that in pure grass stand. Chatterjee et al. (1969) reported that Heteropogon contortus grown in mixture with legume Stylosanthes humilis, S.gracilis or Centrosema pubescens yielded more than the pure grass stand. Whitney and Green (1969a) have carried out extensive field trials on grass-legume mixtures in United States of America. They have also compared the yields of grass grown in association with both inoculated and uninoculated legumes. In their studies, the yield of Pangola grass (*Digitaria decumbens*) grown with inoculated legumes *Desmodium canum* and *D. intortum* were higher than the yield of grass grown with uninoculated legumes.

Bowen (1956) reported that different legumes like *Glycine max*, *G. javanica*, *Pueraria phaseoloides*, *Centrosema pubescens*, *Clitoria ternatea* and *Indigofera hirsuta* responded well to the *Rhizobium* inoculation. On the other hand, Wales (1957) showed that legumes like *Phaseolus lathyroides*, *Dolichos biflorus*, *Centrosema pubescens* and *Lespedeza striata* planted with or without inoculation nodulated equally well.

Most of the work in India on grass-legume association was with applied nitrogen rather than with inoculation of legumes. Further, there are no appropriate rhizobial inoculants for legumes to be grown with associated grass. It was therefore, wished to study the contribution of inoculated and uninoculated legume nitrogen to the associated grass.

Accurate estimates of nitrogen fixation by legumes are very difficult under field conditions. The degree of control of experimental conditions required to arrive at accurate estimates is not possible because of nonspecific fixation of N, N losses through leaching and denitrification, etc.

At best, approximate values of N contribution from legume to associated grass can be assessed indirectly by determining the N in grass tops in cutting experiments. This method is attempted in these studies. Here pure grass plot and uninoculated legume/grass plot would indicate approximately the amount of soil N taken up by the grass and the extent of symbiotic N fixed by naturally nodulated legume and transferred to the grass, respectively and the difference between the sum of these two N values and the total N of the grass grown in association with inoculated legume would give the N fixation due to inoculation.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

1. General

Graham (1951) has studied the various grass-legume associations in pasture for beef production, viz. Panicum maximum var. coloratum / Centrosema pubescens, Melinis minutiflora / Calopogonium muconoides, P.maximum / Stylosanthes guianensis, and M.minutiflora / Pueraria phaseoloides combinations and recommended them for general cultivation. Pyne (1955) reported that the Panicum maximum / Centrosema pubescens association gave the most promising result.

In a grass-legume association study, it was found that the most productive legumes were Pueraria phaseoloides, Centrosema pubescens, Indigofera subulata, I.endecaphylla, Calopogonium muconoides and Dolichos hosii, whereas the best grass-legume mixture was Brachiaria mutica/ Centrosema pubescens (Anonymous, 1956). Patil (1956) used various grass-legume mixtures viz. Alysicarpus rugosus, Indigofera glandulosa, Desmodium uncinatum, D.nicaraguense, Centrosema pubescens, Calopogonium muconoides, Pueraria thunbergiana, P.phaseoloides, Glycine javanica, Desmanthus virgatus and Leucaena glauca on the grass lands of Bombay State and recommended them for cultivation along with grasses.

Neme and Soja (1958) found that Glycine javanica, a perennial creeping species could be grown for fodder either alone or mixed with grasses. Walsh (1958) has reported that the legumes like *Glycine javanica*, *Centrosema pubescens*, *Pueraria phaseoloides*, *Calopogonium muconoides* and *Stylosanthes gracilis* tended to do best when used in association with tall growing vigorous grasses on which they could climb. Andrade *et al*. (1970) have showed that in South America it was preferable to grow *Calopogonium muconoides* in association with grasses.

2. Studies on the nodulation of forage legumes

Samuels and Landrau (1953a), studied the effect of ammonium sulphate and borax on nodulation in *Pueraria phaseoloides*. Ammonium sulphate application at 250 lb per acre had an adverse effect on nodulation which was improved to some extent by liming. Whereas, Borax application at 30 lb per acre had no consistent effect on nodulation.

Bowen (1956) has reported that different legumes like *Glycine max*, *G. javanica*, *Pueraria phaseoloides*, *Centrosema pubescens*, *Clitoria ternatea* and *Indigofera hirsuta* responded well to the *Rhizobium* inoculation, whereas *Vigna sinensis*, *Phaseolus lathyroides* and *Stylosanthes gracilis* were adequately nodulated by the native strains of *Rhizobium*.

Wales (1957) showed that when legumes like *Phaseolus lathyroides*, *Dolichos biflorus*, *Centrosema pubescens* and *Lespedeza striata* planted with or without inoculation, nodulated equally well.

Bowen (1958) reported that Centrosema pubescens showed a considerable degree of specificity in nodule formation. Native rhizobia were consistently found to be effective in nodulation. Bowen (1959a and b) reported that the Rhizobium strains were specific to Centrosema pubescens and C.plumieri. There was a highly significant correlation between weight of the nodule per plant and weight of the above ground parts. The nodulation and nodule senescence were governed more by vegetative growth rather than by flower production.

DE franca and DE Carvalho (1970) studying the effect of nutritional deficiency on the development of legumes like Glycine javanica var. comum, G.javanica var. tinaroo, Macroptilium atropurpureus, Pueraria javanica and Centrosema pubescens on a red-latosol called "fase cerrado" found that the extensive phosphorus deficiency in this soil decreased the nodule weight. Omission of liming caused a decrease in production of ineffective nodules, especially in Pueraria phaseoloides and Centrosema pubescens.

3. Cross-inoculation Studies

Allen and Allen (1939) reported that 54 strains of nodule bacteria isolated from 28 different leguminous plants (including forage legumes) growing in Hawaii were tested for infectivity on 20 members of the cowpea group. Ten test plants were nodulated by all 24 strains and only 13 strains infected all test plants. Plants of the cowpea group furnished nodule bacteria that varied in infectiveness and effectiveness more than did bacteria of other cross-inocula-tion groups.

Galli (1958) while studying the cross-inoculation of the twenty-three rhizobial strains from 13 legume species concluded that (a) there were differences between the morpho-logical characteristics of the nodules of some forage legume species (b) Calopogonium muconoides, Centrosema pubescens, Desmodium adscendens, D.discolor, Indigofera mucronata, I.subulata, I.sumatrana, Stylosanthes guianensis, Tephrosia candida and Teramnus uncinatus belonged to the cowpea group (c) Vicia obscura belonged to the pea group and (d) Leucaena glauca and Cratyli floribunda belonged to other cross-inoculation groups to which no other species belonged. Oblisami and Rangaswami (1972) reported that a forage legume, Clitoria ternatea was nodulated well by the rhizobia from the plants belonging to cowpea and soybean cross-inoculation groups and vice-versa, and was not nodulated by the rhizobia from alfalfa, clover, bean and pea cross-inoculation groups and vice-versa.

4. Effect of grass-legume association on forage yield

Effect of grass-legume association on the fodder yield has been studied by various workers. Roberts and Olson (1942) reported that in several grass-legume mixtures he obtained higher yield per unit area of soil than the pure stand of either grass or legume per half of unit area on dry weight basis.

Vincente-Chandler et al. (1953) reported that when herbage was cut at 10 inches height it produced higher yield as compared to that cut at 4 inches height and the yield was even throughout the year. The grass yield was higher due to utilization of nitrogen fixed by the legumes over a short period of time and it was found proportional to the growth of the legumes. Samuels and Landrau (1953b) reported that when Pueraria phaseoloides grown in association with Pennisetum purpureum var. merkerii produced the highest yield in response to potassium application at the rate of 300 lb per acre. Bumpus (1958) has studied 4 legumes namely Trifolium semipilosum, T. repens, T. rueppellianum, Glycine javanica in combination with Nozia Rhodes grass (Chloris gayana). The grass-legume mixtures yielded no more in the establishment year (1956-57) than the pure grass stand, but in the second and third years the mixtures which included T. semipilosum, T. repens yielded significantly more than the pure grass stand. Among grass-legume associations, Lablab purpureus / Chloris gayana gave higher yield than other grass-legume mixtures.

Lotero (1960) reported that the grass Panicum purpurascens in association with Stylobium deeringianum, Cajanus cajan, Lablab purpureus or Pueraria phaseoloides yielded 2580, 3360, 2280, 2930 kg/ha (average of nine cuttings during two years), respectively. However, S.deeringianum and Lablab purpureus were non-persistent after five cuttings. Walker (1960) showed that application of nitrogen generally produced higher yield on grass land than the nitrogen fixed by legumes. However, in properly established and managed sward, legumes greatly increased herbage production in absence of N-fertilizer. Allen and Cowdry (1961) studied different grass-legume associations for yield. Paragrass/centro (Brachiaria mutica/Centrosema pubescens) yielded 0.65 to 1.1 tons, Rhodes grass/stylo (Chloris gayana / Stylosanthes gracilis) yielded 0.5 to 0.9 tons, Guinea grass / stylo (Panicum maximum / Stylosanthes gracilis) 0.55 to 0.85 tons and Guinea grass / Centro (P.maximum / Centrosema pubescens) yielded 0.4 to 0.75 tons/acre. The pasture was productive at the above rate for ten years. Andrew and Norris (1961) reported that tropical legumes like Desmodium uncinatum, Indigofera spicata, Centrosema pubescens, Stylosanthes bojeri and Phaseolus lathyroides did not show any deficiency symptoms and their yields were 25 to 68 per cent higher than the temperate legumes. Whereas, temparate legumes showed visual symptoms of calcium deficiency and untreated plants yielded 0.2 to 6.5 per cent less than the maxima. Doll et al. (1961) showed that the grass-legume association was superior to N-fertilizer application. Grass-legume association increased fodder yield for longer time than N-fertilizer application. In pasture, where grass was a pure crop, N-fertilizer application was found most essential as compared to grass-legume association. Fernando (1961) reported that the yield of pasture was considerably increased by legume association consisting of Brachiaria brizantha / Centrosema pubescens when compared to pure grass stand.

Hunt and Wagner (1963) showed that height of cutting, frequency and date of first cutting had a marked influence on the grass yield. They preferred 2 inches cutting and longer interval between cuttings which increased the clover-grass yield. Less frequent cutting at a delayed first cutting date maintained good yields of smooth brome grass (Bromus inermis).

Ahlawat et al. (1964) reported that, among pure sown crops on well drained light sandy soil, bajra (Pennisetum typhoideum) and Sudan grass (Sorghum sudanensis) out-yielded jowar (Andropogon sorghum) and two legumes namely, Cyamopsis psoralioides and Vigna catjang in grass-legume mixtures were consistently better than pure sown grasses. Wolf and Smith (1964) studied nine grass-legume mixtures consisting of Medicago sativa, Lotus corniculatus, Trifolium repens with Bromus inermis, Dactylis glomerata or Phleum pratense. Those containing Medicago sativa / Dactylis glomerata yielded the best. Moore (1965) studied the grass-legume association of *Cynodon plectostachyus* and *Centrosema pubescens*. Yield response of 17 and 24 per cent resulted in the first cutting from 50 and 100 lb N per acre respectively and cutting at 2 to 4 inches at 8 weekly intervals produced about 2½ tons of dry matter per acre.

De Wit *et al*. (1966) reported that *Panicum maximum* and *Glycine javanica* grown together in pots containing N-poor sandy soil appeared to be mutually exclusive in absence of *Rhizobium*. Horrell and NewHouse (1966) reported that on soil of low fertility and of pH 5.5 to 6.5, in a grazing trial, unfertilized grass-legume pasture containing *Stylosanthes gracilis* and *Centrosema pubescens* gave yields equal to those pasture receiving 150 lb N per acre. Morrison (1966) conducted an experiment to compare the productivity of cocksfoot, cocksfoot swards with fertilizer N, and swards containing subterranean and Louisiana white clover on a red clay loam soil. Louisiana white clover yielded grass without fertilizer N in both years. In the second year cocksfoot/Louisiana white clover sward gave a dry matter production equivalent to grass receiving 250 lb N per acre. Whitney (1966) studied *Desmodium intortum*, *D. canum* and *Centrosema pubescens* grown alone and in combination with *Pennisetum purpureum* and *Digitaria decumbens* on volcanic soil. *D. intortum* / grass yielded about 1700 lb dry matter per acre. *Centrosema pubescens* in pure stand gave intermediate dry matter yields.

Bleak (1968) reported that herbage production was increased by an average of 144 lb/acre by sowing crusted wheat grass/lucerne. Scateni (1968) reported that a range of mixture of legumes including Glycine javanica cv. CPI 25702, tinaroo and CPI 16830, Macroptilium atropurpureus cv. siratro and lucerne cv. Hunter-River and Hairy Peruvian and of green panic (Panicum maximum var. trichoglume) were compared under infrequent cutting with various rates of N during 1962-63. Dry matter yield response to fertilizer N in the pure grass swards was linear. It was concluded that in the environment of these trials, legumes should be regarded primarily as protein rich herbage. Singh et al. (1968) while studying grass-legume associations of Pennisetum polystachyon with legumes like Stylosanthes gracilis, Centrosema pubescens, Clitoria ternatea, Atylosia scarabacoides and Calopogonium muconoides reported that pure grass stand gave average yields of 63.9 to 97.7 kg/ha in unfertilized plots. P.polystachyon grown in mixture with S.gracilis or C.pubescens, but without fertilizer gave average yields of 64.5 and 92.3 kg/ha, respectively. In the second year, the grass/centro mixture yielded more than pure grass.

Chatterjee et al. (1969) reported that Heteropogon contortus / Stylosanthes humilis mixture gave average yields of 19.5 tons herbage per hectare as compared with 15.6 tons/ha for H.contortus alone. Other mixtures gave lesser yields than pure grass stand. Stobbs (1969a and b) noted that application of superphosphate to a Hyparrhenia rufa / Centrosema pubescens produced marked benefit in herbage production and he also concluded that grass-legume swards significantly yielded higher herbage. Whiteman (1969) tested four legumes (Glycine javanica, Macroptilium atropurpureus cv. siratro, Desmodium uncinatum and Lotononis bainesii) with Rhodes grass. In all respects, the differences between the species were minor, although Glycine javanica showed slightly better persistence in all treatments. Walker (1969) noted that five forage legumes tested in grass-legume mixtures increased yields, mainly due to additional yield from the legumes. Highest yields were obtained from mixtures with Macroptilium atropurpureus or Stylosanthes guianensis and lowest yield was from Glycine javanica mixtures. Whitney and Green (1969a) have carried out extensive field trials on grass-legume mixtures. They have also compared the yields of grass grown in association with inoculated and uninoculated legumes. In their studies, the yields of pangola grass (Digitaria decumbens) grown with and without inoculated Desmodium canum were 7510 kg/ha and 6840 kg/ha, respectively.

Kretschmer (1970) estimated the production of dry matter yields in seven legumes (Macroptilium atropurpureus var. siratro, Centrosema pubescens, Glycine javanica, Stylosanthes humilis, Phaseolus lathyroides, Alysicarpus vaginalis and Desmodium sandwicense) with pangola grass. Among legumes tried, M.atropurpureus yielded the highest (11,610 kg/ha/yr), while D.sandwicense yielded the lowest (7380 kg/ha/yr). However, grass alone yielded 5240 kg/ha/year. DE franca and DE Carvalho (1970) studied the nutrient deficiency of a soil from a "Cerrado" area on five tropical legumes, namely Glycine javanica var. comum, G.javanica var. tinaroo, Pueraria javanica, Macroptilium atropurpureus and Centrosema pubescens and noted that the large phosphorus deficiency decreased dry matter yields of all legumes.

5. Nitrogen fixation by legume component and Nitrogen transfer to the associated grass in grass-legume association

Nitrogen fixation and nitrogen transfer to the associated grass have been studied by various workers. The nitrogen content and nitrogen fixation and transfer varied with type of grass-legume association. Roberts and Olson (1942) reported that greater yields of nitrogen were obtained from mixtures on unit area of soil than from pure stands of grass or legume. Largest gains due to association occurred when a legume with a vigorous growth habit was associated with grass with weak growth habits. Gains in total N were believed to have resulted from spreading of plants with vigorous growth habits over a greater soil area.

Jackobs (1952) showed that when a mixture was predominantly legume (lucerne-grass combination) there was only a slight yield response to N applied as NH_4NO_3, but with Ladinoclover-grass combinations there was a marked response to nitrogen. Vincente-Chandler *et al.* (1953) reported that in a study to estimate the effect of two heights of cutting on the *Pueraria phaseoloides* / *Melinis minutiflora* mixture, grass yields were not depressed by this increase in growth of *Pueraria phaseoloides*. It was assumed that the grass obtained N from the *Pueraria phaseoloides* over short periods of time and more or less in proportion to the growth of the legume.

Moore (1960) reported the comparison made of soil N under a five years old grazed stand of *Cynodon plectostachyus* and a stand of *C.plectostachyus* / *Centrosema pubescens*. There were 2600 lb N/acre (to a depth of 1 foot) in the soil under the mixture and 2040 lb N/acre in the pure grass stand. On an average on annual basis, the *Centrosema pubescens* fixed 112 lb N/acre.

Doll *et al.*(1961) indicated that N fertilizing of grass-legume pastures is inadvisable when total yields and legume content of the mixture are considered. Total uptake of N was closely correlated with yields. Fernando (1961) reported that the yield of pasture herbage was greatly increased by the inclusion of legume. Nitrogen top-dressings gave increased yields of dry matter in the first cut following the application of nitrogen but in the subsequent cuts the effect of nitrogen was virtually lost. Moore (1962) reported about inclusion of *Centrosema pubescens* in a *Cynodon plectostachyus* pasture. Under the pasture containing legume, total N and nitrifiable N in the underlying soil was significantly increased. N content was (250 lb per acre per year) higher than that under the pure grass stand. N content of grass was raised from 1.8 to 2.4 per cent.

Bruce (1965) reported that over 16 year period, *Centrosema pubescens* added 92.00 lb N per acre (6 inches of soil) per annum. Morrison (1966) from Kenya reported that N transfer to cooksfoot grass from subterranean clover was equivalent to 250 lb N per acre. Fixation of 200-300 lbs nitrogen per acre per year was possible at high altitude. Whitney (1966) included three legumes (*Desmodium intortum*, *D.canum* and *Centrosema pubescens*) with two grasses (*Pennisetum purpureum* and *Digitaria decumbens*) to study the amount of N fixation by legumes and the amount of N transferred to grasses grown in association. Total nitrogen fixation during the trial was 340 lb per acre for *D.intortum*, 82 lb per acre for *D.canum* and 156 lb per acre for *Centrosema pubescens.* Transfer of N to grass was about 5 per cent for *D.intortum* and upto 11 per cent for *C.pubescens*.

Birch and Dougall (1967) reported that percentage of N in grass (*Panicum purpureum*, *Tripsacum laxum* and *Panicum maximum*) was significantly increased when they were grown in association with a legume, *Desmodium uncinatum.* Jones *et al*. (1967) in their studies included 11 legumes, grown individually with *Paspalum plicatulum* Mich cv. *hartley*. The best legume treatment gave a N yield equivalent to that from grass fertilized with 170 lb N per acre per year, as urea. N transfer to the associated grass increased with time and was greater for the *Macroptilium atropurpureus* and *Lotononis bainesii.* Oke (1967 a and b) reported that among the several Nigerian legumes tried for N fixing capacity, *Cajanus cajan*, *Centrosema pubescens*, *Stylosanthes gracilis*, *Calopogonium muconoides* and *Pueraria* spp. fixed 14.5, 10.3, 4.6, 3.8 and 9.3 mg of N per day per plant, respectively. Whitney *et al*. (1967)

observed N fixation of 85 lb per acre by Desmodium canum but no N transfer to grasses, whereas Centrosema pubescens fixed 240 lb N per acre in pure stand and 110 lb of N per acre when grown with grasses. In the latter, N transfer ranged from 6 to 11 per cent. Desmodium intortum fixed 340 lb N per acre and transfer noted was about 5 per cent. Whereas, Whitney and Kanchiro (1967) observed in pot culture studies, the significant transfer of N from the more vigorous legumes to the grasses, especially after cutting back.

Singh et al. (1968) estimated the N transfer from legume to the grass at 32 and 74 kg of N per hectare, respectively in the mixtures of Pennisetum polystachyon/ Stylosanthes gracilis and P.polystachyon/Centrosema pubescens. Simpson (1968) reported that the three legumes used performed quite differently. Subterranean clover did not release any nitrogen until senescence and then produced a rapid transference, while the white clover was competitive for nitrogen until the autumn-winter was rapid. However, lucerne released nitrogen gradually over the whole experimental period.

Birch (1969) reported the significant effect of Desmodium uncinatum in increasing N percentages in associated Napier grass (Pennisetum purpureum), Guatemala grass (*Tripsacum laxum*) and Guinea grass (*Panicum maximum*) following their first cutting.

Kretschmer (1970) estimated the nitrogen yields of several tropical legumes in association with pangola grass. Association of grass with *Centrosema pubescens* and *Glycine javanica* fixed 150 and 134 kg N per hectare per year, respectively. Bewg *et al*. (1970) reported that the N-content of the pasture dry matter decreased from 2 per cent in summer to 1 per cent in winter when the grass was grown in association with *Glycine wightii*, *Desmodium intortum*, *D. uncinatum* and *Macroptilium atropurpureus* cv. *siratro*. ODu *et al*. (1971) reported that the nitrogen fixation in *Centrosema pubescens* and *Stylosanthes gracilis* was maximum at pH value of about 6.

6. Crude Protein Production

Many workers have reported on the crude protein production in the grass-legume associations.

Mulder (1949) reported that application of small amounts of N (20 to 40 kg/ha) generally decreased the protein content of herbage due to the depression of clovers. Large dressings of N (80 to 240 kg/ha) increased the protein content considerably, especially when the grass was cut young.

Vincente-Chandler *et al.* (1953) estimated the protein content of the *Pueraria phaseoloides* / *Melinis minutiflora*, and noted that high cutting (10 inches) favoured the *Pueraria phaseoloides* and resulted in a higher yield of protein, and also within yield period the associated molasses grass increased in protein content. Landrau *et al.*(1953) reported that *Pueraria phaseoloides* showed increased protein content with increased nitrogen, principally in the first cutting, when grown in different clay soils.

Washko and Pennington (1956) reported that the protein content of grasses in legume-associations was slightly higher than the grasses in pure stands receiving nitrogen.

Fernando (1961) reported that the yield of herbage was greatly increased by the inclusion of a legume. A significant increase in crude protein was noted due to the presence of legumes in all the three harvests. *Centrosema pubescens* almost invariably gave higher contents of crude protein than other legumes.

Vazquez (1965) estimated the protein content in Guinea grass and påragrass grown alone and in mixture with *Pueraria phaseoloides*. Crude protein content was about equal in pure grass stand and in their mixtures with *Pueraria phaseoloides*. The application of 400 lb N per acre increased the crude protein content by about 2 per cent and 800 lb nitrogen per acre increased it by a further 2 per cent.

Horrell and NewHouse (1966) reported that grass-legume grown in pasture containing Stylosanthes gracilis and Centrosema pubescens gave yields equal to those from grass pasture receiving 150 lb N per acre and gave higher yields of crude protein than the latter. Kynevr (1966) reported that the presence of Glycine javanica generally increased the protein content of the grass component. Morrison (1966) reported that inclusion of clover (Cooks-foot/Lousiana white clover) improved the crude protein content of the associated grass.

Singh et al. (1968) and Singh and Chatterjee (1968a and b) reported that when Pennisetum polystachyon was grown in association with Stylosanthes gracilis or Centrosema pubescens with 80 kg N per hectare, the average protein yield of P.polystachyon was 1056.8 kg per hectare as compared to 729.2 kg for P.polystachyon/S.gracilis mixture and 1210.9 kg for P.polystachyon/C.pubescens mixture. When cut frequently, the grass / Centrosema pubescens mixture yielded 347 kg protein per hectare. Pure grass yielded 189 kg per hectare. Grass grown alone receiving 44 kg N per hectare yielded 383 kg protein per hectare. Chatterjee et al.(1969) reported that Heteropogon contortus/Stylosanthes gracilis mixture gave the highest crude protein yield of 535 kg(on dry matter basis) per hectare, followed by H.contortus/S.gracilis mixture with 435 kg per hectare and H.contortus alone with 342 kg per hectare.

James *et al*. (1969) noted the influence of legume association and nitrogen fertilization on crude protein content of 11 grasses. Seasonal effects, nitrogen sources and differences in kinds of grasses were highly significant in affecting the crude protein content of the forage. Walker (1969) reported that crude protein yields from *Macroptilium atropurpureus* or *Stylosanthes guianensis* mixtures were much higher than those from the nitrochalk treatment (4.5 cwt/acre). Whitney and Green (1969a and b) observed that the percentage of crude protein fluctuated with season, highest being during the cooler months. Crude protein levels were influenced only slightly by low rates of fertilization but were raised by the higher N rates.

Kretschmer (1970) reported that among eight legumes *Centrosema pubescens* and *Glycine javanica* yielded 9.9 and 8.9 per cent crude protein, respectively, whereas Pangola grass alone yielded 6.0 per cent. Febles and Padilla (1970) estimated that the crude protein content of *Pueraria phaseoloides* and *Cajanus cajan* (inoculated with *Rhizobium*) in urea sprayed treatment was increased by 15 per cent. Reid (1970) noted that response of crude protein yield for N application (0 to 600 to 700 lb N per acre) was linear, but inclusion of clover increased the crude protein yields at low N rates.

7. Phosphorus content

Many workers have reported on the phosphorus content and response to applied phosphorus in different grass-legume associations.

Salgues (1938) studied the behaviour of different annual and perennial grasses. Grasses on calcareous soil contained more phosphorus than legumes. Perennial grasses contained more phosphorus than annuals.

Jackobs (1952) noted that when a mixture consisted predominantly of a legume, there was marked response to P_2O_5 application as triple superphosphate. When grass was predominant, there was much smaller response to P_2O_5. The magnitude of the responses of lucerne/grass combinations to P_2O_5 varied with the grass component.

Landrau *et al*. (1953) studied the behaviour of *Pueraria phaseoloides* on different clay soils and reported that application of phosphorus to clay soils was ineffective on *P. phaseoloides*. Vincent-Chandler *et al*.(1953) reported that high cutting to 10 inches favoured phosphorus utilisation of *Pueraria phaseoloides* and high yields of low cut (4 inches) required phosphorus applications. The beneficial effects of phosphorus and potash treatments were largely through the influence of these nutrients on this legume.

Moore (1962) reported that there were no significant differences in phosphorus content of the grass due to its association with the legume (Centrosema pubescens/Cynodon plectostachyus).

Caro-Costas and Vincente-Chandler (1963) reported that application of phosphorus did not affect yields or species balance, but increased the phosphorus content from 0.12 to 0.15 per cent in Pueraria phaseoloides and 0.11 to 0.17 per cent in Melinis minutiflora.

Grof (1966) reported that on various clay soils of the coastal plains Pueraria phaseoloides showed greater vigour and greater response to applied phosphorus than Stylosanthes gracilis did. In the field, a mixed sward of Panicum maximum / Pueraria phaseoloides/ Centrosema pubescens showed significant phosphorus response.

Stobbs (1969a) noted that application of single superphosphate to a Hyparrhenia rufa / Centrosema pubescens swards produced a marked benefit to herbage production. Mean phosphorus content in soil was significantly higher under pasture receiving single superphosphate.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The following forage legumes were selected for the present study. Dharwar hybrid-2 (DH-2) was selected as the associated grass.

1. *Calopogonium muconoides* Desv.
2. *Centrosema pubescens* Benth.
3. *Dolichos biflorus* Linn.
4. *Glycine javanica* Linn.
5. *Lablab purpureus* (Linn.)Sweet
6. *Mimosa invisa* Mart.
7. *Pueraria phaseoloides* Benth.

The forage legume seeds were sown in cement pots (45 x 30 x 28.5 cm) containing Hebbal sandy loam soil mixed with approximately 4 kg of farm yard manure per pot. After 6 to 7 weeks of growth, legumes were well established. Then, the legumes were uprooted and used for the isolation of *Rhizobium* strains. After washing the roots carefully in running tap water, well formed, healthy, pinkish nodules on the tap root were detached and placed in a test tube. These nodules were surface sterilized for one minute by momentary treatment with alcohol and treated with mercuric chloride (0.1 per cent). Then they were washed thoroughly with ten changes of sterile water.

Sterilized nodules were placed in test tube with a few drops of sterile distilled water at the rate of one nodule per tube. Nodules were crushed using a flamed glass rod. One loop-ful of the expunged fluid from the nodules was plated in two or three dilutions using Yeast Extract Mannitol agar. The plates were incubated at 28°C for one week and well isolated colonies suspected to be Rhizobium were picked and sub-cultured onto Yeast Extract Mannitol agar slopes.

Characterization of the Root Nodule Bacteria

Morphological characters: The bacterial isolates from the root nodules of forage legumes were examined microscopically for the cell shape and size and gram reaction as per the procedure given in the Manual of Microbiological Methods (Anonymous, 1957).

Test for purity of Rhizobium: The purity of the isolates was established by the following tests:

YMA-Congored medium: All the isolates were streaked on the YMA-Congored agar medium consisted of Mannitol, 10.0 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCO_3$, 4.0 g; Yeast Extract, 0.4 g; one per cent Congored dye, 2.5 ml per litre; agar, 15.0 g; distilled water, 1000 ml; pH, 6.8 - 7.0. The plates were incubated at 28°C for a week. Isolates that showed pink or red colonies were rejected as not being rhizobia (Hahn, 1966).

Hofer's alkaline broth test: The liquid medium of the following composition was prepared: K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCO_3$, 0.05 g; Yeast Extract, 3.0 g; Mannitol, 10.0 g; distilled water, 1000 ml and pH adjusted to 12.0 by addition of alkali. Ten ml portions of the above medium were dispensed in test tubes and autoclaved. After cooling, the liquid was inoculated with a loop-ful of the culture and incubated at 28°C for 12 days (Hofer, 1935). Absence of any growth in this medium indicated the identity of the isolates as rhizobia.

Ketolactase test: The medium consisted of lactose, 10.0 g; K_2HPO_4, 0.5 g; $CaCl_2$, 0.2 g; Yeast Extract, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; NaCl, 0.2 g; $FeCl_3$, 0.01 g; distilled water, 1000 ml; agar, 15.0 g; pH 6.8 was prepared. The isolates were streaked on to ketolactase agar medium and incubated at 28°C for 7 days. After incubation, the plates were flooded with Benedict's solution. Formation of yellow ring around the colony of the organism was taken as positive test for 3-ketolactase production from lactose which indicated that the isolates were not Rhizobium but probably Agrobacterium (Bernaerts and deLey, 1963).

Growth on glucose peptone agar medium: The medium consisted of glucose, 10.0 g; peptone, 20.0 g; NaCl, 5.0 g; agar, 15.0 g; pH, 7.2. Plates with glucose peptone agar medium were streaked with the isolates and incubated at 28°C.

The isolates that grew well in 24 hours on this medium were rejected as not being rhizobia (Graham and Parker, 1964).

Salt tolerance of Rhizobium: The inoculum was developed by inoculating into yeast extract glucose medium of the following composition. Glucose, 10.0 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCO_3$, 3.0 g; Yeast Extract, 1.0 g and distilled water, 1000 ml. The inoculum thus obtained was added at one per cent level to ten ml of Yeast Extract glucose medium containing NaCl at 3.0, 4.0, 5.0, 7.0, 8.0, 9.0 and 10.0 per cent (W/V). The medium was inoculated with the isolates and incubated at 28°C on a rotary shaker. After 78 hours incubation, the growth of the organisms was recorded by visual observations (Ethiraj et al., 1972).

Cross inoculation studies: Cross inoculation studies were conducted using the modified Leonard's bottle-jar assembly method (Leonard, 1944). The top half of the unit consisted of a round beer bottle (700 ml capacity) that had its bottom cut to provide a level flat ground finish. The lower half (the reservoir) consisted of a jar of such dimensions that the inverted bottle sat snugly on its rim and the neck of the bottle was within 2 to 4 cm above the bottom jar.

A wick made up of absorbent cotton was provided to help the capillary rise of moisture from the reservoir to the top of the growth vessel. The wick was secured in the neck with a wad of cotton wool. Moderately coarse well washed river sand, mixed with $CaCO_3$ (1 g/kg) were filled into the bottle units within 5 cm of the top.

Seedling solution used was that of Thornton (1930). It contained $CaCO_3$, 2.0 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; $FePO_4$, 1.0 g; $FeCl_3$, 0.01 g in a litre of distilled water diluted to one-fifth and a portion of it was used to moisten the sand in the jar. The remaining solution was poured in the bottom container. Total quantity of nutrient solution used was 250 ml per jar. The top of the growth vessel was covered with a petridish half, and the whole unit was secured with moisture proof paper by means of rubber bands. The unit was autoclaved at 120°C for two hours and kept covered until it was used.

Undamaged clean seeds of the seven legumes were selected and rinsed with 95 per cent ethanol and immersed for 3 minutes in 0.2 per cent $HgCl_2$ acidified with 5 ml conc. HCl. The seeds were then washed thoroughly with six to ten changes of sterile distilled water. Then, the seeds were sown, just below the surface of the moist sand and the jars were placed in overhead illumination chamber. When the seedlings were a week old they were inoculated with 2 ml each of 6 days old broth cultures. Light was provided for a minimum period of 16 hours per day throughout the duration of experiment. Uninoculated controls were maintained similarly.

The diluted nutrient solution in the reservoir was replenished thrice during the experiment with usual aseptic precautions. Six weeks after sowing, plants were removed from the jar, washed and observed for nodulation.

Pot Culture Experiment

The experiment was conducted in (45 x 30 x 28.5 cm size) cement pots. Sandy loam soil (pH 6.4) from Main Research Station, Hebbal, was filled to within 2 to 3 cm from the top (40 kg of soil to each pot). Basal application of 60 kg of P_2O_5/ha as single superphosphate, 40 kg of K_2O/ha as muriate of potash were given. No nitrogenous fertilizer was applied. DH-2 grass setts were planted at the rate of one set per pot. Three replications were maintained. The treatments were:

(a) DH-2 + *Calopogonium muconoides* (inoculated)
(b) DH-2 + *Centrosema pubescens* (inoculated)
(c) DH-2 + *Dolichos biflorus* (inoculated)
(d) DH-2 + *Glycine javanica* (inoculated)
(e) DH-2 + *Lablab purpureus* (inoculated)
(f) DH-2 + *Mimosa invisa* (inoculated)
(g) DH-2 + *Pueraria phaseoloides* (inoculated)
(h) DH-2 + *Calopogonium muconoides* (uninoculated)
(i) DH-2 + *Centrosema pubescens* (uninoculated)
(j) DH-2 + *Dolichos biflorus* (uninoculated)
(k) DH-2 + *Glycine javanica* (uninoculated)
(l) DH-2 + *Lablab purpureus* (uninoculated)

(m) DH-2 + *Mimosa invisa* (uninoculated)

(n) DH-2 + *Pueraria phaseoloides* (uninoculated)

(o) DH-2 + DH-2 (pure grass stand)

The *Rhizobium* isolates from the above seven forage legumes were grown on yeast extract mannitol broth in shake culture for 5 days at 28°C. Carboxymethyl cellulose at 100 mg per 100 ml culture was added as a sticker. After 5 days of growth, the legume seeds were treated with appropriate *Rhizobium* inoculant prepared with peat as base and dried in shade before sowing. Seeds were dibbled in each pot around the grass setts. Untreated legume seeds were also dibbled around the grass setts in respective pots. After establishment of grass, irrigation was done once a week.

Grass cuttings were made once in two months for a period of ten months. After recording the green weight, the samples were dried at 60°C for 24 hrs and the dry weights were recorded. Total nitrogen, and phosphorus were estimated in the grass samples. Crude protein in the grass samples was calculated by multiplying total nitrogen values by the factor 6.25. *Dolichos biflorus* seeds inoculated as well as uninoculated were resown in the respective pots after second and fourth cuttings of the grass. Apparent recycling of fixed nitrogen from the legume to its associated grass was estimated by subtracting the N-yield of the pure grass stand from the N-yield of the grass component of each mixture.

Field Experiment - 1.

The experiment was conducted on sandy loam soil, located near Microbiology Department, Agricultural College, Hebbal. A factorial layout and randomized block design was used with three replications. Gross plot size was 2.8 m x 1.5 m.

Treatments consisted of the following:

(a) DH-2 + *Calopogonium muconoides* (inoculated)

(b) DH-2 + *Glycine javanica* (inoculated)

(c) DH-2 + *Pueraria phaseoloides* (inoculated)

(d) DH-2 + *Calopogonium muconoides* (uninoculated)

(e) DH-2 + *Glycine javanica* (uninoculated)

(f) DH-2 + *Pueraria phaseoloides* (uninoculated)

(g) DH-2 + DH-2 (pure grass stand)

Basal application consisted of 60 kg of P_2O_5/ha as single superphosphate and 40 kg of K_2O/ha as muriate of potash. No nitrogenous fertilizers were given. DH-2 grass setts were planted on the ridges 80 cm apart in the row and spacing in between rows was 80 cm. After planting, irrigation was given daily for one week. Legume seeds were treated with appropriate *Rhizobium* culture as detailed earlier, and dibbled around the grass setts. Untreated legume seeds were also dibbled around the grass setts in respective plots, after establishment of grass. Irrigation was continued, once in a week throughout the period of the experiment.

Grass cuttings were made once in two months for one year and the green weight of grass was recorded.

Field Experiment - 2

The experiment was conducted on a sandy loam soil (pH 6.4) at Main Research Station, Hebbal, Bangalore. A factorial layout and randomized block design was used, with three replications. Gross plot size was 3 m x 2 m.

Treatments consisted of the following:

(a) DH-2 + *Calopogonium muconoides* (inoculated)

(b) DH-2 + *Centrosema pubescens* (inoculated)

(c) DH-2 + *Glycine javanica* (inoculated)

(d) DH-2 + *Lablab purpureus* (inoculated)

(e) DH-2 + *Calopogonium muconoides* (uninoculated)

(f) DH-2 + *Centrosema pubescens* (uninoculated)

(g) DH-2 + *Glycine javanica* (uninoculated)

(h) DH-2 + *Lablab purpureus* (uninoculated)

(i) DH-2 + DH-2 (pure grass stand)

Basal application consisted of 60 kg of P_2O_5/ha as single superphosphate and 40 kg of K_2O/ha as muriate of potash. No nitrogenous fertilizers were given. DH-2 grass setts were planted on the ridges at 60 cm apart in the row and spacing between rows was also 60 cm. After planting, irrigation was given daily for one week. Legume seed treatment and planting were carried out as mentioned in the previous experiment. Irrigation was continued once a week throughout the period of the experiment.

The grass was cut once every two months for two years, and green weight was recorded. Phosphorus deficiency was observed during the third cutting. Top dressing with 30 kg of P_2O_5/ha was applied between third and fourth cuttings and the crop recovered from the deficiency. Lablab purpureus being an annual crop, was resown in the respective plots after fifth and tenth cuttings of the grass.

Statistical Analysis

For ascertaining the significance of data on yield, nitrogen, crude protein and total phosphorus content of DH-2 grass in the pot culture studies, the two way analysis of variance was followed (Snedecor, 1961).

The data on the yield were statistically analysed following the procedure of factorial experiment laid as randomized block design (Peterson, 1939).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

Three separate grass-legume association studies (pot culture and two field studies) were made to know the behaviour of forage legumes under different soil conditions and also to know the nitrogen transfer to the associated grass from the nitrogen fixed by the legume. The following combinations of grass-legumes were used in the studies. Dharwar hybrid-2 grass was grown in association with each of the below listed legumes in pot culture and field experiments. Legumes: Calopogonium muconoides, Centrosema pubescens, Dolichos biflorus, Glycine javanica, Lablab purpureus, Mimosa invisa and Pueraria phaseoloides. These legumes were also grown separately for isolation of Rhizobium strains. Morphological and physiological studies were made to know the purity of the isolates.

In pot culture study, all these seven legumes were compared with DH-2 grass, while only four legumes viz. C.muconoides, C.pubescens, L.purpureus and G.javanica were included for the study in cultivated land of fairly high level of fertility, whereas only three legumes viz. C.muconoides, G.javanica and P.phaseoloides were included in freshly opened up uncultivated land. Grass yield in all these three experiments were recorded at periodical intervals. In addition to this, nodulation pattern was also observed in all these legumes. However, in pot culture study, total nitrogen and total phosphorus in the associated grass were determined. Results of these experiments are presented in the respective tables. It may also be noted that pot culture and field experiments were conducted under conditions of natural defoliation of legumes through senescence and only grass was cut at periodical intervals.

Nodulation in Forage Legumes:

Seven forage legumes were grown separately in cement pots for isolation of *Rhizobium*. Nodulation characters of these legumes were observed and the results are presented in table 1.

The nodules were recorded on both tap root and lateral roots of these seven legumes. The nodules were round and smooth surfaced in *C.pubescens*, *D.biflorus*, *L.purpureus* and *P.phaseoloides*, whereas they were rough and wrinkled surfaced in *C.muconoides* and *G.javanica*. Finger like nodules were recorded in *M.invisa* and they were rough surfaced and were found in clusters. The excised nodules exhibited diffuse pink colouration or circular zones of pink colour.

Characterization of Bacterial Isolates from Nodules:

The morphological, cultural, physiological and nodulation characteristics of these isolates were studied following the procedure as given under Material and Methods. Results are presented in table 2.

Table 1

Nodule location and morphology

Legumes	Location of nodule	Shape of nodule	Colour of nodule
Calopogonium muconoides	Both on tap root and the lateral roots	Round, rough and wrinkled surface	Younger nodules were pinkish
Centrosema pubescens	Both on tap root and the lateral roots	Round and smooth surface	Younger nodules were pinkish
Dolichos biflorus	Both on tap root and the lateral roots	Round and smooth surface	Younger nodules were pinkish
Glycine javanica	Both on tap root and the lateral roots	Round, rough and wrinkled surface	Younger nodules were pinkish
Lablab purpureus	Both on tap root and the lateral roots	Round and smooth surface	Younger nodules were pinkish
Mimosa invisa	Both on tap root and the lateral roots	Fingerlike, in cluster and rough surface	Younger nodules were pinkish
Pueraria phaseoloides	Both on tap root and the lateral roots	Round and smooth surface	Younger nodules were pinkish

Table 2

Morphological and Physiological characters of rhizobial isolates

Characters	Isolates from						
	Calopogonium muconoides	*Centrosema pubescens*	*Dolichos biflorus*	*Glycine javanica*	*Lablab purpureus*	*Mimosa invisa*	*Pueraria phaseoloides*
Morphological							
Shape ..	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Gram staining	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative
Cultural and Physiological							
Growth on YMA	Filiform raised	Filiform raised	Filiform raised	Filiform raised	Filiform raised	Filiform raised	Filiform raised
Growth on YMA + Congored	Dye not absorbed	Dye not absorbed	Dye not absorbed	Dye not absorbed	Dye not absorbed	Dye not absorbed	Dye not absorbed
Growth on glucose peptone agar (in 24 hours)	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Growth on alkaline broth (in 12 days)	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Ketolactase test	No yellow ring around the colony	No yellow ring around the colony	No yellow ring aro-und the colony	No yellow ring aro-und the colony	No ye-llow ring around the colony	No yellow ring aro-und the colony	No yellow ring around the colony

The bacterial isolates made from YMA and Congored plates were rod shaped and gram negative. They formed filiform and raised growth on Yeast Extract Mannitol Congo-red agar and did not show any growth on glucose peptone agar within 24 hrs. There was no growth in alkaline broth even after 12 days. When the organisms were tested for ketolactase production, none of the isolates exhibited any ketolactase reaction.

The salt tolerance characteristics of the organisms from seven legumes were studied as detailed under Material and Methods and the results are presented in table 3.

All the seven isolates showed good growth upto 4.0 per cent of NaCl in the medium and there was no growth from 8.0 to 10.0 per cent salt concentration. At 5.0 per cent salt concentration, isolate from L.purpureus showed good growth, while the isolate from C.pubescens showed poor growth and the isolates from the other legumes showed moderate growth. At 7.0 per cent salt concentration, the isolates from C.muconoides, C.pubescens, L.purpureus and P.phaseoloides showed poor growth, while the isolates from other legumes showed no growth.

Cross-inoculation studies:

The cross-inoculation studies were made on all the seven forage legumes viz. C.muconoides, C.pubescens,

Table 3

Salt tolerance of rhizobial isolates

Isolates from	Salt concentrations in per cent						
	3	4	5	7	8	9	10
Calopogonium muconoides	+++	+++	++	+	-	-	-
Centrosema pubescens	+++	+++	+	+	-	-	-
Dolichos biflorus	+++	+++	++	-	-	-	-
Glycine javanica	+++	+++	++	-	-	-	-
Lablab purpureus	+++	+++	+++	+	-	-	-
Mimosa invisa	+++	+++	++	-	-	-	-
Pueraria phaseoloides	+++	+++	++	+	-	-	-

+ = Poor growth
++ = Moderate growth
+++ = Good growth

D.biflorus, G.javanica, L.purpureus, M.invisa and P. phaseoloides by following Leonard's bottle jar assembly method. The results obtained are presented in table 4.

Among the isolates tried, the isolate from C.pubescens was found to be host specific. It caused nodules on its original host only and did not cause nodulation on the other hosts. Generally, the nodules were more in host plant (23 to 36 per plant) than in cross-inoculated legumes (0 to 25 per plant) (table 4). Among the cross-inoculated legumes, isolate from L.purpureus was more effective in nodulating all the plants than the other isolates.

Pot culture Studies:

The experiment was conducted in cement pots filled with sandy loam soil from the Main Research Station, Hebbal. Seven forage legumes namely, C.muconoides, C.pubescens, D. biflorus, G.javanica, L.purpureus, M.invisa and P.phaseoloides were included for the study. Dharwar hybrid-2 grass was grown in association with these legumes.

Grass cuttings were made for a period of ten months at two months interval. Total nitrogen and phosphorus contents of grass were estimated. The moisture content ranged from 74 to 86 per cent in grass samples in each cutting. The mean moisture per cent of 80 was considered for the estimation of dry matter production.

Table 4

Cross-inoculation studies with rhizobial isolates

Isolates from	Nodulation on different legumes													
	C.muconoides		C.pubescens		D.biflorus		G.javanica		L.purpureus		M.invisa		P.phaseoloides	
	T	L	T	L	T	L	T	L	T	L	T	L	F	L
Calopogonium muconoides	+	+++	–	+++	–	+++	–	++	–	++	++	+++	–	++
Centrosema pubescens	–	–	+	+++	–	–	–	–	–	–	–	–	–	–
Dolichos biflorus	+	+++	–	+++	+	+++	–	++	–	++	–	+++	+	+++
Glycine javanica	–	+++	–	++	–	++	+	+++	–	+	–	++	–	++
Lablab purpureus	+	+++	+	+	++	+++	+	+++	+++	+	+	+++	+++	+++
Mimosa invisa	–	+++	–	++	+	+++	–	++	–	++	+	+++	+	+++
Pueraria phaseoloides	+	+++	+	+++	–	++	–	++	–	+++	+	+++	++	+++
Uninoculated control	–	–	–	–	–	–	–	–	–	–	–	–	–	–

T = Tap root
L = Lateral root

– = No nodules
+ = 1-4 nodules

++ = 5-10 nodules
+++ = Above 10 nodules

Apparent recycling of fixed nitrogen from the inoculated and uninoculated legumes to its associated grass was estimated by subtracting the N-yield of the pure grass stand from the N-yield of the grass component of each mixture. N-fixed by the native Rhizobium was calculated by subtracting the N-yield of the pure grass stand from the N-yield of the grass component of each uninoculated mixture. Similarly the N-yield of the grass component of each uninoculated mixture and pure grass stand was deducted from the inoculated mixture to find out the N-fixation due to inoculated strains. The effect of seven forage legumes when inoculated and uninoculated with appropriate rhizobia over the DH-2 grass was studied.

Grass yield: In this experiment, the green weight of the DH-2 grass grown in association with the seven legumes under both inoculated and uninoculated conditions were recorded at each sampling and the results are presented in tables 5a to 5c and in fig. 1.

As a mean over rhizobial treatment, grass grown with M.invisa gave the highest green weight (132.84 t/ha/year), followed by grass with C.muconoides (126.00 t/ha/year), while the green weight of the pure grass stand was the lowest (55.28 t/ha/year) (table 5a). The yield of grass grown with C.pubescens, G.javanica, L.purpureus, D.biflorus and P.phaseoloides were intermediate and ranged from 107.42 to 123.28 t/ha/year.

Table 5(a)

Interaction of legumes and *Rhizobium* inoculation on grass yields (Pot culture studies)

(weight of grass, t/ha/year)

Grass/legume mixtures	Rhizobium inoculated	Rhizobium uninoculated	Mean	Per cent increase over pure grass stand
DH-2/*C.muconoides* ..	162.05	89.94	126.00	128
DH-2/*C.pubescens* ..	150.38	96.17	123.28	123
DH-2/*D.biflorus* ..	137.06	100.80	118.93	115
DH-2/*G.javanica* ..	136.62	82.92	109.77	99
DH-2/*L.purpureus* ..	124.18	90.66	107.42	94
DH-2/*M.invisa* ..	156.96	108.71	132.84	140
DH-2/*P.phaseoloides* ..	144.53	96.62	120.58	118
Mean ..	144.54	95.12		
DH-2/DH-2 (Pure grass stand)			55.28	

	Legumes	Rhizobium inoculation	Interaction
F-Test	N.S.	**	N.S.
S-Em ±	-	8.89	24.89

** Significant at P = 0.01, N.S.= Not significant at P = 0.05

Yield per cutting: The per cutting yields of grass were poor during the first cutting and gradually rose to peak value during the third cutting. Thereafter, there was gradual decline upto the fifth cutting (fig. 1). The green weight of the grass associated with M.invisa was better in most of the cuttings though it reached its peak in the third cutting (36.18 t/ha). The next best mixture which yielded higher grass yield was with C.muconoides which reached its peak at the fourth cutting (36.00 t/ha). In grass with C.pubescens, the yield ranged from 7.30 t/ha in the first cutting to 38.08 t/ha in the fourth cutting. Grass with D.biflorus gave yield of 7.15 t/ha in the first cutting and 36.93 t/ha in the fourth cutting. The grass yield was almost same after the second cutting till the fifth cutting in grass/P.phaseoloides and grass/D.biflorus mixtures. However, the total yield of grass with P.phaseoloides was slightly higher than the grass with D.biflorus. In grass with G.javanica increase in green weight was linear upto the fifth cutting (8.00 t/ha in the first cutting to 28.48 t/ha in the fifth cutting), while in grass with L.purpureus, the yield increased upto the third cutting and then declined (table 5b).

As a mean over legumes, increase in green weight of grass was not appreciable (7.61 to 8.57 t/ha) upto the second cutting. It rose from the third cutting and remained more or less constant till the fourth cutting. The mean grass yield

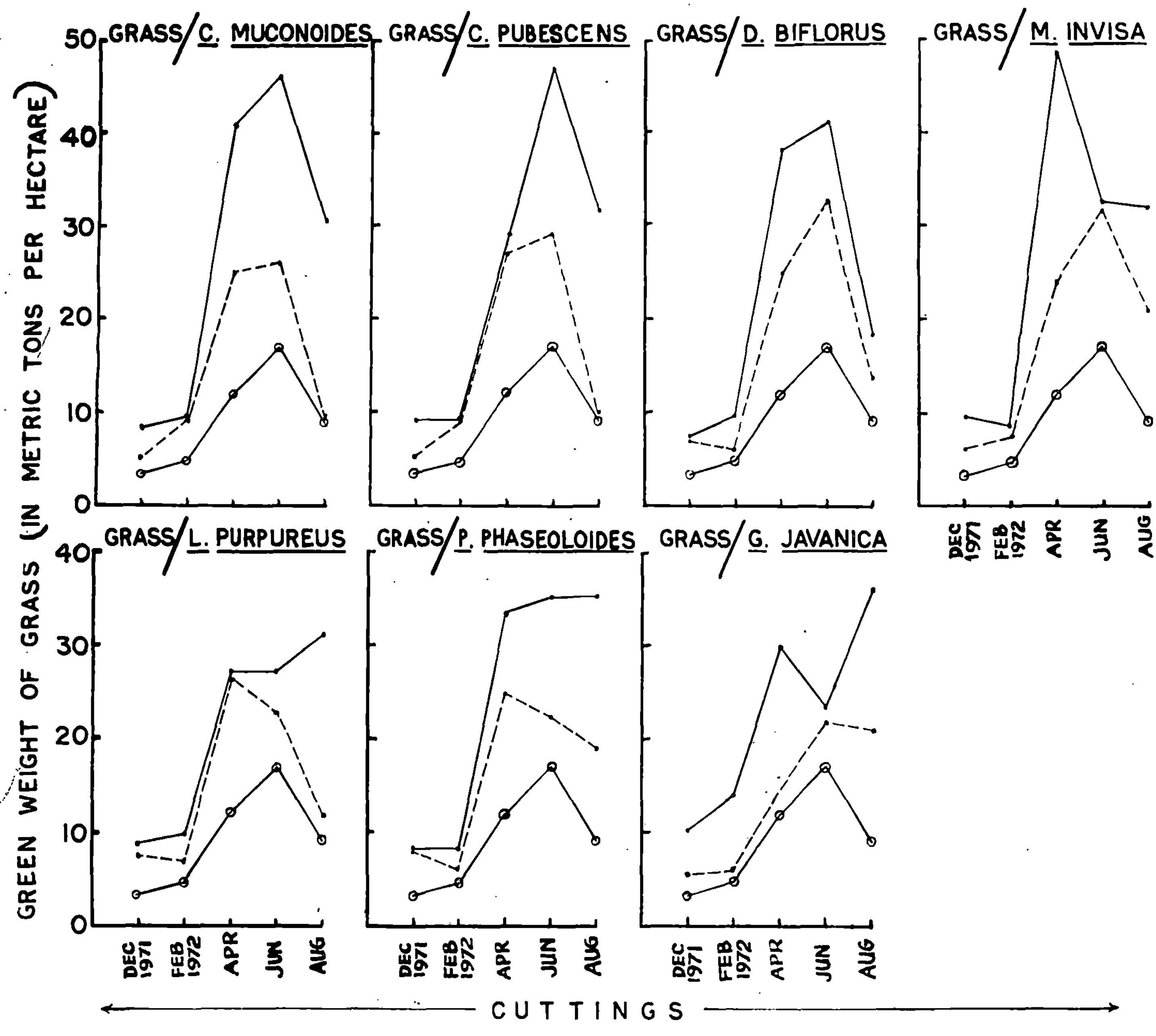

FIG: I RELATION BETWEEN CUTTING DATES AND YIELD OF GRASS IN GRASS-LEGUME MIXTURES (POT CULTURE STUDIES) (DATA OF FIVE CUTTINGS) (GRASS WITH INOCULATED LEGUME •—— GRASS WITH UNINOCULATED LEGUME •---------•, AND PURE GRASS STAND ⊙——⊙).

decreased considerably in the fifth cutting (22.91 t/ha) (table 5b). The increase in yield due to Rhizobium inoculation of associated legume was highly significant as compared to uninoculated legumes. Inoculation of the legumes associated with grass increased the mean grass yield (144.54 t/ha/year) significantly as compared to that of grass associated with uninoculated legumes (95.12 t/ha/year). Grass yield of all mixtures with inoculated legumes was higher in all the cuttings than in the mixtures with uninoculated legumes. Grass yield in pure grass stand was the lowest as compared to that of grass and legume mixtures.

Yield of grass associated with legume differed significantly due to Rhizobium inoculation in all the cuttings except in second cutting (table 5b). The increase in mean green weight of grass associated with inoculated legumes was higher in the third cutting (35.05 t/ha) reaching its peak in the fourth cutting (36.07 t/ha). It was slightly lower in the fifth cutting (30.66 t/ha). Similar trend was observed even in uninoculated legumes-grass mixtures, but the yield at the fifth cutting was considerably lower as compared to the yield of grass at the third and the fourth cutting. Even in pure grass stand, the green weight of grass in different cuttings followed the same trend as in mixtures. The increase in grass yield was only marginal upto the second cutting and was marked in the third and fourth cutting and decreased considerably in the fifth cutting.

Table 5(b)

Effect of Legumes on yield of grass (Pot culture studies)
(weight of grass, t/ha)

Grass-legume mixtures	Cuttings					Total	Total per ha per year
	I Dec. 1971	II Feb. 1972	III Apr.	IV June	V Aug.		
DH-2/*C. muconoides*	6.63	9.45	33.00	36.00	19.93	105.00	126.00
DH-2/*C. pubescens*	7.30	9.15	27.44	38.08	20.78	102.73	123.28
DH-2/*D. biflorus*	7.15	7.67	31.34	36.93	16.04	99.11	118.93
DH-2/*G. javanica*	8.00	10.11	22.26	22.63	28.48	91.48	109.77
DH-2/*L. purpureus*	8.15	8.41	26.63	24.93	21.41	89.52	107.42
DH-2/*M. invisa*	7.82	8.04	36.18	32.11	26.61	110.70	132.84
DH-2/*P. phaseoloides*	8.23	7.19	29.15	28.78	27.15	100.48	120.58
Mean	7.61	8.57	29.43	31.35	22.91	99.86	119.83
F-Test	N.S.	N.S.	N.S.	N.S.	N.S.		
Inoculated	8.83	9.85	35.05	36.07	30.66	120.46	144.54
Uninoculated	6.39	7.29	23.81	26.63	15.16	79.26	95.12
Mean	7.61	8.57	29.43	31.35	22.91	99.86	119.83
F-Test	**	N.S.	**	**	**		
DH-2/DH-2 (Pure grass stand)	3.33	4.67	12.15	17.11	8.81	46.07	55.28

** Significant at P = 0.01,   N.S.= Not significant at P = 0.05

Yields of grass in association with several legumes as affected by Rhizobium inoculation in different cuttings are presented in table 5c. From the data, it could be observed that grass was benefitted by association with inoculated legumes. Further, yield of grass with several legumes either inoculated or uninoculated varied markedly in all the cuttings. The differences in the yields of grass in grass-legume mixtures were marked under inoculated condition than under uninoculated condition.

Total yield: Under inoculated condition, grass with C. muconoides gave higher total yield (162.05 t/ha/year), followed by grass with M.invisa (156.96 t/ha/year), C.pubescens (150.38 t/ha/year) P.phaseoloides (144.53 t/ha/year), D.biflorus (137.06 t/ha/year) and G.javanica (136.62 t/ha/year), while it was comparatively lower in grass with L. purpureus (124.18 t/ha/year).

Under uninoculated condition, the grass was benefitted considerably due to the association of legumes like M.invisa and D.biflorus, while with other legumes the benefit was marginal. Under uninoculated conditions, legumes were also nodulated but nonspecifically by native rhizobia. Nodulation appeared to be poor. Increased yield upto the fourth cutting was recorded in mixtures namely, grass with C.muconoides, C. pubescens, D.biflorus, G.javanica and M.invisa, while in others it was only upto the third cutting.

Table 5(c)

Relation between cutting dates and ield of grass in grass-legume mixtures (pot culture studies)(Weight of grass, t/ha)

Grass-legume mixtures		Cuttings					Total	Green yield per year	Per cent increase over pure grass stand
		I Dc. 1971	II Feb. 1972	III April	IV June	V Aug.			
DH-2/C.muconoides	(inoculated)	8.15	9.63	40.81	45.93	30.52	135.04	162.05	193
DH-2/C.pubescens	(inoculated)	9.33	9.33	27.70	47.26	31.70	125.32	150.38	172
DH-2/D.biflorus	(inoculated)	7.33	9.41	38.00	41.18	18.30	114.22	137.06	148
DH-2/G.javanica	(inoculated)	.0.52	14.15	30.07	23.41	35.70	113.85	136.62	147
DH-2/L.purpureus	(inoculated)	8.74	9.78	26.81	27.11	31.04	103.48	124.18	125
DH-2/M.invisa	(inoculated)	9.41	8.44	48.44	32.44	32.07	130.80	156.96	184
DH-2/P.phaseoloides	(inoculated)	8.30	8.22	33.48	35.18	35.26	120.44	144.53	161
DH-2/C.muconoides	(uninoculated)	5.11	9.26	25.18	26.07	9.33	74.95	89.94	63
DH-2/C.pubescens	(uninoculated)	5.26	8.96	27.18	28.89	9.85	80.14	96.17	74
DH-2/D.biflorus	(uninoculated)	6.96	5.92	24.67	32.67	13.78	84.00	100.80	82
DH-2/G.javanica	(uninoculated)	5.48	6.07	14.44	21.85	21.26	69.10	82.92	50
DH-2/L.purpureus	(uninoculated)	7.55	7.04	26.44	22.74	11.78	75.55	90.66	64
DH-2/M.invisa	(uninoculated)	6.22	7.63	23.92	31.78	21.04	90.59	108.71	97
DH-2/P.phaseoloides	(uninoculated)	8.15	6.15	24.81	22.37	19.04	80.52	96.62	75
DH-2/DH-2 (pure grass stand)		3.33	4.67	12.15	17.11	8.81	46.07	55.28	
F-Test		N.S.	N.S.	N.S.	N.S.	N.S.			

N.S. = Not significant at P = 0.05

Table 5(d)

Monthly average temperature in the field at Hebbal for 1970-72

Months	Temperature in °C
January 1970	21.50
February 1970	22.50
March 1970	25.90
April 1970	27.30
May 1970	26.50
June 1970	25.45
July 1970	24.90
August 1970	23.65
September 1970	24.05
October 1970	22.85
November 1970	21.60
December 1970	18.80
January 1971	21.10
February 1971	22.30
March 1971	24.15
April 1971	26.75
May 1971	26.25
June 1971	23.60
July 1971	23.80
August 1971	23.25

Table 5(d) - Contd.

Months		Temperature in °C
September	1971	23.10
October	1971	24.55
November	1971	20.35
December	1971	18.95
January	1972	19.00
February	1972	22.55
March	1972	23.55
April	1972	26.55
May	1972	25.70
June	1972	25.35
July	1972	23.75
August	1972	23.55
September	1972	23.85
October	1972	23.05
November	1972	21.55
December	1972	20.20

Inclusion of legumes either inoculated or uninoculated, increased the green weight of associated grass. This increase varied markedly among the different legumes and also between inoculated and uninoculated legumes. Inclusion of legumes in various mixtures increased the green weight of the grass by 72 per cent as compared to pure grass stand (55.28 t/ha/year). This increase in green weight of the associated grass was further increased by 52 per cent by inoculating the legumes with Rhizobium as compared to grass with uninoculated legumes (95.12 t/ha/year). The highest increase in green weight of the associated grass as compared to pure grass stand was in mixtures with M.invisa (140 per cent), followed by C.muconoides, C.pubescens, P. phaseoloides and D.biflorus. In mixtures with G.javanica and L.purpureus, the per cent increase in green weight over pure grass stand ranged from 94 to 99 (table 5a).

Dry matter production: Five random grass samples were taken after each cutting and dried. The moisture per cent with grass samples ranged from 74 to 86. The mean moisture per cent of 80 was considered for the estimation of dry matter production. Pattern of dry matter production in different cuttings as well as inoculated and uninoculated legumes followed the same trend of green grass yield.

Nitrogen yield: The effect of all the seven forage legumes namely, *C.muconoides*, *C.pubescens*, *D.biflorus*, *G.javanica*, *L.purpureus*, *M.invisa* and *P.phaseoloides* both under inoculated and uninoculated conditions on the nitrogen yield of the DH-2 grass was studied. The results are presented in tables 6a and 6b.

Nitrogen yield of the grass grown with different legumes varied markedly depending upon the age of the mixtures. In general, as a mean over legumes, N-yield of the grass increased from 33.32 kg/ha in the first cutting to 125.35 kg/ha in the fifth cutting. These differences among the different cuttings were highly marked.

Highest N-yield of the grass was recorded with *G. javanica*(39.77 kg/ha) in the first cutting, with *C.pubescens* in the second cutting, with *M.invisa* in the third cutting, with *C.muconoides* in the fourth cutting and with *P.phaseoloides* in the fifth cutting (table 6a). Nitrogen yield of the grass with *P.phaseoloides* (173.08 kg/ha) at the fifth cutting was markedly higher than other cuttings. The N-yield of the pure grass stand did not differ much between cuttings and it was comparatively lower than grass grown with legumes in all the cuttings.

Total N-yield (total per year) of the grass with *C.muconoides* was the highest, while that of grass with *D.biflorus*

Table 6(a)

Effect of legumes on nitrogen yield of grass (Pot culture studies)
(N - yield of grass, kg/ha)

Grass-legume mixtures	Cuttings					Total	N-yield kg/ha/ year
	I Dec.1971	II Feb.1972	III Apr.	IV June	V Aug.		
DH-2/C.muconoides	28.79	36.78	112.54	144.65	119.98	442.74	531.28
DH-2/C.pubescens	32.59	38.50	84.31	72.05	105.02	332.47	398.98
DH-2/D.biflorus	32.13	26.25	86.37	89.15	73.34	307.24	368.67
DH-2/G.javanica	39.77	37.00	76.54	60.38	150.98	364.67	437.59
DH-2/L.purpureus	31.55	37.62	72.70	58.02	115.04	314.93	377.80
DH-2/M.invisa	34.33	35.82	117.30	80.62	140.01	408.08	489.70
DH-2/P.phaseoloides	34.13	31.13	86.30	52.03	173.08	376.67	451.99
Mean	33.32	34.73	90.86	79.56	125.35	-	-
Inoculated	41.08	42.18	114.48	107.12	179.24	484.10	580.91
Uninoculated	25.57	27.28	67.24	51.98	71.46	243.53	292.24
Mean	33.32	34.73	90.86	79.56	125.35	-	-
DH-2/DH-2 (pure grass stand)	12.20	13.35	30.86	24.30	34.02	114.73	137.68

was the lowest. The N-yield of grass with different legumes was higher than that of the pure grass stand.

Nitrogen yield of the grass grown with different legumes differed markedly with kind of legume in the mixtures. As a mean over inoculation, grass grown in association with C.muconoides gave the highest N-yield (531.28 kg/ha/year), followed by grass with M.invisa (489.70 kg/ha/year), P.phaseoloides (451.99 kg/ha/year) and G.javanica (437.59 kg/ha/year) while, it was the lowest in grass with C.pubescens (398.98 kg/ha/year), L.purpureus (377.80 kg/ha/year) and D.biflorus (368.67 kg/ha/year) (table 6a).

Nevertheless, inclusion of legumes with grass had increased markedly the N-yield of the grass as compared to the pure grass stand. Similarly, Rhizobium inoculation of the associated legumes of the grass further increased the N-yield markedly. As a mean over legumes, grass with inoculated legumes (580.91 kg/ha/yr) gave more N than grass with uninoculated legumes (292.24 kg/ha/yr) (table 6a). Nitrogen yield of grass grown with inoculated as well as uninoculated legumes varied over a wide range. Among inoculated legumes, the nitrogen yield was maximum in grass with C.muconoides (776.14 kg/ha/year) as compared to grass with D.biflorus (449.41 kg/ha/year) in which it was the lowest. Nitrogen yield varied slightly among uninoculated legume mixtures (251.65 in *G.javanica* to 299.51 kg/ha/year in *P.phaseoloides*) (table 6b). The increase in N-yield due to inoculation of the associated legume was more pronounced in grass with *C.muconoides* as compared to other grass-legume mixtures. The lowest increase was observed in grass with *D.biflorus*.

Nitrogen yield of grass grown with inoculated as well as uninoculated legumes was higher over that of pure grass stand in all the cuttings. Nitrogen yield of the grass varied in different cuttings from *Rhizobium* inoculated legumes. As a mean over legumes, there was marked increase in the N-yield of the grass grown with inoculated legumes in all the cuttings. Highest increase of nitrogen was with the inoculated legumes in fifth cutting followed by the third, fourth and second cutting. Crude protein was estimated from the grass samples grown in pot culture. The trend logically with crude protein was similar to the N-yield as crude protein was nothing but total N multiplied by 6.25. The statistical analysis of data would be similar.

Nitrogen fixation due to inoculation and native rhizobia varied markedly between different grass-legume associations. However, inoculation of legumes increased the nitrogen fixation in all the grass-legume combinations. Nitrogen fixing capacity varied with different legumes. *C.muconoides* fixed more nitrogen (489.72 kg/ha/year), followed by *G.javanica* (371.89 kg/ha/year) and the lowest fixation was observed in *D.biflorus* (161.48 kg/ha/year (table 6b).

Table 6(b)

Nitrogen contribution of legumes to the associated grass (Pot culture studies) (N-yield of grass, kg/ha)

Grass-legume mixtures		Cuttings					Total	N-yield kg/ha/ year	N-fixation by native rhizobia	N-fixation due to inoculation
		I Dec.1971	II Feb. 1972	III April	IV June	V Aug.				
DH-2/*C.muconoides*	(inoculated)	36.83	39.29	144.48	241.85	184.33	646.78	776.14	-	489.72
DH-2/*C.pubescens*	(inoculated)	45.73	43.31	90.87	100.19	162.96	443.06	531.67	-	265.38
DH-2/*D.biflorus*	(inoculated)	34.61	34.24	105.64	107.08	92.94	374.51	449.41	-	161.48
DH-2/*G.javanica*	(inoculated)	58.27	55.18	116.09	74.43	215.65	519.62	623.54	-	371.89
DH-2/*L.purpureus*	(inoculated)	35.14	46.26	74.54	73.74	168.84	398.32	477.98	-	200.35
DH-2/*M.invisa*	(inoculated)	42.14	38.84	166.65	84.35	170.63	502.61	603.13	-	226.86
DH-2/*P.phaseoloides*	(inoculated)	34.84	38.15	103.12	68.26	259.36	503.73	604.48	-	304.97
DH-2/*C.muconoides*	(uninoculated)	20.75	34.26	80.59	47.45	55.63	238.68	286.42	148.74	-
DH-2/*C.pubescens*	(uninoculated)	19.46	33.70	77.75	43.91	47.09	221.91	266.29	128.61	-
DH-2/*D.biflorus*	(uninoculated)	29.66	18.25	67.09	71.21	53.73	239.94	287.93	150.25	-
DH-2/*G.javanica*	(uninoculated)	21.27	18.83	36.98	46.32	86.31	209.71	251.65	113.97	-
DH-2/*L.purpureus*	(uninoculated)	27.96	28.99	70.87	42.30	61.24	231.36	277.63	139.95	-
DH-2/*M.invisa*	(uninoculated)	26.51	32.81	67.95	76.90	109.39	313.56	376.27	238.59	-
DH-2/*P.phaseoloides*	(uninoculated)	33.41	24.10	69.48	35.79	86.81	249.59	299.51	161.83	-
DH-2/DH-2 (pure grass stand)		12.20	13.35	30.86	24.30	34.02	114.73	137.68	-	-

Phosphorus content of grass grown with different forage legumes:

Phosphorus content of the grass samples grown with inoculated as well as uninoculated forage legumes and pure grass stand under pot culture conditions was estimated periodically as mentioned under Material and Methods. The results are presented in table 7.

P_2O_5 content (average of five cuttings) of the grass differed significantly at one per cent level both with the inclusion of legumes and Rhizobium inoculation. The interaction effect of legume and Rhizobium inoculation was highly significant. As a mean over Rhizobium inoculation, mean phosphorus content of the associated grass with M.invisa (0.076 g/pot/cutting), C.pubescens (0.074 g/pot/cutting) and P.phaseoloides (0.069 g/pot/cutting) was significantly more than the associated grass with G.javanica (0.052 g/pot/cutting) (table 7). P_2O_5 content of the grass associated with D.biflorus, L.purpureus and C.muconoides was on par with the P_2O_5 content of the grass associated with C.pubescens and P.phaseoloides and was significantly lower than the P_2O_5 content of the grass with M.invisa. Further, P_2O_5 content of the grass increased significantly with the inclusion of legumes as compared to pure grass stand.

As a mean over legumes, P_2O_5 content of the grass grown with inoculated legumes (0.076 g/pot/cutting) was

Table 7

Interaction of legumes and *Rhizobium* inoculation on Phosphorus content of grass (Pot culture studies) (P_2O_5 content of grass, g/pot)

Grass-legume mixtures		*Rhizobium* inoculated	*Rhizobium* uninoculated	Mean
DH-2/*C. muconoides*	(L_1)	0.064	0.060	0.062
DH-2/*C. pubescens*	(L_2)	0.069	0.079	0.074
DH-2/*D. biflorus*	(L_3)	0.073	0.054	0.064
DH-2/*L. purpureus*	(L_4)	0.090	0.036	0.063
DH-2/*G. javanica*	(L_5)	0.061	0.043	0.052
DH-2/*M. invisa*	(L_6)	0.077	0.074	0.076
DH-2/*P. phaseoloides*	(L_7)	0.096	0.042	0.069
Mean		0.076	0.055	-
DH-2/DH-2 (pure grass stand)	(L_0)	-	-	0.045

	Interaction	Legumes	*Rhizobium* inoculation
F-Test	**	**	**
S.Em ±	0.008	0.006	0.004
C.D. at 5%	0.016	0.011	-

** = Significant at P = 0.01

Conclusion   Legumes : $\underline{L_6 \overline{L_2} L_7} \overline{L_3 L_4 L_1} L_5 L_0$

significantly higher than that of the grass with uninoculated legumes (0.055 g/pot/cutting) (table 7). The interaction effect of the legumes with Rhizobium inoculation was also highly significant.

Among the inoculated legumes, P_2O_5 content of the grass with P.phaseoloides was maximum (0.096 g/pot/cutting), while that of the grass with G.javanica was the least (0.061 g/pot/cutting).

Among the uninoculated legumes, P_2O_5 content of the grass grown with C.pubescens was the highest (0.079 g/pot/cutting), while that of the grass associated with L.purpureus was the lowest (0.036 g/pot/cutting).

In general, P_2O_5 content of the grass with inoculated legumes was comparatively higher than the uninoculated legumes with the exception that P_2O_5 content of the grass with uninoculated C.pubescens (0.079 g/pot/cutting) was significantly more than the inoculated C.pubescens (0.069 g/pot/cutting).

Field Experiment 1.

The experiment was conducted on a plot of land hither to uncultivated and freshly opened up near the Agricultural College, Hebbal. Three forage legumes namely, C.muconoides, G.javanica and P.phaseoloides were included in the mixture with Dharwar hybrid-2 grass. After establishment of the grass, inoculated as well as uninoculated legume seeds were dibbled around the grass setts. Grass was cut every two months over a period of one year. Five random samples were taken after each cuttings and dried. The moisture per cent ranged from 74 to 86 in grass samples at each cutting. The mean moisture per cent of 80 was considered for the estimation of dry matter production. Other calculations were made as in pot culture experiment.

Grass yield: The green weight of the grass grown in association with the three legumes under both inoculated and uninoculated conditions were recorded at each sampling and the results are presented below. Rhizobium inoculation of the legumes significantly increased the associated grass yield as compared to the uninoculated legumes. The grass yield differed significantly at one per cent level due to association of legumes with grass. The interaction effect of legume and Rhizobium inoculation caused no significant differences in grass yield.

As a mean over inoculation, grass associated with C. muconoides yielded significantly higher green weight (172.11 t/ha/year), followed by grass with G. javanica (157.08 t/ha/year) and P. phaseoloides (150.57 t/ha/year). It was significantly lower in pure grass stand (73.11 t/ha/year) (table 8a). However, the yield of grass in association with _G.javanica_ and _P.phaseoloides_ did not differ significantly. Nevertheless, yield of grass grown in association with different legumes was significantly higher than that of the pure grass stand.

Yield per cutting: As a mean over legumes, grass yield increased linearly from the first cutting (1.55 t/ha) to the fourth cutting (47.18 t/ha) and then onwards yields declined considerably (table 8a). There were no significant differences in yield of grass associated with different legumes. However, the yield of grass grown with _C.muconoides_ was comparatively higher than those of other associated legumes in all the cuttings except in the second cutting. No such trend in yield differences in cuttings was seen in other mixtures.

Effect of inoculation on associated legumes with grass was not significant in any of the cuttings. However, yields of grass grown with inoculated legumes were consistently more than those of grass grown with uninoculated legumes in all the cuttings. Yield of pure grass stand was significantly lower than the yield of grass in grass-legume mixture in all the six cuttings. The yield of pure grass stand increased rapidly upto the third cutting and then onwards, decreased gradually.

Green weight of the associated grass increased considerably with the inclusion of legumes and this increase varied markedly with different mixtures. The increase in green

Table 8(a)

Effect of legumes on yield of grass (Field Experiment - 1)
(Weight of grass, t/ha)

Grass-legume mixtures	Cuttings						Total yield per year	Per cent increase over pure grass stand
	I Feb.1972	II April	III June	IV Aug.	V Oct.	VI Dec.		
DH-2/*C. muconoides*	1.80	10.64	49.19	51.18	35.11	24.19	172.11	135
DH-2/*G. javanica*	1.41	12.25	41.47	46.82	34.31	20.82	157.08	115
DH-2/*P. phaseoloides*	1.44	12.84	42.27	43.55	29.54	20.93	150.57	106
Mean	1.55	11.91	44.31	47.18	32.99	21.98	-	
F-Test	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
Treatment								
Inoculated	1.68	12.81	48.14	51.38	36.43	25.06	175.50	140
Uninoculated	1.42	11.01	40.47	42.99	29.55	18.90	144.34	97
Mean	1.55	11.91	44.31	47.18	32.99	21.98	-	
F-Test	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
DH-2/DH-2 (pure grass stand)	0.82	6.50	21.21	19.24	14.29	11.05	73.11	

N.S. = Not significant at P = 0.05

weight of the associated grass over pure grass stand was maximum in grass with C.muconoides (135 per cent), followed by that with G.javanica (115 per cent) and it was comparatively lower in grass with P.phaseoloides (106 per cent). Similarly, with the inclusion of inoculated legumes in the mixtures, the grass yield increased by 140 per cent as compared to 97 per cent with uninoculated legumes over pure grass stand.

Grass yields due to different treatments differed significantly in the third, fourth and the fifth cuttings (table 8b). Yields increased generally upto the third cutting and then decreased (fig. 2). In the first cutting, grass yield was higher in grass with inoculated C.muconoides (2.10 t/ha) as compared to that in other mixtures and it was the lowest in pure grass stand (0.82 t/ha). In second cutting the grass yield ranged from 6.50 t/ha in pure grass stand to 14.46 t/ha in grass with inoculated P.phaseoloides and this difference was not significant. Further, in the third cutting grass yield of pure grass stand was significantly lower than the mixtures except that with uninoculated P.phaseoloides. In the fourth cutting, the yield of grass grown with inoculated as well as uninoculated legumes was significantly more than that of pure grass stand. With others, there were no significant differences. Highest yield of 54.36 t/ha/cutting was obtained in grass grown with inoculated

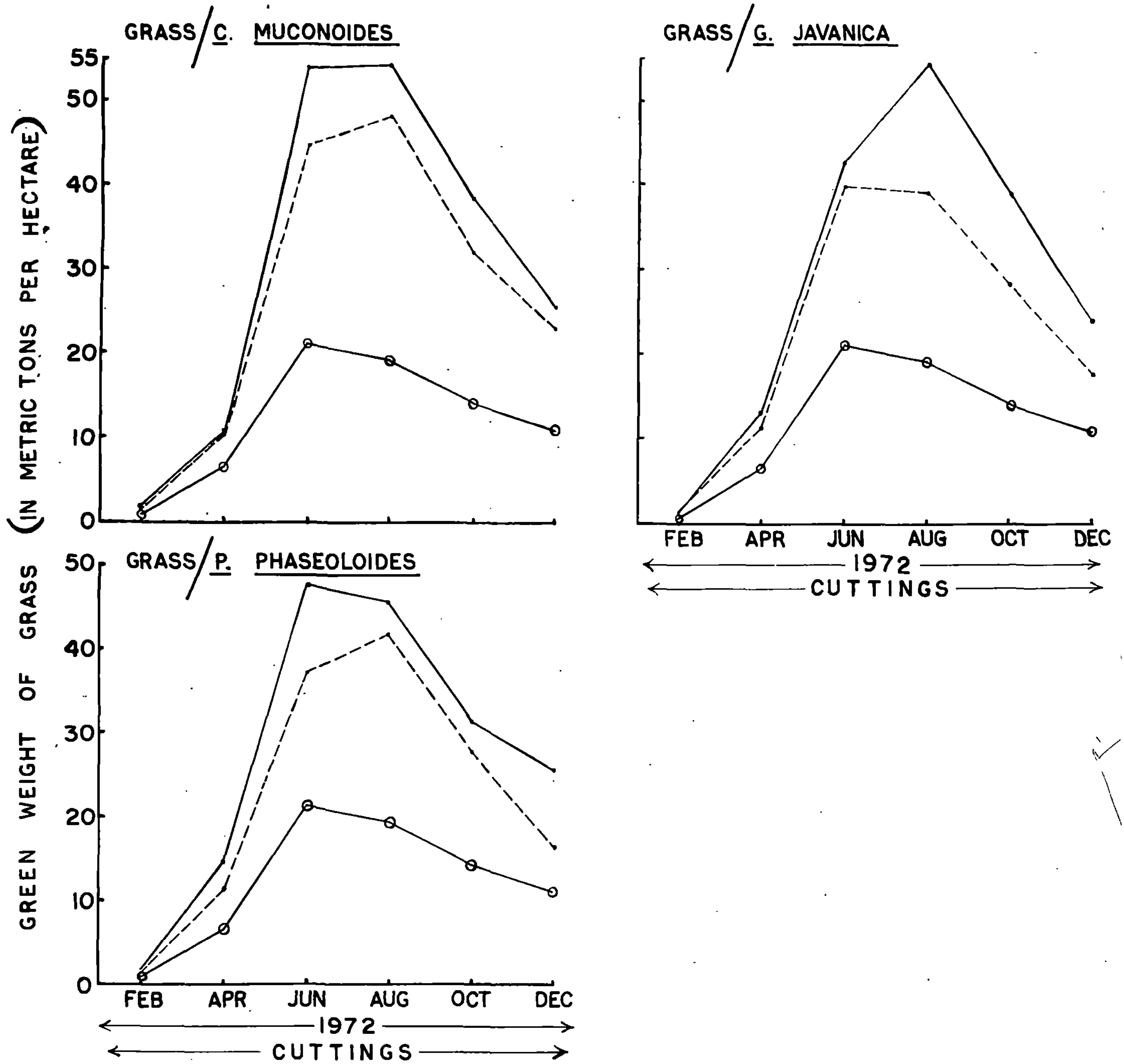

FIG: 2 RELATION BETWEEN CUTTING DATES AND YIELD OF GRASS IN GRASS-LEGUME MIXTURES (FIELD EXPERIMENT-I) (DATA OF SIX CUTTINGS) (GRASS WITH INOCULATED LEGUME •———•, GRASS WITH UNINOCULATED LEGUME •- - - - - -• AND PURE GRASS STAND ⊙———⊙).

Table 8(b)

Relation between cutting dates and yield of grass in grass-legume mixtures (Field experiment - 1) (Weight of grass, t/ha)

Grass/legume mixtures	Cuttings						Total yield per year
	I Feb.1972	II April	III June	IV Aug.	V Oct.	VI Dec.	
Inoculated							
DH-2/*C.muconoides*	2.10	10.76	53.95	54.36	38.29	25.38	184.84
DH-2/*G.javanica*	1.32	13.20	42.86	54.36	39.67	24.19	175.60
DH-2/*P.phaseoloides*	1.63	14.46	47.61	45.43	31.33	25.60	166.06
Uninoculated							
DH-2/*C.muconoides*	1.51	10.51	44.43	48.00	31.93	23.00	159.38
DH-2/*G.javanica*	1.50	11.31	40.07	39.29	28.95	17.45	138.57
DH-2/*P.phaseoloides*	1.26	11.22	36.91	41.67	27.76	16.26	135.08
DH-2/DH-2 (pure grass stand)	0.82	6.50	21.21	19.24	14.29	11.05	73.11
F-Test	N.S.	N.S.	*	**	*	N.S.	
S.Em ±	0.39	2.20	7.71	7.56	5.85	6.07	
C.D. at 5%	-	-	16.81	16.48	12.74	-	

** Significant at P = 0.01
 * Significant at P = 0.05

N.S. = Not significant at P = 0.05

G.javanica and C.muconoides. Again, in the fifth cutting, a similar trend was observed. Grass yield due to inclusion of legumes were higher as compared to pure grass stand. The yields varied from 14.29 t/ha in pure grass stand to 39.67 t/ha per cutting in grass with inoculated G.javanica. There were no significant differences among treatments with respect to yield in the sixth cutting.

Total yield: As a mean over associated legumes, grass yield of DH-2 grass grown with inoculated legumes was significantly more than grass with uninoculated legumes (table 8c). The increase in yield was significant and it was to the extent of 22 per cent as compared to the yield of grass grown with uninoculated legumes.

Among the inoculated legumes, grass yield was the highest when grown in association with C.muconoides (184.84 t/ha/year), while it was the lowest with P.phaseoloides (166.06 t/ha/year). A similar trend was observed in yield of grass with uninoculated legumes. Grass yield with uninoculated C.muconoides was 159.38 t/ha/year, while in grass with P.phaseoloides it was 135.08 t/ha/year. The yields were higher in case of all the grass legume mixtures than the pure grass stand.

Nitrogen yield: The effect of three forage legumes namely, C.muconoides, G.javanica and P.phaseoloides, both under

Table 8(c)

Interaction of legumes and *Rhizobium* inoculation on grass yields (Field Experiment - 1) (Weight of grass, t/ha/year)

Grass-legume mixtures		*Rhizobium* inoculated	*Rhizobium* uninoculated	Mean
DH-2/*C.muconoides*	(L_1)	184.84	159.38	172.11
DH-2/*G.javanica*	(L_2)	175.60	138.57	157.08
DH-2/*P.phaseoloides*	(L_3)	166.06	135.08	150.57
Mean		175.50	144.34	
DH-2/DH-2 (pure grass stand)	(L_0)	-	-	73.11

	Legumes	*Rhizobium* inoculation	Interaction
F-test	**	**	N.S.
S-Em ±	1.39	1.13	1.96
C.D. at 5%	10.17	-	-

Conclusion

L_1 $\underline{L_2 \quad L_3}$ L_0

** = Significant at P = 0.01
* = Significant at P = 0.05
N.S.= Not significant at P = 0.05

inoculated and uninoculated conditions on the nitrogen yield of the associated grass was studied.

There were considerable differences between cuttings in the nitrogen yield of grass in grass-legume mixtures.

As a mean over legumes, N-yield of grass increased from 5.33 kg/ha in the first cutting to 161.98 kg/ha in the fourth cutting and then declined to 75.98 kg/ha in the sixth cutting(table 9a). The increment in N-yield was rapid upto the third cutting and was marginal in the fourth cutting and declined sharply in the subsequent cuttings. Nitrogen yield of the grass varied considerably among the legumes in different cuttings. Nitrogen yield obtained from the grass associated with C.muconoides was higher in all the cuttings except in the second cutting as compared to that from the grass with other two legumes. Between the two grass-legume mixtures, grass with P.phaseoloides yielded more nitrogen in all the cuttings except in the fourth and the fifth cutting. However, N-yield of grass associated with legumes was considerably higher than the N-yield of pure grass stand in all the cuttings.

The nitrogen yield of grass in pure grass stand was the lowest as compared to grass-legume mixtures. N-yield of grass was further increased by inoculating the associated legumes in all the cuttings. The N-yield of the grass increased from the first cutting to the fourth cutting and subsequently declined considerably in both the inoculated and the uninoculated grass-legume mixtures.

Total N-yield of the grass (total per year) differed considerably among the legumes and also between Rhizobium inoculation. As a mean over inoculation, grass with C.muconoides yielded the highest nitrogen (599.47 kg/ha/year), followed by grass with P.phaseoloides (533.15 kg/ha/year), while it was the lowest in grass with G.javanica (516.97 kg/ha/year (table 9a). N-yield was the lowest in pure grass stand as compared to grass-legume mixtures.

The legumes responded markedly to Rhizobium inoculation. Generally, the N-yield of grass was higher due to inoculation of legumes as compared to N-yield of grass with uninoculated legumes. Grass with C.muconoides yielded higher N-yield both under inoculated and uninoculated conditions, while it was moderate with P.phaseoloides and was comparatively lower in grass with G.javanica (table 9a).

Highest N was fixed by native rhizobia in grass with C.muconoides (269.03 kg/ha/year), followed by grass with P.phaseoloides (209.63 kg/ha/year), while this was the lowest in grass with G.javanica (192.76 kg/ha/year). Similarly, this N fixation by different legumes was increased by Rhizobium inoculation. Nitrogen fixation by inoculation was the highest (242.73 kg/ha/year) in grass with C.muconoides

Table 9(a)

Effect of legumes on Nitrogen yield of grass (Field Experiment-1)
(N-Yield of grass, kg/ha)

Grass-legume mixtures	Cuttings						Total per year
	I Feb.1972	II April	III June	IV Aug.	V Oct.	VI Dec.	
DH-2/*C.muconoides*	6.35	36.74	171.85	177.99	122.55	83.99	599.47
DH-2/*G.javanica*	4.55	40.15	135.25	154.80	113.38	68.84	516.97
DH-2/*P.phaseoloides*	5.11	45.59	150.04	153.17	104.13	75.11	533.15
Mean	5.33	40.83	152.38	161.98	113.35	75.98	-
Inoculated	6.42	48.63	183.46	194.94	138.10	95.29	666.84
Uninoculated	4.25	33.03	121.30	129.03	88.60	56.67	432.88
Mean	5.33	40.83	152.38	161.98	113.35	75.98	-
DH-2/DH-2(pure grass stand)	2.35	18.59	60.67	55.02	40.86	31.59	209.08

followed by grass with *G.javanica* (230.26 kg/ha/year), while it was the lowest in grass with *P.phaseoloides* (228.89 kg/ha/year) (table 9b).

Field Experiment 2.

The experiment was conducted on a sandy loam soil of fairly high level of fertility (0.088 per cent nitrogen) at the Main Research Station, Hebbal, Bangalore. Four forage legumes namely, *C.muconoides*, *C.pubescens*, *G.javanica* and *L.purpureus* were included in the mixture with Dharwar hybrid-2 grass. After the establishment of grass, legume seeds inoculated as well as uninoculated were dibbled around the grass setts. Grass was cut every two months over a period of two years. Five random samples were taken after each cutting and dried. The moisture per cent ranged from 74 to 86. The mean moisture per cent of 80 was used for the estimation of dry matter production. The effect of four legumes when inoculated and uninoculated with appropriate rhizobia over the DH-2 grass was studied.

Grass yield: Green weight of the grass grown in association with the four legumes under both inoculated and uninoculated conditions were recorded at each samplings and the results are presented below.

Table 9(b)

Nitrogen contribution of legumes to the associated grass (Field experiment - 1)

(N-Yield of grass, kg/ha)

Grass-legume mixtures	Cuttings						Total N-yield per year	N-fixation by native rhizobia	N-fixation due to inoculation
	I Feb.1972	II April	III June	IV Aug	V Oct.	VI Dec.			
noculated									
DH-2/*C.muconoides*	8.18	41.97	210.41	211.99	149.31	98.98	720.84	-	242.73
DH-2/*G.javanica*	4.75	47.51	154.28	195.68	142.80	87.08	632.10	-	230.26
DH-2/*P.phaseoloides*	6.34	56.39	185.68	177.17	122.20	99.82	647.60	-	228.89
ninoculated									
DH-2/*C.muconoides*	4.51	31.52	133.29	144.00	95.79	69.00	478.11	269.03	-
DH-2/*G.javanica*	4.35	32.78	116.21	113.93	83.96	50.61	401.84	192.76	-
DH-2/*P.phaseoloides*	3.89	34.78	114.40	129.17	86.06	50.41	418.71	209.63	-
DH-2/DH-2 (pure grass stand)	2.35	18.59	60.67	55.02	40.86	31.59	209.08	-	-

<u>Yield per cutting</u>: Yield of grass due to legumes differed significantly in the fourth, fifth and the ninth cuttings only. As a mean over associated legumes, yield of grass was maximum in the second cutting(44.07 t/ha), while it was the lowest in the first cutting(11.05 t/ha) (Table 10a). The yield varied in between these two ranges in other cuttings. The yield of grass grown with <u>C.muconoides</u> was higher than that with the other associated legumes in the first, second third and the sixth cuttings, while the yield of grass with <u>L.purpureus</u> was comparatively higher than that with the other legumes in rest of the cuttings (fig.3). Yield of grass grown with <u>Rhizobium</u> inoculated legumes was significantly superior over that with uninoculated legumes in majority of the cuttings. The yield of the pure grass stand was the least when compared with the other treatments in all the cuttings. Maximum yield recorded was in the eleventh cutting (20.76 t/ha) and the lowest in the fifth cutting (4.26 t/ha) (table 10a). In the first cutting, there were not many differences in the grass yields between the treatments (table 10b). The first cutting yield ranged from 4.93 t/ha in the pure grass stand to 13.80 t/ha in the grass with inoculated <u>C.muconoides</u>. In the second cutting, the yield ranged from 18.92 t/ha in pure grass stand to 56.68 t/ha in the grass with inoculated <u>C.muconoides</u>. Grass yields with uninoculated <u>C.muconoides</u> were comparatively higher than in the other treatments, except those with inoculated

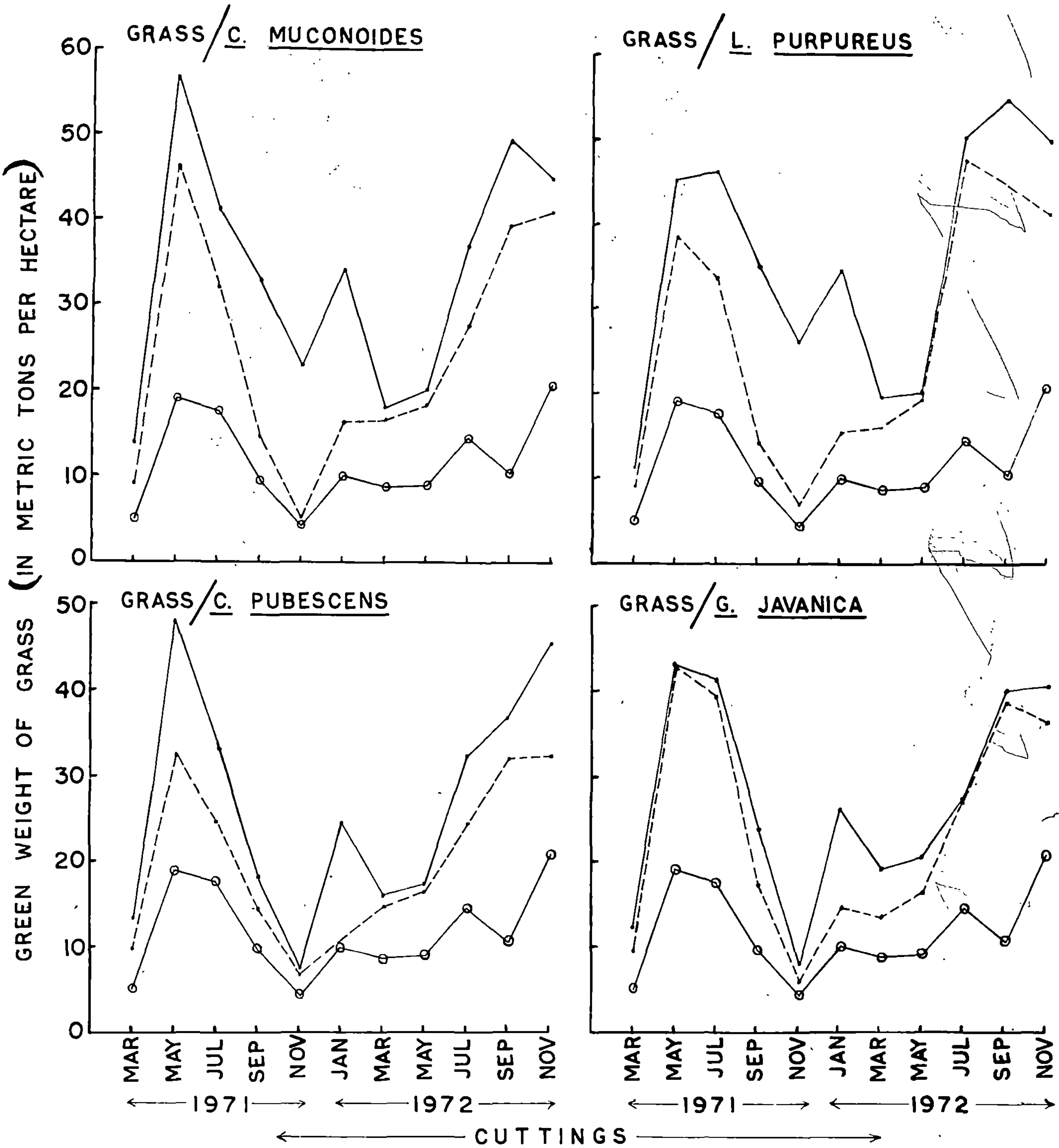

FIG: 3 RELATION BETWEEN CUTTING DATES AND YIELD OF GRASS IN GRASS-LEGUME MIXTURES (FIELD EXPERIMENT-2) (DATA OF ELEVEN CUTTINGS) (GRASS WITH INOCULATED LEGUME ———, GRASS WITH UNINOCULATED LEGUME •- - - - -• AND PURE GRASS STAND ⊙———⊙).

Table 10(a)

Effect of legumes on yield of grass (Field experiment - 2)
(Weight of grass, t/ha)

Grass-legume mixtures	Cuttings I Mar.1971	II May	III July	IV Sep.	V Nov.1971	VI Jan.1972	VII Mar.	VIII May	IX July	X Sep.	XI Nov.	Total	Total yield per year	Per cent increase over pure grass stand
DH-2/C.muconoides	11.54	51.41	36.71	23.73	14.04	25.27	17.28	19.08	32.60	44.55	43.06	319.27	174.15	148
DH-2/C.pubescens	11.52	40.07	29.24	16.12	7.04	17.77	15.34	16.87	28.05	34.45	38.66	255.13	139.16	98
DH-2/L.purpureus	10.12	41.94	40.01	26.94	16.45	24.99	17.84	19.67	49.02	49.73	45.39	342.10	186.60	166
DH-2/G.javanica	11.03	42.85	40.34	20.77	6.76	20.41	16.12	18.27	27.09	39.30	38.45	281.39	153.48	118
Mean	11.05	44.07	36.57	21.89	11.07	22.11	16.64	18.47	34.19	42.00	41.39	-		
C.D. at 5%	N.S.	N.S.	N.S.	*5.79	**2.64	N.S.	N.S.	N.S.	**7.56	N.S.	N.S.			
Treatment														
Inoculated	12.71	48.20	40.69	27.57	16.02	29.80	18.09	19.41	36.91	45.35	45.08	339.83	185.36	164
Uninoculated	9.39	39.95	32.46	16.20	6.12	14.43	15.20	17.53	31.47	38.66	37.70	259.11	141.33	101
Mean	11.05	44.07	36.57	21.89	11.07	22.11	16.64	18.47	34.19	42.00	41.39	-		
F-Test	**	**	N.S.	**	**	**	N.S.	N.S.	*	*	N.S.			
DH-2/DH-2 (pure grass stand)	4.93	18.92	17.64	9.58	4.26	10.07	8.73	9.06	14.40	10.45	20.76	128.80	70.25	-

** Significant at P = 0.01, * Significant at P = 0.05, N.S.= Not significant at P = 0.05

Table 10(b)

Relation between cutting dates and yield of grass in grass-legume mixtures (Field experiment - 2)
(Weight of grass, t/ha)

Grass-legume mixtures	Cuttings I Mar.1971	II May	III July	IV Sept.	V Nov.	VI Jan. 1972	VII Mar.	VIII May	IX July	X Sept.	XI Nov.	Total	Total yield per year
Inoculated													
DH-2/C.mucunoides	13.80	56.68	41.10	33.06	23.00	34.18	17.92	19.86	37.50	49.72	44.88	371.70	202.74
DH-2/C.pubescens	13.30	47.92	34.02	18.05	7.35	24.45	16.10	17.36	32.50	36.95	45.10	293.10	159.87
DH-2/L.purpureus	11.33	45.28	46.26	35.00	26.02	34.48	19.58	20.15	50.42	54.74	49.77	392.98	214.35
DH-2/G.javanica	12.43	42.92	41.38	24.18	7.72	26.10	18.75	20.28	27.23	40.00	40.56	301.55	164.48
Uninoculated													
DH-2/C.mucunoides	9.27	46.15	32.32	14.40	5.08	16.35	16.65	18.30	27.70	39.[illegible]	41.23	266.83	145.54
DH-2/C.pubescens	9.73	32.22	24.45	14.19	6.73	11.10	14.58	16.38	23.[illegible]	[illegible]	32.22	217.55	118.66
DH-2/L.purpurea	8.90	38.60	33.76	18.88	6.88	15.56	16.10	19.19	47.6[illegible]	44.73	41.00	291.23	158.85
DH-2/G.javanica	9.63	42.79	39.30	17.35	5.80	14.72	15.49	16.25	26.9[illegible]	38.60	35.35	261.23	142.49
DH-2/DH-2 (pure grass stand)	4.93	18.92	17.64	9.58	4.26	10.07	8.73	9.06	14.40	10.45	20.76	12[illegible].80	70.25
F-Test	*	**	N.S.	**	**	**	N.S.	*	**	**	N.S.		
S.Em ±	2.27	6.28	9.82	3.88	1.77	4.04	3.62	2.54	5.[illegible]	6.67	9.12		
C.D.at 5%	4.80	13.32	-	8.22	3.73	8.57	-	5.38	10.[illegible]	14.13	-	-	

** = Significant at P=0.01, * = Significant at P = .0.05, N.S. = Not significant at P = 0.05

C.muconoides and C.pubescens. However, yield of grass grown with inoculated C.pubescens was on par with the yield of pure grass stand. During the third cutting, grass yield due to treatments did not differ significantly. The yields ranged from 17.64 t/ha in pure grass stand to 46.26 t/ha in grass with inoculated L.purpureus. In the fourth cutting, grass yields ranged from 9.58 t/ha in pure grass stand to 35.00 t/ha in grass with inoculated L.purpureus and the differences in yield due to treatments were significant, while the yields of grass with uninoculated C.pubescens and grass with uninoculated C.muconoides were on par with the yield of pure grass stand. During the fifth cutting, grass yield with inoculated L.purpureus and that with inoculated C.muconoides was significantly more than the yields of other treatments. Yields in other treatments were on par with the pure grass stand (4.26 t/ha). In the sixth cutting, the grass yields due to different treatments were comparatively higher than the fifth, seventh and the eighth cuttings. The yields ranged from 10.07 t/ha in pure grass stand to 34.48 in grass with inoculated L.purpureus and this difference in yield due to treatments was highly significant. During the seventh cutting, grass yields did not differ significantly due to treatments. They ranged from 8.73 t/ha in pure grass stand to 19.58 t/ha in grass with inoculated L.purpureus. In the eighth cutting, grass yield of pure grass stand was significantly lower as compared to that in other treatments. The grass yields in other treatments ranged from 16.25 t/ha in grass with uninoculated G.javanica to 20.28 t/ha in grass with inoculated G.javanica. In the ninth cutting, the grass yields ranged from 14.40 t/ha in pure grass stand to 50.42 t/ha in inoculated L.purpureus. During the tenth cutting, the yields ranged from 10.45 t/ha in pure grass stand to 54.74 t/ha in grass with inoculated L.purpureus and the differences among treatments were significant. In the last cutting, the yields did not differ significantly. They ranged from 20.76 t/ha in pure grass stand to 49.77 t/ha in grass with inoculated L.purpureus.

Total Yield: Rhizobium inoculation of legumes significantly increased the associated grass yield as compared to the uninoculated legumes. The grass yield differed significantly at one per cent level due to associated legumes. The interaction effect of legume and Rhizobium inoculation caused no significant differences in grass yield.

As a mean over Rhizobium inoculation, total grass yield (total per year) with L.purpureus was significantly more than the total grass yields of mixtures, namely with G.javanica and C.pubescens, while it was on par with the yield of grass in association with C.muconoides (table 10c). Total yield of grass with C.muconoides was on par with grass grown in association with G.javanica but was significantly more than the yield of grass with C.pubescens. However, the yield of grass with legumes was significantly more than the yield of the pure grass stand. The increase in total (total per year) green weight of the associated grass over pure grass stand was maximum with L.purpureus (166 per cent), followed by that with C.muconoides (148 per cent) and it was comparatively lower in grass with C.pubescens (98 per cent). Similarly, with the inclusion of inoculated legumes in the mixture the grass yield increased by 164 per cent as compared to 101 per cent in uninoculated legumes over pure grass stand.

As a mean over associated legumes, yield of grass grown with Rhizobium inoculated legumes was significantly higher than that with uninoculated legumes and the increase was to the extent of 31 per cent (table 10c). Grass with legumes did not interact significantly with Rhizobium inoculation.

Among the Rhizobium inoculated legumes, yield of grass was the highest in association with L.purpureus (214.35 t/ha/year) and lowest with C.pubescens (159.87 t/ha/year).

A similar trend was observed in grass yield even when associated legumes were uninoculated. When legumes were uninoculated, grass yield was the highest with L.purpureus and the least with C.pubescens. Yield of pure grass stand

Table 10(c)

Interaction of legumes and *Rhizobium* inoculation on grass yields (Field experiment - 2) (Weight of grass, t/ha/year)

Grass-legume mixtures		*Rhizobium* inoculated	*Rhizobium* uninoculated	Mean
DH-2/*C. muconoides*	..	202.74	145.54	174.14
DH-2/*C. pubescens*	..	159.87	118.66	139.26
DH-2/*G. javanica*	..	164.48	142.49	153.48
DH-2/*L. purpureus*	..	214.35	158.85	186.60
Mean	..	185.36	141.38	
DH-2/DH-2 (pure grass stand)		-	-	70.25

	Legumes	*Rhizobium* inoculation	Interaction
F-Test	**	**	N.S.
S.Em ±	5.12	3.63	0.72
C.D. at 5%	25.61	-	-

** Significant at P = 0.01

N.S. = Not significant at P = 0.05

was the lowest as compared to that grown with different legumes and this difference was highly significant (table 10c).

Pattern of dry matter production of grass in different cuttings in inoculated and uninoculated legume followed the same trend as in green grass yield.

Nitrogen yield: Effect of all the four forage legumes namely *C.muconoides*, *C.pubescens*, *G.javanica* and *L.purpureus*, both under inoculated and uninoculated conditions on the N-yield of the DH-2 grass was studied.

There were considerable differences in nitrogen yields among the kind of legumes and between cuttings and this variation was dependent on the age of the mixtures. Generally the trend of N-yield was inconsistent. As a mean over legumes, N-yield increased from 39.06 kg/ha in the first cutting to 156.19 kg/ha in the second cutting and then onwards declined gradually upto the fifth cutting (40.01 kg/ha). From the seventh cutting, N- yield increased gradually upto the tenth cutting (148.45 kg/ha) and then declined in the eleventh cutting (table 11a). The legumes differed in their capacity to contribute nitrogen to the associated grass. Nitrogen yield obtained from the grass associated with *C.muconoides* was higher in all the cuttings except the third, fourth, fifth, ninth and the tenth cuttings as compared to other three grass-legume mixtures. Among the other three grass-legume mixtures, grass with *L.purpureus* yielded more nitrogen in all the cuttings except in the first cutting. Nitrogen yield of the grass with *G.javanica* was higher in all the cuttings except in the first, fifth, ninth and the eleventh cuttings. However, the nitrogen yield of grass associated with legumes was considerably higher than that of pure grass stand in all the cuttings.

Total N-yield of grass (total per year) differed considerably among the legumes as also between uninoculated and inoculated grass-legume mixtures. As a mean over inoculation, N-yield of grass with *C.muconoides* and *L.purpureus* was highest, followed by that with *G.javanica* and lowest in grass with *C.pubescens.* Further, the N-yield was the lowest in pure grass stand as compared to the grass legume mixtures.

The legumes responded markedly to *Rhizobium* inoculation. Generally the N-yield of grass was higher due to inoculation of legumes as compared to N-yield of grass with uninoculated legumes (table 11a). Due to inoculation of the associated legumes, the increase in N-yield was to the extent of 51 per cent. Nitrogen yield in grass with *C.muconoides* was higher both under inoculated and uninoculated conditions. It was moderately higher with *L.purpureus*, medium in grass with *G.javanica* and comparatively low with *C.pubescens*.

Legumes differed in their contribution of N to the associated grass. Relatively higher nitrogen was fixed

Table 11 (a)

Effect of legumes on Nitrogen yield of grass (Field experiment - 2)

(N-yield of grass, kg/ha)

Grass-legume mixtures	Cuttings											Total	N-yield per year
	I Mar.1971	II May	III July	IV Sep.	V Nov.	VI Jan.1972	VII Mar.	VIII May	IX July	X Sep.	XI Nov.		
DH-2/*C.muconoides*	43.86	196.73	140.40	90.93	54.16	96.75	65.84	72.73	124.55	170.19	164.40	1220.54	665.75
DH-2/*C.pubescens*	38.93	136.01	98.89	54.28	23.49	61.36	51.34	56.33	94.87	115.48	130.86	861.86	470.10
DH-2/*G.javanica*	37.59	143.55	135.61	71.07	23.07	70.93	55.15	62.07	90.76	131.97	129.72	951.49	518.99
DH-2/*L.purpureus*	35.87	148.46	142.01	96.15	59.31	89.46	63.20	69.43	173.24	176.15	160.77	1214.05	662.21
Mean	39.06	156.19	129.23	78.11	40.01	79.62	58.88	65.14	120.85	148.45	146.44	-	
Inoculated	47.80	181.29	152.83	103.72	60.27	111.98	67.93	72.90	138.29	170.33	169.09	1276.43	693.23
Uninoculated	30.32	131.09	105.63	52.50	19.75	47.27	49.84	57.37	103.42	126.56	123.79	847.54	462.30
Mean	39.06	156.19	129.23	78.11	40.01	79.62	58.88	65.14	120.85	148.45	146.44	-	
DH-2/DH-2 (pure grass stand)	14.11	54.10	50.43	27.41	12.20	28.79	24.98	25.88	41.18	29.84	59.34	368.26	200.87

by native Rhizobia in grass with C.muconoides and L.purpureus, while it was moderate in grass with G.javanica and least in grass with C.pubescens (table 11b). Nitrogen fixation by different legumes was increased by Rhizobium inoculation. Nitrogen fixation by inoculation was the highest in grass with C.muconoides (249.66 kg/ha/year) and L.purpureus (244.41 kg/ha/year) and was comparatively low in grass with C.pubescens (229.77 kg/ha/year) and the lowest in grass with G.javanica (211.84 kg/ha/year).

Table 11(b)

Nitrogen contribution of legmes to the associated grass (Field experiment -2) (N-yield of grass, kg/ha)

Grass-legume mixtures	Cuttings											Total	N-yield per year	N-fixation by native rhizobia	N-fixation by inoculation
	I Mar.1971	II May	III July	IV Sep	V Nov.	VI Jan.1972	VII Mar.	VIII May	IX July	X Sep.	XI Nov.				
Inoculated															
DH-2/*C. mucunoides*	53.82	221.00	160.29	128.89	89.70	133.25	69.87	77.41	146.25	193.89	175.04	1449.41	790.59	-	249.66
DH-2/*C. pubescens*	48.67	175.38	124.50	66.06	26.84	89.42	58.93	63.50	118.95	135.17	165.07	1072.49	584.99	-	229.77
DH-2/*G. javanica*	47.24	163.08	157.25	91.83	29.32	99.18	71.25	77.01	103.42	152.00	154.09	1145.67	624.91	-	211.84
DH-2/*L. purpureus*	41.48	165.68	169.27	128.10	95.22	126.06	71.67	73.69	184.53	200.26	182.15	1438.11	784.42	-	244.41
Uninoculated															
DH-2/*C. mucunoides*	33.91	172.47	120.51	52.98	18.63	60.25	61.81	68.05	102.85	146.48	153.76	991.70	540.93	340.06	-
DH-2/*C. pubescens*	29.19	96.65	73.29	42.50	20.15	33.30	43.75	49.15	70.80	95.80	96.65	651.23	355.22	154.35	-
DH-2/*G. javanica*	27.93	124.02	113.97	50.31	16.82	42.68	39.05	47.12	78.10	111.94	105.36	757.30	413.07	212.20	-
DH-2/*L. purpureus*	30.26	131.24	114.75	64.20	23.40	52.87	54.74	65.17	161.95	152.04	139.40	990.02	540.01	339.14	-
DH-2/DH-2 (pure grass stand)	14.11	54.10	50.43	27.41	12.20	28.79	24.98	25.88	41.18	29.84	59.34	368.26	200.87		

DISCUSSION

V. DISCUSSION

The role of legumes in adding symbiotically fixed nitrogen to the soil/plant system is not easy to assess though much of the soil N might result from such fixation. In the present studies, attempts were made to assess the accrual of symbiotically fixed N to the grass in grass-legume association by measuring the recovery of N in grass tops.

Both dry matter and green weight yields of grass grown in association with inoculated and uninoculated tropical species of legumes were determined over a period of time in cutting trials. Pure grass stand served as a control and N recovered in tops represented the soil N. Nitrogen recovered in grass tops in the grass-legume mixtures represented the symbiotically fixed N (inoculated and uninoculated) and soil N. The data on legume N fixation in these studies are only approximations because, non symbiotically fixed N, N accruing to the soil through precipitation, irrigation water etc., are not easily measurable.

In the present study, the identity of the rhizobial isolates were made through series of biochemical, physiological and cultural tests. Identification through serological methods were attempted but they were not successful because of poor titres of antisera obtained. The identity of the organisms were however conclusively established in Leonard's bottle jar assembly method on respective host legumes.

The isolates were able to form nodules both on tap root and on lateral roots. Nodules formed in Mimosa invisa were finger like clusters and others were spherical with smooth or rough surface.

Cross-inoculation studies indicated that the isolate from Centrosema pubescens alone was host specific. The other legumes could be easily nodulated by all the isolates. This is in agreement with the reports of Bowen (1958 and 1959a) who also observed C.pubescens to be highly specific in its bacterial requirement.

Comparable estimates of N-fixation and transfer to hybrid grass of the type used in the present studies have not been made with tropical legumes. The grasses on which much of the work is reported, belong to short statured grasses like paragrass, pangola grass, thin napier and Sudan grass. For instance, Whitney and Green (1969a) have observed dry matter yields of 3.78 t/ha/year in pure grass stand, 5.08 t/ha/year in grass with uninoculated legume and 5.46 t/ha/year in grass with inoculated legumes in Pangola grass/Desmodium canum and D.intortum mixtures. Likewise, Kretschmer (1970) has reported an average grass yield of 5.24 t/ha/year in pure pangola grass stand, 9.49 and 9.44 t/ha/year in pangola grass with C.pubescens and Glycine javanica, respectively. These yields of grass are not comparable to 70 to 214 t/ha/year yields of hybrid grass obtained in the present studies with hybrid grass-legume mixtures.

Dharwar hybrid-2 grass used in this study was evolved in the year 1966 by effecting cross between elephant grass (*Pennisetum purpureum*) and cultivated bajra cultivar Golgeri-1 (*Pennisetum typhoides*). It is more nutritious. It tillers extensively and is easier to propagate. It is very vigorously growing type and responds to irrigation and mineral nitrogen application. It is known for its heavy yields especially under adequate irrigation and fertilizer application. As much as 475 t/ha/year of green matter can be expected with application of 180 kg N, 90 kg P_2O_5 and 50 kg K_2O per hectare (personal communication, P.C.Hiremath, Agrostologist, Regional Research Station, Dharwar-5).

In the year of establishment, yields in the first cutting were uniformly low with all the grass-legume mixtures and the pure grass stand. Here again, the grass associated with *Mimosa invisa* reached a peak production during the third cutting and with other legumes during the fourth and subsequent cuttings. In all the mixtures there was a gradual decline after reaching peak production.

These fluctuations in yields were related to seasonal changes in temperature. Green weight of the grass increased with the increase in mean temperature. Grass yield was higher during late summer and early monsoon and decreased during late monsoon and winter when the mean temperatures were usually low (table 5d). In pot culture studies, the grass yield in association with the inoculated and uninoculated legumes as well as pure grass stand increased with increase in temperature. April and June cuttings gave the highest green weight of grass followed by August cutting. Green yields of December and February cuttings were lower (table 5b). Mean temperatures during April and June, 1972 were higher (25.35 and 26.55°C, respectively) than during December, 1971 (18.95°C), February (22.55°C) and August, 1972 (23.55°C) (table 5d). This relationship between grass yields and mean temperatures indicated seasonal effect on grass growth. In uncultivated field (Field experiment - 1) trial, yields were higher in cuttings made during June and August followed by October and December, while they were lowest during February and April. This indicated that higher temperatures caused better forage production. Similarly, in cultivated field trial (Field experiment - 2) the yield increased with the increase in temperature during first year. The yields were higher during May and July (Second and third cutting) as compared to September and January (fourth and sixth cuttings). The yields reached a maximum after four months of planting and thereafter they fluctuated depending upon the temperatures prevailing during that period. This observation is in conformity with the findings of Vincent-Chandler et al. (1953), Whitney (1969) and Whitney and Green (1969a) who also observed an increase in yield with increase in mean temperatures.

This relationship between increase in temperature and increase in yield observed in the first year of the experiment was absent during the second year. Such phenomenon was also noted by Whiteman and Lulham (1969). However, fluctuations in per cutting yields of the grass during the second year were not correlated with seasonal changes. Compared with first year, second year per cutting yields were by and large higher with few exceptions. The reason for this is unknown at the moment. It was however, likely that there was build-up of fertility during the second year due to defoliation and decay of dead leaves, stems and roots of the legumes and this could have been responsible for fairly uniform higher yields during the second year.

In pot culture studies, grass associated with M. invisa gave the highest green yield (132.84 t/ha/year), while the green weight of pure grass stand was the lowest (55.28 t/ha/year). The yield of grass with other legumes was intermediate (107.42 to 123.28 t/ha/year). Under uncultivated field condition Calopogonium muconoides yielded the highest green weight (172.11 t/ha/year) and the least with P. phaseoloides (150.57 t/ha/year). Grass yield was considerably lower in pure grass stand (73.11 t/ha/year) as compared to grass-legume mixtures. However, grass with Lablab purpureus and C.muconoides yielded higher green weight (186.60 and 174.15 t/ha/year, respectively) than the pure grass stand (70.25 t/ha/year).

Grass yields in different grass-legume associations were comparatively lower in pot culture studies as compared to field trials (field experiments 1 and 2). This might be due to the restricted growth of roots and shorter duration of the experiment.

Green weight increased significantly to the extent of 72 per cent in pot culture studies and 97 to 101 per cent in field trials by mere inclusion of legumes in the grass mixture as compared to pure grass stand. Whitney and Green (1969a) have reported an increased grass yield of 34 per cent when the Desmodium canum and D.intortum were grown with Pangola grass as compared to pure grass stand. Kretschmer (1970) have also reported an increased grass yield of 77 per cent in Pangola grass grown in association with twelve tropical legumes as compared to pure grass stand.

The inclusion of inoculated legumes increased green weight by 52 per cent in pot culture studies and 22 to 31 per cent in field trials as compared to grass with un-inoculated legumes. This is in conformity with the results obtained by Whitney and Green (1969a) wherein they have also observed an increase in yield of the associated grass with inoculated legumes referred above.

Again among the inoculated trials the response to inoculation was marked in field trials than in pot culture studies. However, in the uninoculated trials the yields in the uninoculated conditions were superior in the field conditions to the corresponding yields under pot culture conditions. This might be due to better nodulation in field grown legumes by native rhizobia.

Although grass grown with M.invisa gave the highest response in pot culture, it was not included in field experiments hence response to inoculation or nodulation by native rhizobia could not be assessed for this legume under field condition. L.purpureus and grass mixture responded better to inoculation and was well nodulated by native rhizobia in field experiment as compared to pot culture studies.

Nitrogen fixation due to Rhizobium inoculation varied markedly in different grass-legume mixtures. Nitrogen fixation increased with Rhizobium inoculation. N fixation was higher in Rhizobium inoculated C.muconoides-grass mixtures in all the three experiments as compared to other mixtures.

In pot culture studies, C.muconoides fixed more nitrogen (489.72 kg/ha/year) followed by G.javanica (371.89 kg/ha/year) and the lowest was in grass with D.biflorus (161.48 kg/ha/year). The probable reason for the low fixation of N in case of D.biflorus may be due to the shorter period of growth and resowing.

In field trials, C.muconoides fixed relatively higher nitrogen (242.73 to 249.66 kg/ha/year) than L.purpureus (244.41 kg/ha/year) and C.pubescens (229.77 kg/ha/year) while G.javanica fixed the lowest among the legumes tried (211.84 to 230.26 kg/ha/year). Similar variations have been observed in capacity of legumes to fix nitrogen (80-264 kg/ha/year) by Whitney and Green (1969a) in their studies on inoculated and uninoculated D.canum and D.intortum with Pangola grass.

Nitrogen yield and crude protein yield varied markedly among different grass-legume mixtures. N-yield increased with the inoculation of the associated legumes with respective strains of Rhizobium. Among the different mixtures, grass with C.muconoides yielded more N in all the three trials.

Nitrogen fixation due to native rhizobia varied markedly among the mixtures in all the three studies. In pot culture studies, nitrogen fixation due to native rhizobia was maximum in grass with *Mimosa invisa* (238.59 kg/ha/year) and was comparatively lower in *G.javanica* (113.97 kg/ha/year). The N-fixation in other grass-legume mixtures varied in between. The N-fixation due to native rhizobia was less in pot culture studies as compared to field trials. This was perhaps due to low native rhizobial population and restricted volume of the soil used in the experiment. The N-fixation due to native rhizobia in uncultivated land was maximum in *C.muconoides* (269.03 kg/ha/year) as compared to grass grown with *G.javanica* or *P.phaseoloides* (192.76 to 209.63 kg/ha/year). Similarly, in cultivated field trial (field experiment-2), nitrogen fixation was again more in grass grown with *C.muconoides* (340.06 kg/ha/year), closely followed by *L.purpureus*/grass mixture (339.14 kg/ha/year) and was the lowest in *C. pubescens*/grass mixture (154.35 kg/ha/year). Between two field trials, N-fixation was maximum in cultivated field. This might be due to the presence of more native rhizobial population as a consequence of earlier leguminous crop particularly inoculated soybean. The probable reason for the low fixation of N due to native rhizobia in case of *C.pubescens* may be due to its specific bacterial requirement. In uncultivated land, no legumes were grown previous to the experiment and hence N-fixed was low probably due to the presence of inadequate and non-specific native rhizobia.

Nevertheless, the legumes exhibited wider range of fixing nitrogen through nodulation by native rhizobia.

Studies carried out in India have been on grasses with uninoculated legumes. For instance, Singh et al. (1968) have reported that grass grown alone gave an average yields of 63.9 to 97.7 kg/ha/year in unfertilized plots whereas, grass (Pennisetum polystachyon) grown with uninoculated Stylosanthes gracilis and Centrosema pubescens yielded 64.5 and 92.3 kg/ha/year, respectively. In the second year the grass/Centro mixture yielded more than the pure grass stand. Chatterjee et al. (1969) have also reported that, when Heteropogon contortus/Stylosanthes humilis grown in mixture yielded 19.5 t/ha/year as compared to the pure grass stand (15.6 t/ha/year). No published work in India is available on hybrid grasses grown as mixtures with legumes. The yield increases noted by these workers did not go beyond 20 t/ha/year. The results obtained in these studies indicated a large potential for increasing grass yields through growing them as mixtures with a variety of tropical forage legumes and through inoculation of associated legume with efficient strains of rhizobia.

Further, the results indicated the possibility of developing an integrated system of use of symbiotically fixed nitrogen and fertilizer nitrogen. Further studies on this line might lead to large reduction in the quantity of fertilizer nitrogen, yet keeping the same high yields obtainable with fertilizer nitrogen application alone.

Tropical soils are known to harbour a large variety of rhizobia with low symbiotic efficiency and this requires further studies on the symbiotic response of sown forage legumes to inoculation with highly efficient strains of rhizobia and the studies on saprophytic competence of inoculant strains of rhizobia under a wide variety of ecological situations obtainable in the country.

SUMMARY

VI. SUMMARY

Ability of legume *Rhizobium* symbiosis to add N to the soil/plant system in Indian Agriculture has been made use of traditionally by growing legumes in rotation with cereal crops and as green manures. In these practices assumption has all along been that nodulation and nitrogen fixation by native rhizobia were adequate to provide the needed nitrogen.

In the present studies, nitrogen contribution of seven forage legumes to the associated grass both under inoculated and uninoculated condition was assessed. The nitrogen transference from legumes to associated grass was determined using the growth of the grass in pot culture and field trials.

Of the seven legumes only *C.pubescens* appeared to have specific rhizobial requirement and others could be cross inoculated by each other' rhizobial isolates.

In pot culture studies, green weights of grass in association with the legumes were significantly higher than that of pure grass stand and those of the *Rhizobium* inoculated legumes were superior to the uninoculated legumes. Inclusion of legumes in various mixtures increased the green weight of the grass by 72 per cent as compared to pure grass stand (55.28 t/ha/year). Green weight of grass

increased by 52 per cent in the inoculated grass/legume mixtures over that in the uninoculated grass-legume mixtures. There were marked variations in the yield of grass with different legumes. Association of legumes like C.muconoides, C.pubescens, D.biflorus and M.invisa gave better yield of grass as compared to the association of grass with G.javanica, P.phaseoloides or L.purpureus. The grass yield increased linearly upto the fourth cutting in majority of the legumes and then declined.

Under uncultivated field condition, the grass was studied in association with three legumes namely, C. muconoides, G.javanica and P.phaseoloides under inoculated and uninoculated conditions over a period of one year. The green weight of the grass was consistently higher in grass legume mixtures over that of pure grass stand. Significantly better yields of grass were recorded with Rhizobium inoculated legumes as compared to those with uninoculated legumes. Inclusion of legumes in various mixtures increased the green weight of the grass by 97 per cent as compared to pure grass stand (73.11 t/ha/year). Green weight of the grass increased by 22 per cent in the inoculated grass-legume mixtures as compared to grass with uninoculated legumes (144.34 t/ha/year). Among the legumes, G.javanica and C.muconoides were found to be superior to P.phaseoloides in nitrogen transfer. The green weight of the grass increased progressively upto the fourth cutting in G.javanica and C.muconoides grass mixtures while in P.phaseoloides/grass mixture the yield increased upto third cutting and there-after it declined.

Under cultivated field trial, the grass was studied in association with four legumes namely C.muconoides, C. pubescens, G.javanica and L.purpureus with and without inoculation over a period of two years. The grass production was significantly higher in grass-legume mixtures than in pure grass stand. Better yields of grass were recorded in inoculated legume-grass mixtures as compared to uninoculated legume - grass mixtures. The inclusion of legumes in various mixtures increased the green weight of the grass by 101 per cent as compared to pure grass stand (70.25 t/ha/year). Green weight of grass was increased by 31 per cent by inoculating the legumes with Rhizobium as compared to grass with uninoculated legumes (141.38 t/ha/year). Among the legumes, C.muconoides and L.purpureus were superior to other grass-legume mixtures. The grass yields reached a peak in the second cutting, then declined upto fifth cutting and once again progressively increased upto tenth cutting.

The nitrogen content and crude protein of the grass in association with the legumes under pot culture studies were significantly higher than the pure grass stand. N-yield and crude protein of the grass grown in association with the inoculated legumes were significantly more than those of grass grown with uninoculated legumes.

Nitrogen fixation due to Rhizobium inoculation varied markedly in different grass-legume mixtures. N-fixation was higher in Rhizobium inoculated C.muconoides-grass mixture in all the three experiments as compared to other mixtures. In pot culture studies, C.muconoides fixed more nitrogen (489.72 kg/ha/year), followed by G.javanica (371.89 kg/ha/year) and the lowest was in grass with D.biflorus (161.48 kg/ha/year). While in field trials, C.muconoides fixed relatively higher N (242.73 to 249.66 kg/ha/year) than L.purpureus (244.41 kg/ha/year) and C.pubescens (229.77 kg/ha/year), and the lowest was with G.javanica (211.84 to 230.26 kg/ha/year).

The results indicated the possibility of obtaining adequate yields of grass without resorting to nitrogen fertilizer application. However, these yields were not comparable to the high yields obtainable with nitrogen fertilizer application.

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