Yield and yield components of sainfoin (Onobrychis viciaefolia Scop.) seed and an evaluation of its use as a protein supplement
by Raymond Lee Ditterline

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:
Yield and yield components of sainfoin seed, and protein quality of sainfoin seed were studied in separate experiments. The first experiment was a study of the components of seed yield in sainfoin and their heritabilities. Components studied included stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, weight per 100 seeds, and seed yield. Crude protein content of the seed, plant height and lodging were also studied. Significant differences between clones existed for most of the traits studied. Seed per raceme, which is a function of percent seed set and the number of florets per raceme was closely associated to seed yield and should be a useful tool when screening for high seed yielding plants. Stems per plant were positively associated with percent protein, indicating that plants with large vegetative skeletons were better able to supply developing seeds with the necessary nutrients for protein production. A positive non-significant relationship was found between seed yield and percent protein, suggesting that selection for high seed yield should not adversely affect the protein content of the seed. Heritability estimates obtained by parent-progeny correlations were lower than those obtained by regression of offspring on the female parent; however, both estimators indicated a high degree of association between parents and progeny for stems per plant, racemes per stem, seed weight and percent protein. Lower heritability estimates were obtained for racemes per plant, seed per raceme, percent seed set and seed yield.

The second experiment included one swine and two rat feeding trials to evaluate sainfoin seed as a source of protein for monogastric animals. Sainfoin seed had approximately 36% crude protein and its essential amino acid composition was similar to soybean meal. Weanling pigs performed better on soybean meal than on sainfoin seed; however, weanling rats on 20% protein diets performed equally well on sainfoin seed and soybean meal. When the protein content of the diets was restricted to 11% protein, weanling rats fed sainfoin seed gained as well as rat's fed soybean meal; however, they did not utilize the protein quite as efficiently as rats fed soybean meal or casein. The assay for trypsin inhibitor revealed that raw sainfoin was high in inhibitory activity, and that this activity was drastically reduced or nullified when the seed was autoclaved. Performance and pancreas data indicated the inhibitor did not have a detrimental effect on the feed value of sainfoin seed. Pancreases from rats fed raw sainfoin were not enlarged.

YIELD AND YIELD COMPONENTS OF SAINFOIN (*Onobrychis viciaefolia* Scop.)
SEED AND AN EVALUATION OF ITS USE AS A
PROTEIN SUPPLEMENT

by

RAYMOND LEE DITTERLINE

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

Clee S Cooper
Chairman, Examining Committee

K Goering
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1973

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the following:

Doctors C. S. Cooper, L. E. Wiesner and A. E. Carleton, who have served as my major Professors, for their encouragement, advice and guidance during this course of study and the preparation of this manuscript.

Dr. C. W. Newman for his assistance in conducting the animal feeding trials. Without Doctor Newman's advice, and the use of the Animal and Range Science facilities, these feeding trials could not have been conducted.

Professor R. F. Eslick for his timely advice, words of encouragement, and assistance in the preparation of this manuscript.

Doctors J. R. Sims and E. L. Moody for serving on my graduate committee.

Doctor K. D. Hapner for performing the amino acid analyses and trypsin inhibitor assays reported in this manuscript.

The Montana Agricultural Experiment Station and the Plant and Soil Science Department for providing me with an assistantship. Without this assistance, attending graduate school would have been impossible.

Mrs. Sharon L. Ditterline, my wife, for her endless patience, cooperation, encouragement and sacrifice throughout my graduate work.

And to my three children, Colleen, Shannon and Mandy, who have done without so many things in order for me to attend graduate school.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Yield and yield components of sainfoin seed, and protein quality of sainfoin seed were studied in separate experiments. The first experiment was a study of the components of seed yield in sainfoin and their heritabilities. Components studied included stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, weight per 100 seeds, and seed yield. Crude protein content of the seed, plant height and lodging were also studied. Significant differences between clones existed for most of the traits studied. Seed per raceme, which is a function of percent seed set and the number of florets per raceme was closely associated to seed yield and should be a useful tool when screening for high seed yielding plants. Stems per plant were positively associated with percent protein, indicating that plants with large vegetative skeletons were better able to supply developing seeds with the necessary nutrients for protein production. A positive non-significant relationship was found between seed yield and percent protein, suggesting that selection for high seed yield should not adversely affect the protein content of the seed. Heritability estimates obtained by parent-progeny correlations were lower than those obtained by regression of offspring on the female parent; however, both estimators indicated a high degree of association between parents and progeny for stems per plant, racemes per stem, seed weight and percent protein. Lower heritability estimates were obtained for racemes per plant, seed per raceme, percent seed set and seed yield.

The second experiment included one swine and two rat feeding trials to evaluate sainfoin seed as a source of protein for monogastric animals. Sainfoin seed had approximately 36% crude protein and its essential amino acid composition was similar to soybean meal. Weanling pigs performed better on soybean meal than on sainfoin seed; however, weanling rats on 20% protein diets performed equally well on sainfoin seed and soybean meal. When the protein content of the diets was restricted to 11% protein, weanling rats fed sainfoin seed gained as well as rats fed soybean meal; however, they did not utilize the protein quite as efficiently as rats fed soybean meal or casein. The assay for trypsin inhibitor revealed that raw sainfoin was high in inhibitory activity, and that this activity was drastically reduced or nullified when the seed was autoclaved. Performance and pancreas data indicated the inhibitor did not have a detrimental effect on the feed value of sainfoin seed. Pancreases from rats fed raw sainfoin were not enlarged.

INTRODUCTION

Most legume varieties have been released for their high forage yielding ability with little consideration being given to seed yield. As a result, many of the current legume varieties have poor seed yields, and breeders are beginning to emphasize seed yield in their breeding programs. 'Uinta', for example, is a variety of alfalfa (Medicago sativa L.) that was bred for both high seed and forage yields. This variety is similar to the variety 'Ranger' in forage yield, but produces sixty-seven percent more seed than does Ranger (50).

Sainfoin (Onobrychis viciaefolia Scop.) is a newly reintroduced forage legume that is gaining popularity in the Northern Rocky Mountain States and in Canada. Varietal release in this crop has been from selection within and among adapted plant introductions. Although the seed yields of sainfoin have been relatively good, it was felt selection pressure should be placed on its seed yielding ability in the early stages of the breeding program in order to circumvent the problem that has arisen in other legume species.

Seed yield is generally recognized to be a very complex character. It is the end result of the activities of the plant acting within its genetic potential in a given environment. When one or more environmental factors vary, differences in yield may result. Seed yield differences have also been noted among sainfoin varieties and experimental lines grown under the same environmental conditions. These differences indicate that seed yield is also controlled by genetic factors. Since seed yield is a complex character resulting from the interaction of a number of separate characters. yield components should be studied separately, as well as in combination with each other. Knowledge of the components of seed yield and their relative importance can aid plant breeders in selecting superior genotypes based on their phenotypic appearance and behavior, and to determine which component or components should be subjected to the greatest selection pressure for most rapid progress.

The objectives of Experiment I were to: 1) study the components of seed yield in sainfoin; 2) determine their relationships with each other; 3) determine their heritabilities; and 4) determine, if possible, which components were the best indices of seed yield under the environmental conditions found at Bozeman, Montana.

A second series of experiments were initiated to investigate the possible use of sainfoin seed as a protein supplement. Sainfoin seed has 36% protein, and seed yields of this plant are high enough that it might be economically grown as a protein supplement.

Protein supplement is the most expensive ingredient in livestock rations. Soybean meal is commonly used as a protein supplement because of its high protein content and essential amino acid balance.

It is one of the best quality plant proteins available for livestock feeding.

In 1963, soybean meal sold commercially for $83 a ton. In June of 1972, the cost of soybean meal had risen to $131 a ton and in August of 1973, the cost had risen to $334 a ton. These rapidly rising costs have caused investigators to look for other sources of protein.

Woodman and Evans (65) reported lambs did very well on sainfoin seed, and suggested the lambs would have performed better if the seed pod had been removed. Holden (25) found that rats fed sainfoin seed had higher feed consumptions and lower feed efficiencies than rats fed soybean meal. He surmised that sainfoin seed with the pod removed would compare favorably with soybean meal.

The purpose of Experiment II was to compare milled sainfoin seed with soybean meal as a source of protein for monogastric animals.

REVIEW OF LITERATURE

Morphology and Types

Sainfoin is a long-lived, deep rooted perennial (51). The root system consists of a main tap root with several large and numerous fine lateral roots (58). Tap roots may be 5 cm in diameter (51), and extend to a depth of 1-10 m (2,58). Most nodules occur on the fine lateral roots, but a few may also occur on the young tap root (58).

Sainfoin has a branched crown from which numerous erect stems arise. Leaves, born on a petiole, are pinnately compound with 11-29 leaflets per leaf (51,58). The inflorescence is born on an erect raceme with 5-80 florets (6,51). The seed is born in single-seeded pods which are brown, indehiscent, lenticular and reticular on the surface (51). The seed is kidney shaped, with the hilum situated in the middle of the concave edge. It is 2.5 mm long, 2.0-3.5 mm wide and 1.5-2.0 mm thick. Seed color ranges from olive to brown or black (58). The weight of 1000 milled seeds ranges from 13.2-16.8 g (58), and the seed pod comprises approximately 30% of the seed weight of unmilled seed (6).

Sainfoin consists of several types with differing growth and adaptation characteristics. In Russia the three types generally grown are common, sand, and transcaucus. Common sainfoin is distributed in the forest-steppe belt of the Ukraine. It has moderate drought tolerance and winterhardiness, slow recovery after cutting, and is used as a one-cut crop. Sand sainfoin is also adapted to the Ukraine. It has excellent drought tolerance and winterhardiness, and its one-cut growth habit resembles that of common. Transcaucus sainfoin is grown in the Transcaucasus region, and exceeds common sainfoin in winter-hardiness, drought tolerance and yield. It grows rapidly, has good recovery after harvesting, and gives 2-3 cuttings a year under irrigation. The transcaucus type is shorter-lived than common sainfoin (2,55).

Common and Giant are the two types of sainfoin grown in Great Britain. Common sainfoin has limited stem elongation the year of establishment, and rarely flowers until the second year. The stems are 60-90 cm long at flowering, and are decumbent at the base. The leaves of Common are smaller and have fewer leaflets than Giant. Common sainfoin produces one hay crop a year. Giant is a multicut, short-lived sainfoin. Stem elongation and flowering occur the first year. The stems of Giant are longer and less decumbent than Common. Two hay crops are usually obtained from Giant (28).

History

Sainfoin is not a new crop. Shain (55) reported that sainfoin was used as a forage crop in Russia over 1,000 years ago. Four hundred years ago sainfoin gained recognition in southern France, where it was first cultivated in 1582, and its culture first described in 1629. It was grown in Germany in the 17th Century and in Italy in the 18th Century (51). Its use in Russia and Europe was primarily on dry calcareous soils where other forage legumes did not thrive (2,51,55,58).

Sainfoin's popularity in Europe and Russia has been attributed to its drought tolerance, winterhardiness, disease resistance, forage quality and its non-bloating characteristics (2,11,51,58).

Although sainfoin is known to have been grown in North America prior to 1900, it gained little recognition as a forage crop until 1964 when the variety 'Eski' was released by the Montana Agricultural Experiment Station (14). Eslick (13) attributed sainfoin's slow rise in popularity to its being tested on soils where it was not adapted, overlooking its non-bloating characteristics, and to investigators being misled by visual notes on coarseness, leafiness and probable palatability. Since the release of Eski, sainfoin has been evaluated in most of the western states, and breeding programs have been initiated in New Mexico and intensified in Canada.

Sainfoin for Hay or Pasture

Alfalfa (*Medicago sativa* L.) has long been the major forage legume in Montana. In recent years, its production was limited by alfalfa weevil infestations. This caused many growers to look for alternative hay crops. Sainfoin is resistant to the alfalfa weevil which suggested that it might be a suitable alternate (4,7,62).

As a hay crop, sainfoin's performance has been variable. It has yielded less than, the same as, and more than alfalfa, depending on the location and environmental conditions (7,19,42,52). In general, sainfoin has yielded more in the first cutting and less in the second cutting than alfalfa (7). Yields of sainfoin have been reduced in the third and fourth years due to a reduction in stand. This reduction in stand has been attributed to root and crown rot organisms (7,38). Persistence is also poor under irrigation or on soils with high water tables (2,7,13,55).

The nutritive value of sainfoin hay is high. It is lower in crude protein, crude fiber and ash than alfalfa, but is higher in nitrogen free extract (27). Cattle feeding trials in Nevada have shown sainfoin to be equal to alfalfa for average daily gains, feed consumption, feed efficiency and disgestability (27), and swine feeding trials in Montana have indicated that 3% ground sainfoin in the diet is equivalent to 3% ground alfalfa in the diet (44). Thus, sainfoin may be a suitable hay crop for areas not suited for alfalfa.

Pasture trials have shown that sainfoin is a very palatable and nutritious non-bloating legume. Cattle and sheep have shown a definite preference for sainfoin over alfalfa, cicer milkvetch (Astrogulus cicer L.), birdsfoot trefoil (Lotus corniculatus L.) and crested wheatgrass (Agropyron desertorum Fisch.)(20,24,63). Yearling steers grazed on sainfoin had superior gains and beef production over steers grazed on other pastures, although the stocking rate of sainfoin was lower. The lower stocking rate of sainfoin was attributed to the uneven seasonal distribution of forage from the variety Eski. In May and June, forage production of Eski is very high, but later in the season it is low. Consequently, the stocking rate had to be varied from 6-8 to 2-4 animals per hectare (32,33). The variety 'Remont', a multicut variety released in 1971, has a seasonal yield distribution similar to alfalfa which should help to alleviate this problem (5).

Seed Production

Sainfoin seed production has been relatively good. It has been estimated the average yield of sainfoin seed in Montana is 500 kg per hectare (4). Seed yields have been reported in excess of 700 kg per hectare in Nevada (27), 1,000 kg per hectare in Idaho (42) and Canada (19), and 1,300 kg per hectare in Montana (8).

Cultural practices. Uniform, firm and well prepared seed beds are essential to obtaining good stands. On most soils, a planting depth of one-half inch has provided satisfactory results. Despite its large seed size, sainfoin does not emerge well from deep plantings (19,27).

Significant responses have been obtained from the use of N fertilizer on sainfoin for both forage yield (27,56) and seed yield (56).

It has not been shown to respond to P fertilization (27,53,56). Sainfoin's response to N fertilization has been attributed to the lack of an effective N fixing rhizobia (3,19,27,41,56).

In alfalfa, maximum seed yields have been obtained from plants that were slightly water stressed during flowering and seed set. An irrigation at early bloom was sufficient to restore moisture to the root zone and provide enough moisture to mature the crop. Irrigation at the time of full bloom stimulated vegetative growth and decreased seed yields (9,59,60,61). Although the effect of soil moisture on sainfoin seed production has not been studied, irrigation during flowering and seed set have not been observed to stimulate vegetative growth and was not felt to be detrimental to seed yield (64).

There has been general recognition among alfalfa investigators that lower plant densities result in higher seed yields. Planting rates of 1-2 kg per hectare in 60 cm rows, or 3-5 kg per hectare broadcast resulted in the highest alfalfa seed yields (9,30,31,39).

Plant density-seed yield studies in sainfoin have not been in agreement. Jenson and Sharp (27) reported the highest seed yields were obtained with 90 cm row spacings and the lowest with 15-30 cm row spacings. Seeding rates from 2-10 kg per hectare had no appreciable effect on seed yields. Hanna et al. (19) obtained maximum seed yields from sainfoin planted in 60-90 cm rows seeded at the rate of 6-9 kg per hectare. Carleton and Wiesner (6), however, obtained maximum seed yield from sainfoin seeded in 7 cm rows. Increasing row spacing to 15 cm decreased seed yields 50%. Seeding rates did not appreciably affect yield. These investigators attributed sainfoin's higher seed yield at narrow row spacings to sainfoin's inflorescence being born at the apex of an erect stem which resulted in maximum exposure of the inflorescence to pollinating insects, and to an increased number of inflorescences per unit area (6).

The common recommendation for harvesting alfalfa for seed is when one-half to two-thirds of the pods are brown to black (40,48,61). In Canada, it was recommended that sainfoin be cut for seed when the pods at the base of the raceme have turned brown (19). Montana data indicates that the relationship of pod color to seed maturation varies with locations and environmental conditions. Maximum seed yields were obtained when the seed was harvested at 40% moisture. This moisture percentage occurred when 10, 33, and 65% of the pods were brown at Moccasin, Bozeman, and Kalispell, Montana, respectively. Earlier harvests, prior to 40% moisture, resulted in poor quality seed, and delayed harvests resulted in seed loss due to shattering. These investigators recommended that sainfoin be swathed at 40% moisture, allowed to dry in the windrow before threshing, and that the seed be air-dried to 12% moisture prior to storage (8).

Components of seed yield. Seed yield is a function of the number of seeds per unit area and seed weight (49). Seeds per unit area may be further subdivided into its individual components which include: plants per unit area, stems per plant, racemes per stem, florets per raceme, and percent seed set.

Seeds per raceme, percent seed set (used as a measure of self- and cross-fertility) and seed weight have been studied in sainfoin. Hanna (18) reported cross-fertility ranged from 3.7-78.3% with a mean of 35.6%, and self-fertility ranged from 0-21.4% with a mean of 4.8%. Self- and cross-fertility were highly correlated (r = .65**). Carleton and Wiesner (6) found a positive relationship between cross-fertility and seed yield, and a negative relationship between seeds per raceme and seed weight. They stated selection for large seeded plants could result in selection for low cross-fertility.

In alfalfa, a number of components and traits have been studied to determine which may be used as indices of seed yield. Factors studied include date of initial bloom, stems per plant, racemes per stem, raceme length and width, florets per raceme, flower color, plant height, forage yield, self- and cross-fertility, seeds per pod, curls per pod, and seed weight per plant. The importance of these factors varied with locations and environmental conditions. None have been universally accepted as good indices of seed yield in alfalfa, although one or more may be considered indicative of seed yield at a particular location (45,54).

Sainfoin Seed as a Protein Supplement

In 1947, Woodman and Evans (65) compared the chemical composition and nutritive value of rye grass, clover, alfalfa and sainfoin seed meal. They indicated that lambs fed unmilled sainfoin seed performed well and would have equalled the gains of lambs fed alfalfa and clover seed meal if the seed pod had been removed.

Holden (25) compared the protein quality of unmilled sainfoin seed and pigweed seed with soybean meal in rat feeding trials. He reported no significant difference in weight gain of rats fed sainfoin seed and soybean meal, although rats fed sainfoin seed consumed more feed and had lower feed efficiencies than rats fed soybean meal. He surmised milled sainfoin seed would compare favorably with soybean meal as a source of protein.

For many years commercial soybean meal has been recognized to be an excellent protein supplement for livestock and has recently been used in increasing quantities as a protein source for humans. Soybean meal's popularity as a protein source may be attributed to its high protein content and essential amino acid balance. Research has indicated that the most limiting amino acid was methionine (22), but even without supplemental methionine, soybean meal is one of the best quality plant proteins available (45).

In 1936, Hayward, Steenbock, and Bohstedt (23) determined that the heating process associated with commercial extraction of oil from soybeans resulted in a definite improvement in the feeding value of soybean meal. Recent research indicates the poor feeding quality of raw soybean meal may be attributed to the presence of heat labile trypsin inhibitors and a deficiency of sulfur containing amino acids (17, 28,31,36,66). The inhibitors present in unheated soybean meal results in hypertrophy of the pancreas, increased synthesis of trypsin and chymotrypsin, and decreased synthesis of amylase (17,31,36). Hypertrophy of the pancreas is detectable in four days (17). Excess levels of trypsin and chymotrypsin are lost to the animals through excretion. These enzymes are high in sulfur containing amino acids and their excretion is thought to create a deficiency for these amino acids. In addition, the inhibitors have a disproportionate amount of cystine, which is not available to the animal and may accentuate deficiencies (28).

Although heating soybean meal has been shown to be an effective means of inactivating the activity of these inhibitors, there is also evidence that genetic variability exists in soybeans for decreased trypsin inhibitor activity. Yen (66) reported that rats fed experimental line 661 excreted less fecal nitrogen, retained more nitrogen, had better gains, and had smaller pancreases than rats fed Clark and Harsoy varieties. Although 661 was superior to the other two varieties, it was inferior to heated soybean meal.

METHODS AND MATERIALS EXPERIMENT I

Ten clones were selected for study on the basis of high 1967 seed yields in a space-planted nursery. Seed yield of these clones ranged from 248 to 391 g. Clonal propagules (So) and open-pollinated (OP) progeny from these ten clones were used to study the components of seed yield in sainfoin and their heritabilities.

In the fall of 1970, crown bud cuttings and seedlings from OP seed from these clones were started and maintained individually in the greenhouse. In the spring of 1971, they were transplanted to the field. A split plot randomized complete block design with three replications was used. Clones were assigned to mainplots and generations (So,OP) assigned to subplots. The rows were 3 m long and .6 m apart. Each row contained 9 plants.

The plants became established during the 1971 growing season. Data were collected in 1972 on stems per plant, racemes per plant, racemes per stem, florets per raceme, seed per raceme, percent seed set, weight per 100 seeds, seed yield, crude protein content of the seed, plant height, and lodging.

Stems per plant were obtained by selecting three plants at random in each row and counting the number of basal stems. The total number of racemes on each of these plants were also counted to obtain racemes per plant. Racemes per stem were derived by dividing racemes per plant by stems per plant.

Florets per raceme were determined by randomly selecting ten racemes per row and counting the number of florets on each raceme. These racemes were tagged and the number of florets counted recorded on the tag. Prior to harvesting for seed, the tagged racemes were collected and the number of seeds per raceme counted. Percent seed set was calculated by dividing seeds per raceme by the number of florets that had been available for pollination and multiplying by 100.

Seed yield was obtained by hand harvesting the plants in each row and weighing the cleaned seed to the nearest g. A random sample of 100 seeds from the plants in each row was weighed to the nearest mg to obtain weight per 100 seeds. Crude protein was determined by Kjeldahl method (N x 6.25)(1) from another random sample of seed from the plants in each row by the Chemistry Station Analytical Laboratory at Montana State University. The analyses were performed on seed contained in the pod.

Plant height was determined and lodging was estimated just prior to seed harvest. Plants were measured, to the nearest cm, from the base of the crown to the tip of the longest stem at three randomly selected points along a row. Lodging was estimated on a one to five scale. Rows with all of the plants standing erect were rated one, and rows with all of the plants prostrate were rated five.

Analysis of variance was used to detect significant differences for each of the traits studied. Row means for each of the characters were used for these analyses. When the F test indicated significance, Duncan's New Multiple Range Test was used to separate means (12). Narrow sense heritability estimates were determined for traits in which significant differences existed by parent-progeny correlations (15) and by regression of OP on So (35). All possible simple correlations between traits were also obtained for the So and OP generations.

Good growing conditions prevailed during this experiment. The field was sprinkler irrigated as necessary to keep the soil moist and allow the plants to grow vigorously. Irrigation was stopped when plants were in the early pod stage to minimize seed loss through shattering. The plants were sprayed at weekly intervals with malathion from early bloom to full bloom to minimize insect damage, particularly from Lygus spp. Although beehives were not placed around the field, honeybees were abundant when the plants were blooming.

RESULTS AND DISCUSSION EXPERIMENT I

Seed yield is a function of the number of seeds per unit area and seed weight. The number of seeds per unit area may be further subdivided into its individual components, i.e., stems per unit area, racemes per stem, florets per raceme, and percent seed set. This study was designed to determine which components were good indices of seed yield in sainfoin, their relationship to each other, and the heritability of each of these components under the environmental conditions at Bozeman, Montana in 1972.

Stems Per Plant

Clones differed significantly for the number of stems per plant (Table 1). Clones A-33 and A-67 had the greatest number of stems per plant with 57.9 and 55.2, respectively. Clone A-40 had the fewest stems per plant with 31.6. Clone A-63, which had the highest seed yield, was intermediate for this character. Significant differences between generations were not detected, and the clone x generation interaction was not significant.

Stems per plant of the So generation were negatively correlated (Table 2) with racemes per stem (r = -.71*), and positively correlated with crude protein content of the seed (r = .76*). Stems per plant of the OP progeny were negatively correlated with racemes per stem (r = -.74*) and positively correlated with percent seed set (r = .66*).

Table 1. Clone and Generation Means for Number of Stems Per Plant

Clone	Generation	Generation mean	Clone mean
A-33	So	66.0	57.9 a[1]
	OP	49.9	
A-67	So	58.1	55.2 a
	OP	52.2	
A-34	So	54.6	49.4 b
	OP	44.3	
A-93	So	51.3	48.7 b
	OP	46.0	
A-10	So	48.2	47.6 b
	OP	47.0	
A-63	So	47.9	46.1 bc
	OP	44.6	
A-55	So	43.0	42.8 c
	OP	42.6	
A-54	So	44.8	42.2 c
	OP	39.6	
A-70	So	33.1	37.9 d
	OP	42.7	
A-40	So	31.6	31.6 e
	OP	31.6	
Mean	So	47.9	
	OP	44.1	

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

Stems per plant were not significantly correlated with any of the other traits studied, including seed yield (Table 2).

Table 2. So, OP, and Total Population Correlation Coefficients for Stems/Plant and Other Traits Studied.

Component	So	OP
Racemes/plant	.41	.41
Racemes/stem	-.71 *	-.74 *
Florets/raceme	-.38	-.10
Seed/raceme	-.06	.52
% Seed set	.14	.66 *
Seed yield	-.07	.28
Wt/100 seed	-.27	-.33
% Protein	.76 *	.51
Plant height	.10	-.23
Lodging	.40	.42

* - P<.05

These data indicate that differences among clones exist for stems per plant, but that this character is not indicative of seed yield. Plants with many stems had fewer racemes per stem, but the total number of racemes per plant was not significantly affected by this relationship (r = .41). Increased stems per plant were also associated with increased protein content in the seed for the So generation. This association was not found for the OP generation (Table 2), although the correlation coefficient did approach significance (r = .51). These relationships suggest that plants with a large vegetative skeleton are able to supply developing seed with the essential nutrients for protein production.

Stems per plant of the OP progeny were significantly related to percent seed set. The meaning of this relationship is not known. It is possible that OP progeny with more stems had less natural stripping of florets, increased pollination, or less shattering. Any of these factors can significantly affect percent seed set. It is also possible that this is a chance relationship and has no biological significance.

Racemes Per Plant

Significant differences occurred among clones and between generations for the number of racemes per plant; however, the clone x generation interaction was significant, negating meaningful interpretation of main effects (Table 3). This interaction resulted from the So of clones A-33 having more racemes per plant than the OP progeny, and the OP progeny of clones A-54, A-70, and A-93 having more racemes per plant than the So's. Differences among generations within the other clones studied were not significant.

Racemes per plant of the So generation were positively correlated (r = .70*) with protein content of the seed (Table 4), whereas racemes per plant of the OP progeny were associated (r = .68*) with

Table 3. Clone and Generation Means for the Number of Racemes Per Plant.

Clone	Generation	Generation mean	Clone mean
A-55	So	251.6 a[1]	240.3 a[2]
	OP	229.1 a	
A-34	So	233.1 a	227.7 ab
	OP	222.3 a	
A-67	So	212.8 a	221.7 b
	OP	230.7 a	
A-70	So	193.9 b	220.6 b
	OP	247.3 a	
A-33	So	242.8 a	220.5 b
	OP	198.2 b	
A-10	So	206.4 a	218.3 b
	OP	230.1 a	
A-63	So	222.0 a	210.0 b
	OP	198.0 a	
A-54	So	160.7 b	189.2 c
	OP	217.7 a	
A-93	So	149.3 a	170.7 d
	OP	192.0 b	
A-40	So	165.3 a	169.0 d
	OP	172.7 a	
Mean	So	208.8 b[3]	
	OP	213.8 a	

1 - Generation means within a clone followed by different letters differ significantly ($P<.05$).

2 - Means in the same column followed by different letters differ significantly ($P<.05$).

3 - Generation means followed by different letters differ significantly ($P<.05$).

a higher incident of lodging. Racemes per plant were not correlated with seed yield.

Table 4. So, OP, and Total Population Correlation Coefficients for Racemes Per Plant and Other Traits Studied.

Component	So	OP
Stems/plant	.41	.41
Racemes/stem	.34	.28
Florets/racemes	.14	.30
Seed/racemes	.22	.10
% Seed set	.25	-.04
Seed yield	.49	-.11
Wt/100 seed	-.47	-.45
% Protein	.70 *	.29
Plant height	.30	-.27
Lodging	.16	.68 *

* - P<.05

<u>Racemes Per Stem</u>

Clones A-70, A-55, and A-40 had the highest number of racemes per stem, and clones A-67, A-33 and A-93 had the least (Table 5). Clones A-34, A-10, A-63, and A-54 were intermediate for this character. When measured across all clones, the OP progeny had significantly more racemes per stem than the So's. The clone x generation interaction was not significant.

Table 5. Clone and Generation Means for the Number of Racemes Per Stem.

Clone	Generation	Generation mean	Clone mean
A-70	So	5.8	5.8 a[1]
	OP	5.7	
A-55	So	6.0	5.7 a
	OP	5.4	
A-40	So	5.3	5.4 a
	OP	5.6	
A-34	So	4.5	4.7 b
	OP	5.0	
A-10	So	4.4	4.6 b
	OP	4.9	
A-63	So	4.6	4.5 b
	OP	4.5	
A-54	So	3.7	4.4 b
	OP	5.1	
A-67	So	3.6	4.0 c
	OP	4.3	
A-33	So	3.7	3.9 c
	OP	4.1	
A-93	So	3.1	3.6 c
	OP	4.2	
Mean	So	4.5 b[2]	
	OP	4.9 a	

1 - Means in the same column followed by different letters differ significantly (P<.05).

2 - Generation means followed by different letters differ significantly (P<.05).

Racemes per stem were negatively correlated with stems per plant for the So (r = -.71*) and OP generations (r = -.74*), but were not correlated with any other trait (Table 6). These correlations suggest a negative relationship between racemes per plant and vegetative growth. The number of racemes per plant did not affect seed yield.

Table 6. So, OP, and Total Population Correlation Coefficients for Racemes Per Stem and Other Traits Studied.

Component	So	OP
Stems/plant	-.71 *	-.74 *
Racemes/plant	.34	.28
Florets/raceme	.49	.39
Seed/raceme	.15	.35
% Seed set	-.04	-.61
Seed yield	.23	-.35
Wt/100 seed	-.10	-.02
% Protein	-.24	-.40
Plant height	.13	-.05
Lodging	-.24	.04

* - P<.05

<u>Florets Per Raceme</u>

Clone A-10 had the most florets per raceme with 62.27. Clones A-93, A-67, and A-33 had the fewest with 54.05, 53.33, and 52.98, respectively (Table 7). Generations had similar numbers of florets per raceme. The clone x generation interaction was significant and occurred because the OP progeny of clone A-63 had fewer florets per raceme than the So's. Significant differences between generations of the other clones were not detected.

Florets per raceme were associated with the number of seed per raceme for the So's (r = .70*)(Table 8). Florets per raceme of the OP progeny were not significantly correlated with any of the traits studied. These data indicate that clones differ in the number of florets per raceme but that this component was not indicative of seed yield.

Seed Per Raceme

Seeds per raceme differed significantly among clones and between generations (Table 9). The clone x generation interaction was significant, negating meaningful interpretation of main effects. This interaction occurred because the OP progeny of clones A-63, A-34, and A-54 had fewer seed per raceme than the So's. Differences between generations of the other clones were not significant.

Seeds per raceme of the So's were correlated (Table 10) with florets per raceme (r = .70*), percent seed set (r = .91**), and seed yield (r = .71*). Seeds per raceme of the OP progeny were associated with percent seed set (r = .89**) and seed yield (r = .70*). Seed

Table 7. Clone and Generation Means for the Number of Florets Per Raceme.

Clone	Generation	Generation mean	Clone mean
A-10	So	65.8 a[1]	68.3 a[2]
	OP	70.7 a	
A-63	So	69.4 a	65.6 b
	OP	61.8 b	
A-70	So	64.6 a	63.6 c
	OP	62.6 a	
A-34	So	65.8 a	62.1 cd
	OP	58.4 a	
A-55	So	63.4 a	61.4 d
	OP	59.4 a	
A-54	So	61.9 a	59.2 e
	OP	56.6 a	
A-40	So	56.4 a	58.1 e
	OP	59.8 a	
A-93	So	52.1 a	54.0 f
	OP	56.0 a	
A-67	So	51.7 a	53.3 f
	OP	55.0 a	
A-33	So	49.3 a	53.0 f
	OP	56.3 a	
Mean	So	60.0	
	OP	59.7	

1 - Generation means within a clone followed by different letters differ significantly ($P<.05$).

2 - Means in the same column followed by different letters differ significantly ($P<.05$).

Table 8. So, OP, and Total Population Correlation Coefficients for Florets Per Raceme and Other Traits Studied.

Component	So	OP
Stems/plant	-.38	-.10
Racemes/plant	.14	.30
Racemes/stem	.49	.39
Seed/raceme	.70 *	.54
% Seed set	.34	.10
Seed yield	.32	.54
Wt/100 seed	-.45	-.03
% Protein	-.19	-.53
Plant height	.60	.15
Lodging	.06	.41

* - P<.05

Table 9. Clone and Generation Means for the Number of Seed Per Raceme.

Clone	Generation	Generation mean	Clone mean
A-10	So	29.6 a[1]	27.6 a[2]
	OP	25.5 a	
A-63	So	34.1 a	26.3 a
	OP	18.6 b	
A-70	So	22.5 a	21.3 b
	OP	20.1 a	
A-34	So	25.8 a	21.0 b
	OP	16.1 b	
A-67	So	20.2 a	20.6 b
	OP	21.1 a	
A-33	So	19.1 a	20.2 b
	OP	21.2 a	
A-40	So	21.4 a	19.6 bc
	OP	17.8 a	
A-54	So	23.1 a	18.2 c
	OP	13.3 b	
A-93	So	14.1 a	16.8 d
	OP	19.6 a	
A-55	So	17.7 a	16.2 d
	OP	14.7 a	
Mean	So	22.8 a[3]	
	OP	18.8 b	

1 - Means in the same column followed by different letters differ significantly ($P<.05$).

2 - Generation means within a clone followed by different letters differ significantly ($P<.05$).

3 - Generation means followed by different letters differ significantly ($P<.05$).

per raceme of the OP progeny were not correlated with florets per raceme, although the correlation coefficient approached significance (r = .54).

These data suggest the number of seed per raceme is indicative of seed yield and two of its components, florets per raceme, and percent seed set. Seed per raceme should be a useful selection trait when screening for high seed yielding plants.

Table 10. So, OP, and Total Population Correlation Coefficients for Seed Per Raceme and Other Traits Studied.

Component	So	OP
Stems/plant	-.06	.52
Racemes/plant	.22	.10
Racemes/stem	.15	-.35
Florets/raceme	.70 *	.54
% Seed set	.91 **	.89 **
Seed yield	.71 *	.70 *
Wt/100 seed	-.50	-.08
% Protein	-.10	-.12
Plant height	.47	-.22
Lodging	-.19	.08

* - P<.05

** - P<.01

Percent Seed Set

Clones and generations differed significantly for percent seed set (Table 11). The clones x generation interaction, however, was significant, which negated meaningful interpretation of main effects. This interaction resulted because the OP progeny of clone A-63, A-34, and A-54 had fewer seed per available floret than the So's.

Percent seed set for the So's was correlated with seed per raceme and seed yield (Table 12). Percent seed set of the OP progeny was associated with stems per plant and seed per raceme. These data revealed that So plants which set more seeds per available floret have more seed per raceme and higher seed yields. OP progeny that set more seed per available floret also had more seed per raceme. The correlation coefficient for percent seed set and seed yield approached significance.

The average percent seed set for this population of sainfoin was 34.3%. With ideal environmental conditions, percent seed set is a good measure of fertility. Ideal conditions are rarely encountered in the field, and it is doubtful the true fertility level of sainfoin is only 34%. Factors such as lack of pollination, natural flower drop, insect damage, and shattering also affect percent seed set. Visual observations indicate shattering is a very important factor affecting percent seed set in sainfoin. Percent seed set probably reflects shatter resistance better than fertility.