Physiological factors of sainfoin (Onobrychis viciaefolia Scop.) as they relate to yield
by Charles Spurgeon Straley

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and soil Science
Montana State University

Abstract:
Specific leaf weights (SLW = mg/cm2) of eight sainfoin clones were determined from material grown in a breeding nursery, and in a replicated space-planted nursery. Specific leaf weights of progeny from these eight clones were determined on plants grown in a solid-seeded nursery and in a space-planted nursery. Repeatable differences in SLW among clones within environments were found. Differences in SLW among clones were not the same across environments. There was no correlation of SLW among clones with yield. Specific leaf weights were lower in the solid-seeded nursery and did not differ among the progeny within an environment. The heritability estimate of SLW was low (.08) in the solid-seeded nursery but was .54 for space-planted clones and progeny.

Photosynthetic rates (P) of the eight clones used in the SLW study were determined over a period of two years and from material grown in a breeding nursery, a growth chamber, in a replicated space planted nursery and the greenhouse. Differences in P among clones were not repeatable across environments. Average P for the eight clones were lowest in the space-planted nursery and highest in the greenhouse. Differences in P within an environment were not related to yield. There was some relationship between P averaged across environments and yield.

Ribulose diphosphate (RuDP) carboxylase activity plus chlorophyll and protein content of four clones with different. P were determined for plants grown in the field and greenhouse. RuDP carboxylase activity among clones within an environment was the same There was 40% more RuDP carboxylase activity in the greenhouse material which was related to the 40% higher P in the greenhouse. The clone with the lowest P had the highest chlorophyll and protein content. Chlorophyll and protein were 40% higher in plants grown in the greenhouse than in the field.

Yield components of twenty-four clones and their progeny were determined. Mother clone leaf and stem weights were correlated to progeny yield. Stem weight differences of mother clones were most closely related to yield of the progeny. The heritability estimate of leaf weight was .56 and for stem weight was .70.

PHYSIOLOGICAL FACTORS OF SAINFOIN (ONOBRYCHIS VICIAEFOLIA SCOP.) AS THEY RELATE TO YIELD

by

CHARLES SPURGEON STRALEY

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Crop and Soil Science

Approved:

K. C. Feltner
Head, Major Department

Jarvis H. Brown
Chairman, Examining Committee

Henry L Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

March, 1973

ACKNOWLEDGEMENTS

I should like to express my sincere appreciation to Dr. Scott Cooper for his advice and encouragement to me in this study. I acknowledge my indebtedness to Dr. Gary Strobel for advice throughout my graduate studies.

Many thanks are extended to Dr. Scott Cooper, Dr. J. Brown, and Professor Robert Eslick for reviewing the manuscript. I am grateful to Dr. D. Mathre in allowing me access to his laboratory and to Dr. S. Kent for advice on various laboratory procedures.

I am also grateful to my wife Mary Lou for her cooperation and support throughout my graduate career.

I also acknowledge the financial assistance provided by the Montana Experiment Station in the form of an assistantship.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Specific leaf weights (SLW = mg/cm^2) of eight sainfoin clones were determined from material grown in a breeding nursery, and in a replicated space-planted nursery. Specific leaf weights of progeny from these eight clones were determined on plants grown in a solid-seeded nursery and in a space-planted nursery. Repeatable differences in SLW among clones within environments were found. Differences in SLW among clones were not the same across environments. There was no correlation of SLW among clones with yield. Specific leaf weights were lower in the solid-seeded nursery and did not differ among the progeny within an environment. The heritability estimate of SLW was low (.08) in the solid-seeded nursery but was .54 for space-planted clones and progeny.

Photosynthetic rates (P) of the eight clones used in the SLW study were determined over a period of two years and from material grown in a breeding nursery, a growth chamber, in a replicated space-planted nursery and the greenhouse. Differences in P among clones were not repeatable across environments. Average P for the eight clones were lowest in the space-planted nursery and highest in the greenhouse. Differences in P within an environment were not related to yield. There was some relationship between P averaged across environments and yield.

Ribulose diphosphate (RuDP) carboxylase activity plus chlorophyll and protein content of four clones with different P were determined for plants grown in the field and greenhouse. RuDP carboxylase activity among clones within an environment was the same. There was 40% more RuDP carboxylase activity in the greenhouse material which was related to the 40% higher P in the greenhouse. The clone with the lowest P had the highest chlorophyll and protein content. Chlorophyll and protein were 40% higher in plants grown in the greenhouse than in the field.

Yield components of twenty-four clones and their progeny were determined. Mother clone leaf and stem weights were correlated to progeny yield. Stem weight differences of mother clones were most closely related to yield of the progeny. The heritability estimate of leaf weight was .56 and for stem weight was .70.

INTRODUCTION

Sainfoin is a new forage legume which does not cuase bloat and is not affected by the alfalfa weevil. Very little breeding work has been done, however, to increase forage yield in sainfoin. Therefore it is important that traits related to yield in sainfoin be determined.

Recent workers have found that several physiological as well as morphological traits are related to increased yield within a cultivar (21). Since photosynthesis is the principle means of dry matter production it appears feasible to look for genetic differences in photosynthetic rates (P) as a basis for increasing forage yields. There is evidence that sink capacity is also an important determinant of yield. It has been shown that relative contributions of yield components are independent of changes in leaf area and P (32). Leaves and stems may be unable to accept all of the potential output of photosynthate. Presumably the sink capacity in forages depends on the number of organs such as stems and leaves, acting as sinks, as well as on internal factors that control their growth.

The feasibility of using physiological or morphological traits to increase the yield in sainfoin were studied to determine:

1. The heritability of specific leaf weight (SLW = mg/cm^2) and its relationship to yield.
2. The relationship of photosynthesis to dry matter

production.

3. The factors that are related to differences in P such as ribulose diphosphate carboxylase activity and chlorophyll content.

4. The heritability of yield components and their relationship to yield.

REVIEW OF LITERATURE

Dry matter production is derived almost exlusively from CO_2 assimilation. Hence yield within a plant community involves the biochmeistry and translocation of the assimilated CO_2. It would appear feasible then to look for genetic differences in CO_2 assimilation rates. Finding genetic differences in photosynthesis by a single leaf could be one way of increasing yield potential. The magnitude of leaf photosynthetic rate (P) in soybeans ranges from 29.4 to 43.4 mg CO_2 $dm^{-2}hr^{-1}$ at light saturation (3,4,6,14). High yielding ability of dry beans has been correlated to high P (21). Lupton (24) found that increased yield in a higher yielding wheat variety was associated with greater net CO_2 assimilation.

Many studies, however, have not been able to associate increased yield potential with increased P on an area basis. In cotton El-Sharkawy (16) and in corn Hanson (19) found dry matter production was related to yield components, but not to increased P of leaves. In physiological studies on the evolution of wheat, it has been shown that modern cultivated varieties have been selected for larger leaf area and larger grain size. The P per unit area, however, has decreased with increasing leaf size (17). Apparently leaf area expansion is more important than leaf P as a possible determinant of higher yields.

Thus at present increased yield potential is not clearly

associated with an increase in P. Leaf P is just one of the parameters that determine total dry matter production of a plant.

Growth analysis is a technique that has been used in an attempt to explain dry matter production. Watson (32) found that although net assimilation rate (NAR, g $dm^{-2}day^{-1}$) varies between species, very small differences occurred within a species. Large differences in dry matter accumulation between species are associated with differences in the rate of leaf area development (16,32). Thus high yielding cultivars have a high leaf area index (LAI). Chandler (8) found that the least productive varieties of rice had a lower optimum LAI than the most productive varieties.

By using LAI, the entire canopy can be taken into account. In contrast, when a single leaf is used to determine the photosynthetic capacity of a canopy, factors such as light penetration and mutual shading are ignored. Shaded leaves fix CO_2 less rapidly than brightly illuminated leaves, but leaves within a canopy are saturated at lower light intensities than leaves at the top of a canopy (18). Therefore increased yields are related to many factors besides P, such as leaf area development.

The size of a photosynthetic sink is also important in determining dry matter production. The supply of photosynthate and its requirement by growing tissues are not always in balance. The balance of these two variables depends on external conditions and

on internal growth factors and one of these two variables may be in excess. If one compares the growth of C_3 and C_4 species, the C_4 plant having a higher P, any inherent superiority of the C_4 plant in net assimilation rate is hard to demonstrate. Maximum rates of dry matter accumulation in sugarcane occurs at the time of year when photosynthetic activity is similar to that of C_3 plants (7). The same relationship exists between a C_3 species (Atriplex naptata) and a C_4 species (A. spongiosa). In early stages of growth the C_4 species had the highest growth rate but near the end of the growth period the C_3 species had the fastest growth rate because this species put more of its photosynthate into new leaf production (29). These studies indicate that overall crop growth rate is primarily controlled by leaf area.

Less work has been done in forages than in cereals to determine why varieties differ in dry matter production. Since the entire above ground portion of growth is harvested in forages, fewer variables may be determining yield in forages than encountered in seed crops. Yield component compensation has been studied in seed crops but not in forages (1). Very little physiological work has been done to determine why forage cultivars differ in yield.

Since most forage crops are open-pollinated, genetic traits vary widely within a cultivar. Therefore, in order to study

physiological differences within such a variable system, the factors causing those differences should be isolated and studied separately. This approach makes it possible to deduce the nature and function of the individual components affecting yield. Thus if we are investigating photosynthetic efficiency, we should identify the component or components that produce or are associated with photosynthetically efficient genotypes. Dornhoff and Shibles (14) found that leaf diffusive resistance was largely responsible for varietal differences in P in soybeans. Other workers have found that leaf thickness was correlated with P (26). The relative activity of the carboxylating enzyme ribulose-1,5 diphosphate carboxylase has also been related to differences in P (4,5,25). Workers have found that soybeans grown in different light environments have different P that are positively correlated to differences in amounts of the carboxylating enzyme (5). Bjorkman (4) has also found that the level of carboxylating activity was related to P since shade plants have lower P and carboxylase activity than do sun plants.

Yield components have been used to explain varietal differences in yield of seed crops (12). However, very little is known about yield components in relation to yield of forage crops. Cope et al. (12) found that large-leaved plants of crown vetch had higher yielding progeny than small-leaved plants. When crosses were made between the two plant types heterosis for yield occurred. Cope

(11) also found that heterosis for yield was expressed to a greater degree in solid-seeded nurseries than in space-planted nurseries. There would not be an optimum LAI in space-planted nurseries, whereas in closely spaced nurseries the LAI would be an important factor in determining yield. Since there is not an optimum LAI in space-planted nurseries a vital determinant of dry matter production would never be expressed which could explain why yield components are usually independent in space-planted nurseries (1). It must be recognized that LAI in forages in an important determinant of yield and the plant breeder must learn to exploit the idea of leaf area development.

MATERIALS AND METHODS

Plant Material

In 1970 twenty-four clones of 'Eski' parentage with a wide range in general combining ability were selected for study of specific leaf weight (SLW, mg/cm^2) and its heritability. The clones were in an unreplicated breeding nursery and spacing between plants was 3 dm. Eight of these clones were selected on the basis of differences in SLW and yield (31), and were maintained for study of relationships between SLW, photosynthesis, and the amount of ribulose diphosphate (RuDP) carboxylase.

Cuttings from these clones were made in September, 1970, and maintained in the greenhouse for the duration of these studies. Six plants of each clone and 20 open pollinated progeny from each of seven clones were also transplanted to the field in May, 1970, in space-planted rows. The spacing between rows and between plants was 9 dm.

Photosynthetic Measurements

Photosynthetic rates (P) were calculated from measurements of the decrease in CO_2 concentration in a stream of air passing over leaves. Measurements were made with a Backman 215 A infra-red gas analyzer. Air containing 300 ± 10 ppm of CO_2 was prepared by first passing compressed air through calcium carbonate to remove water, then through Ca-apatite to remove naturally occuring CO_2, and finally mixing the air with the appropriate volume of pure CO_2. The air was passed

through water and brought to a temperature of 20C.

Photosynthetic rate measurements were made by placing leaves in a round plexiglas tube 35 cm long and 7 cm in diameter. Leaves were held upright in the center of the chamber by placing the leaves in a test tube that was mounted in the center of a removable cap. Air was passed from the lower to the upper end of the chamber across the leaf surfaces at a rate of 2 ℓ/min. The chamber was placed horizontally under a light in a box mirrored on four sides and the bottom. The cylinder could be rotated to insure maximum light interception by the leaf. A light intensity of 0.32 ly min^{-1} was provided by a 400 watt lucalox lamp and two 500 watt incandescent bulbs. Lower light intensities of 0.23, 0.16, 0.08 and 0.04 ly min^{-1} were produced by placing metal screen between the light sources and the leaf chamber. Measurements of P were made at each light intensity.

Five measurements of P were made during a period of 2 years on sainfoin plants grown in five different environments.

1. January, 1971 - 8 clones from growth chamber
2. June, 1971 - 8 clones from replicated spaced plants
3. July, 1971 - 8 clones from breeding nursery
4. August, 1971 - 4 clones from replicated spaced plants
5. February, 1972 - 4 clones from greenhouse

Photosynthetic rate measurements were made on the youngest most fully expanded leaves at the pre-bud stage. Leaves were

detached the evening prior to P measurements and placed on a mist bench in the dark. Leaves were placed in the light 30 minutes prior to P measurements. Photosynthetic rates were made individually on 3 to 6 plants from each clone depending on the availability of plant material.

RuDP Carboxylase Assay

Preparation of Extracts

One gram of leaf material was ground with a mortar and pestle in 10 ml solution of 100 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1 mM DTT and 0.25 mM $MgCl_2$. The tissue was then placed in a smooth surfaced tissue homogenizer and pulverized thoroughly. The tissue was then strained through 4 layers of cheesecloth into a new Pyrex fine ground glass homogenizer and homogenized for a minimum of 5 minutes. The above procedures were carried out at 4C. This extract was the source of enzyme and was used for the protein and chlorophyll determination.

Enzyme Activity

The enzyme activity was relatively measured 15 min. after the start of homogenization. The reaction was initiated by the addition of 0.2 ml of enzyme extract to 0.2 ml of a freshly prepared mixture (34) containing (in u mol); Tris-Cl pH 8.0, 100; $MgCl_2$, 5; DTT, 3; RuDP, 0.25; and $NaH^{14}CO_3$, 25, with a specific activity of 0.2 uc/ u mole. The sodium salt of ribulose-1, 5 diphosphate was obtained

from the barium salt by a method described elsewhere (33).

After a 2 minute incubation period at 30C the reaction was terminated by the addition of 0.1 ml of 12 N acetic acid. A 0.1 ml aliquot was pipetted into a scintillation vial and dried at 90C for 30 minutes. One-tenth milliliter of water, 0.5 ml hydroxide of hyamine 10x and 14.5 ml of toluene containing 4 g of PPO plus 50 mg POPOP per liter was added to the contents of each vial. The amount of radiolabelled $^{14}CO_2$ incorporated into nonvolatile product was measured with a Packard Tri-Carb model 3320 Liquid Scintillation Spectrometer. Four assays were made for each plant. Variation between replicates was less than 10%.

Chlorophyll and Protein

Five milliliters of the leaf extract was divided into acetone soluble and insoluble fractions. Total chlorophyll in the acetone soluble fraction was then determined by the method of Arnon (2). The acetone precipitate was resuspended in 5 ml of water and precipitated two times with acetone to remove polyphenols. The acetone precipitate was then solubilized by boiling 5 minutes in 0.1 N NaOH. Total protein was estimated by the method of Lowry (23).

Yield Components

In 1970, yield component variation was determined on 24 three-year old space-planted clones in a breeding nursery and their two-year old open-pollinated progenies. The clones had been selected

to represent a wide range in general combining ability for forage yield. Clones were spaced on 3 dm centers and the open pollinated progenies were seeded in single 3 m rows spaced 27 cm apart in three replications.

Ten stems per clone and 20 stems per progeny row were collected. The stems were dried and leaf and stem material were separated and weighed. All possible correlations between clones and progeny measurements were made. Heritability estimates were made from parent-progeny regression data according to the procedures suggested by Kneebone (22).

RESULTS

Specific Leaf Weights

Specific leaf weight (SLW = mg/cm^2) of eight sainfoin clones grown in a space-planted breeding nursery in 1970 and 1971 and in replicated space-planted rows in 1972 is shown in Table 1. Specific leaf weight differed significantly among clones but not always in the same order among years and plantings. The correlation coefficient for SLW of clones measured in 1970 and 1971 in the same nursery was $r = 0.84$. The correlation of average SLW values of clones for the two years in the breeding nursery, on 3 dm centers, with SLW of clones in replicated rows, on 9 dm centers, in 1972 was $r = 0.05$. Specific leaf weight of progeny within space-planted or within solid-seeded nurseries did not differ significantly (Table 2) but SLW was greater in spaced than in solid-seeded nurseries. Heritability of SLW for space-planted clones and solid-seeded progeny was only 0.08, but for space-planted clones and space-planted progeny it was 0.54.

Several workers (5,26) have suggested that SLW should be related to dry matter production since SLW is correlated with photosynthesis. In this study there was no relationship between SLW of clones and dry matter production of progeny in solid seedings. This was not unexpected since there was no difference in SLW among progeny. These data indicate that SLW cannot be used as a means of selecting high yielding sainfoin genotypes for solid-seeded environments.

Table 1. Specific leaf weights of eight sainfoin clones grown under different environments.

Clone No	Progeny yield T(metric)/ha	SLW (mg/cm^2) Breeding nursery 3 dm centers 1970	1971	Replicated spaced rows 9 dm centers 1971	Avg.
88	2.2	12.5a*	8.5a	8.0a	9.6
56	2.7	5.7b	5.2d	6.4bc	5.8
27	2.9	6.8b	7.0bc	7.1ba	7.0
78	3.6	5.9b	6.2c	8.2a	6.8
23	4.2	6.9b	6.5c	8.7a	7.4
81	4.2	7.2b	7.1a	5.5c	6.6
97	4.7	11.7a	7.0a	6.9b	8.5
66	5.1	12.0a	8.5a	6.9b	9.1
AVG	8.6	7.0	6.4	7.6	

* Values within a given environmental regime not followed by letters in common are significantly different at the 5% level of probability (15).

Table 2. Average specific leaf weights for progeny from each of seven sainfoin clones grown under different environments,* progeny ranked from high to low in dry matter yield.

Progeny from Clone No	SLW (mg/cm^2) Solid seeded 1970	1971	Space planted 1971
56	5.8	4.0	7.8
27	5.3	4.1	7.2
78	7.0	4.1	7.3
23	5.8	4.1	7.8
81	7.7	4.3	6.5
97	4.9	4 4	6.7
66	7.0	4.1	7.1
AVG	6.2	4.2	7.2

* Values within a given environmental regime are not significantly different.

Interpretation of lower SLW in solid plantings as compared to spaced plantings indicate that the light environment within a canopy affects SLW of new leaves formed. Our studies of the effect of shading the lower portion of alfalfa plants on SLW of new leaves formed have found that this is the case (31). Specific leaf weight of sainfoin leaves formed in full sunlight at the top of plants with mature leaves shaded (3.6 mg/cm^2) was lower than SLW of leaves formed at the top of plants grown without shading (4.7 mg/cm^2). Likewise leaves of alfalfa formed in the sun as a result of basal shading appeared to take on characteristics of shade leaves. Thus, it appears that the light environment neutralizes the genetic mechanism which produces differences in SLW in space planted clones of sainfoin.

Photosynthesis

Photosynthetic rates differed significantly among eight sainfoin clones grown in different environments but not always in the same order (Table 3). Photosynthetic rates of clones were not correlated among environments and were lowest when space planted in the field (avg 8.9 mg CO_2 $dm^{-2}hr^{-1}$) and highest when grown in the greenhouse (24.5 mg CO_2 $dm^{-2}hr^{-1}$). The lack of correlation in P between environments is in contrast to work in alfalfa where the relative rank in P was maintained across environments (26). Only two sainfoin clones were consistent in relative rank across

environments. Clone 81 had low P and 97 had high P in all environments.

Table 3. Photosynthetic rates of eight sainfoin clones grown in different environments. (mg CO_2 $dm^{-2}hr^{-1}$).

Clone No	Space planted 9 dm centers June 1971	Breeding nursery 3 dm centers July 1971	Growth chamber 1971	Space planted Aug. 1971	Greenhouse Feb. 1972
97	11.4a*	14.9a	16.7b	16.1a	27.8a
23	10.7a	9.5c	6.4d		
88	9.4ab	17.2a	11.2bc		
78	9.0b	12.7b	14.3bc		
27	8.7b	12.9b	22.4a		
66	8.2b	14.5a	14.1bc	12.3b	27.0a
56	7.2c	11.3b	14.4bc	16.1a	25.0a
81	6.8c	11.0b	9.8cd	12.0b	16.1a
AVG	8.9	11.8	13.6	14.1	24.5

* Values within a given environmental regime not followed by letters in common are significantly different at the 5% level of probability (15).

Photosynthetic rates in sainfoin appear to be influenced by the environment. These data indicate that P increase during the summer months. No explanation can be offered as to why P should increase during the summer, however several factors which change as the summer progresses are light intensity, day length, and temperature. Light intensity and temperature increase while day length decreases through the summer. The highest P (24.5 mg CO_2 $dm^{-2}hr^{-1}$) occurred in the greenhouse during February which is a period of low light intensity, short days, and high temperature.

Isolation of RuDP Carboxylase

The chloroplast must be disrupted in order to extract and study the chloroplast enzymes. Standard procedures for breaking chloroplasts such as grinding with a mortar and pestle or sonication were effective in breaking alfalfa chloroplasts but were not effective in breaking sainfoin chloroplasts (Table 4).

Table 4. RuDP carboxylase activity using different techniques to break sainfoin (S) and alfalfa (A) chloroplasts. (mg CO_2 $g^{-1}hr^{-1}$).

Time (min)	Mortar & pestle		Sonified tissue		New glass Homogenizers	
	S	A	S	A	S	A
1	0.0	0.0	0.0	1.3	0.0	2.4
2	0.0	0.0	0.0	7.7	0.9	12.3
3	0.0	1.1	0.0	16.7	2.6	19.0
4	0.0	2.2	0.9	18.0	6.4	20.0
5	0.0	4.8	2.0	18.0	6.6	20.0

An alternate technique had to be devised for preparing enzymes from sainfoin chloroplasts. Leaf tissue was ground in a cold mortar and pestle and the tissue was placed in a smooth tissue homogenizer and pulverized throughly. This homogneate was strained through cheesecloth into a new, fine-ground Pyrex tissue homogenizer and homogenized for a minimum of 5 minutes (Table 4). The tissue homogenizers had to be discarded after grinding 12 samples since they became ineffective in breaking the sainfoin chloroplasts.

The rate of ^{14}C incorporation into non-volatile products was linear up to two minutes and then declined with increasing incubation

time. Enzyme activity decreased within 30 minutes after homogenization. The rate of ^{14}C incorporation in the absence of added ribulose diphosphate was less then 1% of the rate in the presence of the substrate.

RuDP Carboxylase

Ribulose diphosphate carboxylase activity in four sainfoin clones grown in two environments is shown in Table 5. The differences in P among clones within an environment were not due to differences in the amount of the carboxylating enzyme. Both carboxylase activity and P were 40% greater in plants grown in the greenhouse than in plants grown in the field.

Table 5. Environmental effects on photosynthesis and RuDP carboxylase activity in four sainfoin clones.

	mg CO_2 $dm^{-2}hr^{-1}$	
	Photosynthetic rate	RuDP carboxylase
Sainfoin	Greenhouse	
97	28.0	22.0
66	27.0	20.6
56	22.0	21.5
81	18.0	21.6
AVG	23.8	20.3
Alfalfa	28.0	21.7
Sainfoin	Field	
97	16.2	10.3
66	12.3	10.6
56	16.2	12.4
81	12.7	12.4
AVG	14.3	11.4

Photosynthesis and RuDP carboxylase activity were similar between greenhouse grown sainfoin and alfalfa. It appears that the low P in field grown sainfoin was related to the low level of carboxylase activity.

Several workers have found that when plants are grown under low light intensities, P and RuDP carboxylase activity decrease. This was not true in sainfoin since the field material grown under high light intensities had the lowest P and RuDP carboxylase activity.

Chlorophyll

Chlorophyll content of four sainfoin clones is shown in Table 6. Photosynthetic rate differences found for the four clones

Table 6. Chlorophyll content of four clones of sainfoin grown in the field and greenhouse and of alfalfa.

	mg Chlorophyll per g fresh wt	mg Chlorophyll per dm^2
Sainfoin	Greenhouse	
81	2.5a*	5.0a
56	2.0b	3.9b
66	1.9b	3.2c
97	2.6a	4.6a
AVG	2.2	4.4
Alfalfa	3.1	3.4
Sainfoin	Field	
81	1.5a	2.9a
56	1.4a	2.5b
66	1.4a	2.5b
97	1.3a	2.4b
AVG	1.4	2.6

* Values within a column not followed by letters in common are significantly different at the 5% level of probability (15).

within an environment were not related to differences in chlorophyll content. Clone 81 had the lowest P but the most chlorophyll. Both P and chlorophyll were 40% greater in the greenhouse grown sainfoin than in the same clones grown in the field. But plants in both environments were near saturation at the same light intensity (Figure 1). This indicates that the 40% higher P in the greenhouse was not due completely to the increased amount of chlorophyll.

Protein

Protein content of four sainfoin clones is shown in Table 7.

Table 7. Protein content of leaves of four clones of sainfoin grown in the field and greenhouse and of alfalfa.

	mg Protein per	
	g fresh wt	dm^2
Sainfoin	Greenhouse	
81	53.7a*	109.6a
97	51.8a	94.2b
56	50.5a	97.4b
66	49.8a	85.6c
AVG	51.4	96.7
Alfalfa	74.8	83.1
Sainfoin	Field	
81	33.4a	65.6a
97	33.7a	57.1b
56	31.8a	57.8b
66	34.1a	57.9b
AVG	33.2	59.7

* Values within a column not followed by letters in common are significantly different at the 5% level of probability (15).

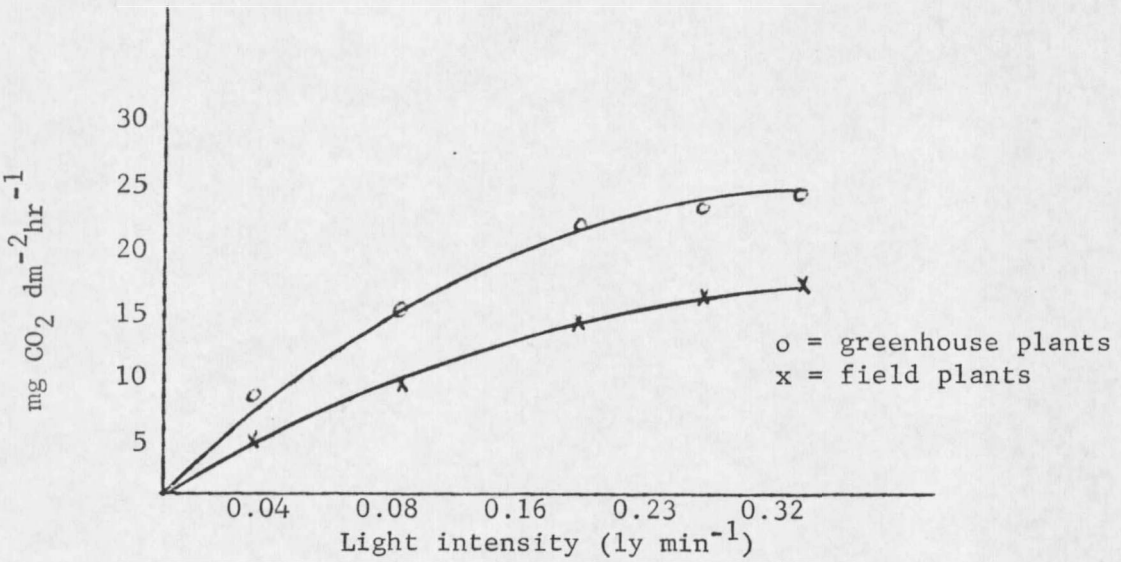

Figure 1. Average light saturation of photosynthesis in leaves of four sainfoin clones grown in the field and greenhouse.

There was a significant difference among clones in protein content on an area basis. Clone 81, which had the highest chlorophyll content, also had the highest protein content. This finding is in contrast to the work of Bjorkman (4) who reported that plants with high chlorophyll content had less protein. The average protein content was 40% higher in the greenhouse plants than in field grown sainfoin.

Yield Components

Differences in mother clone leaf and stem weights were significantly correlated to differences in progeny yield (Appendix Table 1). The leaf to stem ratio of the clones was negatively correlated to progeny yield (Table 8). This indicates that progeny from clones with large heavy stems were the highest yielding. It also indicates that stem weight was the most important factor in determining yield. The heritability estimates of the three factors were high; for leaf weight (.56), stem weight (.70), and the ratio of leaf weight to stem weight (.88). One should be able to increase progeny yield

Table 8. Correlation coefficients for yield component values and yield between twenty-four mother clones and their progeny.

	Correlation coefficients mother clone yield components		
Progeny	Stem wt	Leaf wt	Leaf/stem wt
Yield	.60**	.40*	-.25
Stem wt	.36*	---	---
Leaf wt	---	.34	---
Leaf/stem wt	---	---	.53*

* Significant at .05 level of probability
** Significant at .01 level of probability

by selecting only clones with large stems. But such a program could have an adverse effect on forage quality since the resulting cultivars would have a high percentage of stems.

DISCUSSION

Specific leaf weight has been proposed as a method of selecting plants for high P (26). In these experiments heritability of SLW was very low and would not appear to be useful as a selection tool in a sainfoin breeding program. Specific leaf weight of space-planted plants, where all leaves were exposed to high light intensities was greater than solid-seeded or greenhouse plants where leaves were exposed to lower light intensities. Therefore light intensity is one of the factors determining SLW. Other factors such as competition for water and nutrients could also affect SLW since SLW estimates were repeatable within a nursery but not between nurseries. In contrast, differences in SLW among alfalfa clones were consistent across environments (26).

Relative differences in P among clones were not repeatable across environments. Photosynthetic rates for clones for a given environment were not correlated with progeny yield. When P were averaged across environments however there was some relationship between P in clones and progeny yield. Photosynthetic rate of a clone may vary between environments for several reasons. Photosynthetic rate is related to SLW so when SLW changes across environments P will also change. It was also observed that plants varied in the length of time it took to reach maximum rates of photosynthesis. Therefore when one determines P after a given period of exposure to a light source plants may not have reached maximum rates. Most studies