Some components of seedling vigor in birdsfoot trefoil (Lotus corniculatus L.)
by James Douglas McElgunn

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
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ABSTRACT

Components of seedling vigor were investigated, using 120 randomly selected clones of Tana birdsfoot trefoil (*Lotus corniculatus* L.). Seedlings were grown for four weeks before harvest for growth measurements.

Final cotyledon area, final leaf area and net assimilation rate were found to affect final dry weight significantly and were proposed as factors which could produce a more vigorous seedling in that they accounted for 88.4 percent of the variation in final dry weight of the seedlings. These three factors were shown to be independent contributors to seedling dry weights.

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INTRODUCTION

Birdsfoot trefoil (Lotus corniculatus L.) has shown its adaptability to areas of the United States by establishing itself in the Atlantic states and the north-western states following its introduction from Europe in the late 1800's.

Agronomically, birdsfoot trefoil has many desirable characteristics (12, 13, 16, 20, 24) but two traits have succeeded in suppressing its wide utilization as a forage crop. One trait is that of seed shattering, which has caused seed to remain relatively high in price. Several investigators have proposed methods to overcome this trait by use of cultural practices (16, 20, 24, 25, 32).

The other objectionable trait, its lack of ability to establish a stand, presents the most serious hindrance to its use (9, 12, 15, 16, 20, 22, 24, 25, 27, 30). Cultural practices (9, 14, 16, 20, 22, 26, 27, 30) have been devised that will aid the "weak" seedling of birdsfoot trefoil to establish, but these methods are not entirely adequate to assure a vigorous stand.

The purpose of this investigation was to examine some of the early vegetative growth characteristics of birdsfoot trefoil as they relate to seedling vigor. An assumption was made that seedlings which accumulate greater dry weight are more vigorous. This assumption placed emphasis on measurements which were assumed to contribute to dry matter accumulation.

LITERATURE REVIEW

Extensive writings dealing with the history, botanical and agronomic characteristics, and agricultural uses and practices applicable to birdsfoot trefoil (Lotus corniculatus L.) production have been prepared by MacDonald (20), Midgley (24), and Hughes et al. (16). Hughes et al. (16) have characterized birdsfoot trefoil as to its advantages and disadvantages as a forage crop.

Advantages: Deep-rooted perennial, heat and drought resistant --- wide soil tolerance, both as to fertility and acidity ---produces seed abundantly, reseeding even when close grazed --- vigorous, with high acre production when well established --- palatable and with high feed value --- able to maintain itself in competition with sod forming grasses, such as bluegrass --- can survive severe grazing abuses ---winter-hardy --- no bloat.

Weaknesses: Slow to establish --- seed cost high, owing to difficulties in seed harvest --- weak stems, it lodges easily when grown alone --- starts rather late in spring --- slow recovery after harvest.

In yield trials, birdsfoot trefoil has been shown to produce sizeable amounts of forage especially when grown as a component of simple pasture mixtures. Cooper 1/ has recently reviewed literature concerning its ability to produce sizeable forage yields in grazing and simulated grazing trials. Its persistence, once a stand was established, was better than Ladino clover (Trifolium repens L.) in grazing trials using lambs (13). The non-bloating characteristic of birdsfoot trefoil also enhances its desirability as a forage.

1/ Cooper, C. S. Factors affecting establishment, survival, and production of birdsfoot trefoil (Lotus corniculatus L.) and alfalfa (Medicago sativa L.). Ph.D. Thesis. Oregon State University. 1964.

Holden [2] states that "the right legume for Montana would combine the characteristics of high nutrient production, non-bloating, palatability, competitive ability, seedling vigor, ample seed production, long life, disease and drought resistance, winter hardiness and adaptability to our soil." These characteristics of an ideal forage crop are undoubtedly not restricted to Montana and could well be intended to include the northern United States and southern Canada. Holden's [2] most serious rejection of birdsfoot trefoil is on the basis of its seedling establishment or seedling vigor. This poor establishment trait of birdsfoot trefoil is the major deterrent to its use in the above mentioned agricultural areas.

Seedling vigor of various forage plants has received considerable attention but only recently has the seedling vigor of birdsfoot trefoil been studied.

The effect of seed size on seedling vigor, whether vigor is defined as seedling dry weight, height of seedling, or actual establishment of seedlings is generally agreed upon as being substantial. Lawson and Rossiter (19) have reported one of the two serious conflicts in the literature as to the effect of seed size on vigor. They (19) found that seed size had no effect on growth of swards of subterranean clover (Trifolium subterranean L.) when sown at the same rate of pure live seed per acre. Whitney [3] found that seed size had no effect on the growth per se of birdsfoot trefoil after emergence.

2/ Holden, J. N. Agronomic potentials of sainfoin (Onobrychis viciaefolia) for Montana. M.S. Thesis. Montana State College. 1963.

3/ Whitney, A. S. The influence of some seed characteristics and depth of sowing on the germination, emergence, and early growth of birdsfoot trefoil (Lotus corniculatus L.). M.S. Thesis. Cornell University. 1958.

The duration of the effect of seed size on seedling growth varies with the crop investigated and with the experimental conditions used. Black (4, 5, 6, 8) found that seed size effects extended throughout the initial growing season in subterranean clover. Smith (29) found that the effect of seed size had diminished one month after planting alfalfa (Medicago sativa L.) and red clover (Trifolium pratense L.). Kittock and Patterson (17), in a study of seed size and seedling vigor of native dry-land grasses, state that "if any differences in seedling vigor for seeds of various weights, beyond emergence ability exists, it is apparently short lived." The study lasted only three weeks and at this time, initial seed size effects were not related to differences in seedling dry weights.

Lin's 4/ data suggest that the critical period for trefoil seedling growth is during early vegetative growth, possibly not more than three weeks after emergence. Seed size can, therefore, be expected to be operative in determining seedling growth during all or at least a part of this critical period.

The superior vigor of seedlings derived from large seeds is manifested by seedlings that are greater in height, leaf area, dry weight or some other factor or factors that enable the seedling to establish a better stand than those seedlings produced from small seeds.

Black's (4, 5, 6) studies with subterranean clover represent an extensive amount of work to determine the effect of seed size on seedling vigor. Consistently he has shown that larger seeds of the same variety or

4/ Lin, Chuang-Sheng. Seedling vigor in birdsfoot trefoil. M.S. Special Problem. Montana State College. 1963.

seeds of a large-seeded variety produce seedlings of greater dry weight than do smaller seeds of the same variety or seeds of small-seeded varieties.

Smith (29) noted differences in seedling height between plants produced from different seed sizes of alfalfa and of red clover. Later in the season, the differences in plant height due to seed size had disappeared.

Beveridge and Wilsie (3) found that seed size and vigor (expressed as seedling dry weight) were significantly correlated in alfalfa.

Kittock and Patterson (17) as well as Kneebone and Cremer (18) have reported that seedling emergence is favored by larger seeds in grasses. The rate and percentage of emergence were found to be greater for larger seeds.

Increased seed size definitely enhances dry matter production of trefoil seedlings during early vegetative growth. Henson and Tayman (15) showed increased dry matter accumulation in trefoil due to greater seed size during 30-and-60-day studies. Using six types of trefoil and three seed sizes of each, they (15) showed that seed size significantly affected plant height and root weight.

Shibles and MacDonald (28) found that differences between trefoil strains at emergence could be accounted for by differences in seed size between the strains. The strains differed in dry weight and cotyledon area due to seed size.

Black (4) found that two strains of subterranean clover with the same seed weight had different dry matter production, but he later resolved this apparent contradiction to the effect of seed size by showing differences in seed size distribution within the two populations.

Lin [4] has conclusively demonstrated the superiority of large seeds in the development of trefoil vigor. He showed that, regardless of variety, large seeds produce seedlings of greater dry weight at two weeks of age.

In the proposed explanation of why large seeds produce this superior vigor an obvious but erroneous hypothesis is that large seeds store more food reserve to aid establishment (4). Black (4) has shown that no correlation exists between cotyledon weight and vigor. However, Black (4) points out that the cotyledon area, regardless of cotyledon weight, is correlated with vigor and cotyledon area is also correlated with seed size. To demonstrate this, seeds of similar size were planted at various depths with the result that the emerging cotyledons varied in weight but were constant in area.

The removal of cotyledon areas has been shown to produce a proportional reduction in final dry weight of seedlings. McElgunn [5] has shown that removal of one or both cotyledons in alfalfa and trefoil at three days and seven days after emergence reduced seedling dry weight. The removal of both cotyledons at seven days after emergence reduced the dry weight to 39 and 22 percent of the check in alfalfa and trefoil, respectively. Harvest was made three weeks after the cotyledons were removed. The difference observed between the percentage reduction in dry weight may be accounted for by earlier initiation of the first leaf in alfalfa. The first leaf was present on the third day after emergence in alfalfa but not until the sixth day was the first leaf present in trefoil. If the cotyledons were removed

[5] McElgunn, J. D. Unpublished data. 1963.

at four weeks after emergence, no reduction in dry weight as compared to the check was found. Aexeman (1), working with soybean and cucumber, has shown that removal of cotyledon area reduces the dry weight of seedlings.

Shibles and MacDonald (28), in their work with trefoil, have reported that no difference in maximum photosynthetic rate exists between cotyledons and leaves of equal area.

The cotyledon, being the first photosynthetic tissue, is important to seedling growth but later the significance of this photosynthetic tissue may be over-shadowed by the greater area of the true leaves. To maintain the superiority derived from large seeds that produce greater cotyledon area, seedlings must remain superior in photosynthetic area, or be more efficient in the rate of producing photosynthates per unit of leaf area, that is, net assimilation rate must be greater, or be superior in both (31).

Shibles and MacDonald (28) reported that similar sized seeds of the Viking and Empire varieties of trefoil produced the same dry weight and cotyledon area at emergence. However, the dry weight and leaf area 200 hours after emergence were not the same. Viking had both greater dry weight and photosynthetic area than did Empire. The net photosynthetic rate, or net assimilation rate, determined manometrically by measuring oxygen evolution, showed no differences between varieties. The differences in dry weight between the two varieties were accounted for by differences in relative growth rate of leaf area. Shibles and MacDonald's results were subject to unequal seed size distribution within their population.

Contrary to the findings of Shibles and MacDonald are the date of Lin 4/. Lin, using seeds of similar size, found that the relative growth rate of leaf area was the same among Viking, Empire and Tana varieties of trefoil at three light intensities. The net assimilation rate, however, was found to be different among the varieties. The net result is the same as the Shibles and MacDonald studies; that is, Viking produced more dry weight than Empire. Tana was intermediate in dry matter production.

Cooper [6/] has compared the relative growth rates, relative growth rates of leaf area and net assimilation rates of alfalfa and birdsfoot trefoil over an 11-week interval. The 11-week averages were similar for relative growth rate between the two genera. Trefoil had a slightly lower net assimilation rate but a slightly higher relative growth rate in leaf area. He attributes the greater growth of alfalfa to the larger initial photosynthetic area and not to a greater relative growth.

[6/] Cooper, C. S. Annual Report. 1963 Irrigated Pasture Research. U.S.D.A. Agronomist, Montana State College. 1963.

Table I summarizes the findings of Shibles and MacDonald (28), Lin 4/, Cooper 6/, and Black (4).

Table I. A comparison of the results reported in four papers pertaining to relative growth rate (dry weight), relative growth rate of leaf area, and net assimilation rate.

Investigator	Time Measured	Relative Growth Rate Leaf Area *	Dry Weight *	Net Assimilation Rate *
Shibles and MacDonald[1]	2 weeks	DS	DS	S
Lin[1]	2 weeks	S	DS	DS
Cooper[2]	11 weeks	DS	S	DS
Black[3]	Several weeks	S	S	S

* S = similar; DS = dissimilar

1. between varieties and/or strains of birdsfoot trefoil with same seed size.

2. between Vernal alfalfa and Tana birdsfoot trefoil.

3. between varieties of subterranean clover with same seed size.

MATERIALS AND METHODS

Seed grown at Bridger, Montana, from 120 randomly selected clones of Tana birdsfoot trefoil was used. Seed of each clone was harvested by two methods in 1963. One method consisted of hand-picking mature pods and allowing them to dehisce in paper bags. The other method involved harvesting the entire plant and drying it at room temperature. These individual plants were then threshed by hand.

One hundred seeds selected at random from each clone from each harvest were then weighed to the nearest 0.0001 gm. on an analytical balance.

Due to lack of ample growth chambers, the seedlings were grown in a glass box in the greenhouse. The 4 by 2 by 1 foot box had a capacity of seventy-two 4 by 4 inch pots.

Twenty scarified seeds from the 1963 hand-picked harvest were planted 1/4 inch deep in a 4 by 4 inch plastic pot in coarse, washed sand. A solution which supplied nitrogen, potassium and phosphorus was used to water the pots every two to three days. The temperature within the box varied. The day temperature ranged from 68° F. to 80° F., and the night temperature was 60° F. The relative humidity at 12 noon averaged 91 percent. Seedlings were illuminated with four fluorescent and one incandescent lights which supplied 400 foot candles at 1 foot above the pots. The day length was 15 hours with not more than three hours per day being supplied by artificial light alone.

Because the box was capable of holding a maximum of only 72 pots, the 120 strains could not be grown at one time. The number of plants grown at one time was also limited by the procedure utilized in measuring cotyledon and leaf area. Plantings of 15 strains plus 3 checks (Tana, Viking and Empire) were made at one-week intervals until the box was full. At each removal or harvest of seedlings, 18 pots were replaced to maintain 72 pots in the box.

The seedlings were grown for 30-days after emergence. Emergence rate was relatively uniform within each pot, and the day of emergence was considered to occur when 10 seedlings were present per pot. The first 10 seedlings were marked with toothpicks and any later emerging seedlings were removed.

At harvest the 30-day-old seedlings were removed from the pots and the roots washed free of sand. The seedlings were stored by wrapping them in wet paper towels and then placing them in glass cake plates. They were stored in a cold room at approximately 5° C. Seedlings stored in this manner could be kept for one week, and longer if necessary, until data on leaf area were gathered.

Leaf area was determined by removing both cotyledons and all leaves from each plant and projecting them onto 1/4" x 1/4" quadrille paper and tracing the enlarged (x5) image. The number of squares and portions of squares occupied by the image was used to determine the area.

Entire seedlings were then bagged and dried for 48 hours at 70 degrees C. The dry weight of the seedlings was measured to the nearest 0.0001 gms.

Data on fall establishment and spring stand were made available by the Eastern Montana Branch Station at Sidney, Montana. The 120 strains were planted in June of 1963 in 10-foot rows spaced one foot apart. Seeding rate was based on 8-pounds of seed per acre. Check varieties, Tana and Empire, were planted alternately every fifth row. The number of seedlings in a 2-foot segment of each row was counted in the fall of 1963 and in the spring of 1964. The same portion of the row was not counted in the spring and fall determinations. As a result, some spring readings exceed their corresponding fall readings. When this occurred, the percent spring survival was considered to be 100 percent.

Data on 1962 seed weights per 100 seeds and seed yield per plant were made available by R. F. Eslick of the Plant and Soil Science Department at Montana State College.

MATHEMATICAL AND STATISTICAL PROCEDURES

Most calculations were made on an I.B.M. 1620 digital computer at the Montana State College Computer Laboratory.

The only manual calculations were made in arriving at the correction factors to adjust cotyledon area, leaf area and dry weight so that they would be comparable between different planting dates. These factors were calculated by dividing the average of the three checks at a given planting date into the overall experiment check average. The correction factors for area also included the conversion factor to convert areas in $(1/4)^2$ inches to mm^2.

From the data present on the initial cards the computer made 13 mathematical calculations. They were:

1. Initial cotyledon area = 6.53 + 9.57 (1963 initial seed weight/100 seed). This formula is based on data collected by Lin (41). This equation accounts for 69 percent of the variability in initial cotyledon area.
2. Corrected final cotyledon area = true cotyledon area x correction factor.
3. Corrected final leaf area = true leaf area x correction factor.
4. Total final photosynthetic area = corrected final cotyledon area corrected final leaf area.
5. Corrected dry weight = true dry weight x correction factor.
6. Relative growth rate of cotyledon area = [$\log_e$ of corrected final cotyledon area − $\log_e$ of initial cotyledon area] /30.

7. Relative growth rate of leaf area = [$\log_e$ of corrected final leaf area − $\log_e$ of initial cotyledon area] /30.

8. Relative growth rate of photosynthetic area = [$\log_e$ of corrected final photosynthetic area − $\log_e$ of initial cotyledon area] /30.

9. Relative growth rate (dry weight) = [$\log_e$ of final corrected dry weight − $\log_e$ of (1963 100 seed weight/100)] /30.

10. Net assimilation rate = [(corrected final dry weight − 1963 100 seed weight/100) ($\log_e$ of corrected final photosynthetic area − $\log_e$ of initial cotyledon area)] [(corrected final photosynthetic area − initial cotyledon area) (30)].

11. Corrected fall establishment = true fall establishment X correction factor.

12. Corrected spring stand = true spring stand X correction factor.

13. Percentage winter survival = (corrected spring stand/corrected fall establishment) (100).

Simple correlation coefficients were calculated between all variables except the true data and the correlation factors. It is obvious that some correlations may be meaningless in that one or more variate has been used to calculate another. For example, a correlation between seed weight and initial cotyledon area may be meaningless because initial cotyledon area was calculated from seed size.

RESULTS

A relatively uniform emergence time at all planting dates was observed. In only rare instances were there less than 10 seedlings present per pot four days after planting. Percentage germination was variable, but in only 14 cases did 20 scarified seeds fail to supply 10 seedlings per pot.

The check varieties, grown at each planting date, varied in leaf area, cotyledon area and dry weight probably due to the variation in temperature and light during the experimental period. No detailed data were recorded on the temperature in the glass box where the seedlings were grown.

Table II has been presented to describe briefly the population used in this study. A relatively large range existed for all characteristics determined, except for initial cotyledon area.

Seed Weight

The 100-seed-weight correlations between harvest methods and seed yield per plant in two years are presented in Table III. The 100-seed-weights were not changed by hand-picking mature pods in 1963. Sieving somewhat altered the 100-seed-weights as compared to the bulk harvested 100-seed-weights in 1962.

The bulk harvested 100-seed-weights produced in different years were correlated. The yields of seed per plant produced in different years were not correlated.

Cotyledon Area

Seed weight and initial cotyledon area were correlated (r 1.00) but this was expected because initial cotyledon area was calculated from seed weight. Initial cotyledon area did not affect cotyledon area at four