A study of the early growth characteristics of birdsfoot trefoil (Lotus corniculatus L.) as they relate to seedling vigor
by Mickey Gene Qualls

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in AGRONOMY
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Abstract:
Seedling vigor of 4 varieties of birdsfoot trefoil was studied during the heterotrophic and autotrophic stages of development at various temperatures.

In the heterotrophic stage the variety Leo had a greater germination, elongation and emergence speed than the varieties Tana, Viking and Empire. This characteristic of Leo was not due to differences in respiration rates nor was it due to a more rapid water absorption rate, Respiration rates were the same for all varieties at each of 6 different ages. Respiration rates of all varieties increased significantly with increasing temperature levels of 60, 70 and 80° F.

In the autotrophic stage Leo had a greater relative growth rate (RGR) than the other varieties at 70° F. When compared to Viking and Tana the greater RGR of Leo was a result of Leo having a greater leaf area to plant weight ratio (LAR). Net assimilation rates were similar for these three varieties. The greater RGR of Leo as compared to Empire, however, was due to Leo having a greater net assimilation rate (NAR). Differences which occurred in LAR were due to changes in the leaf area to leaf weight ratio (LALW) since the ratio of leaf weight to plant weight (LWR) was similar for all varieties. At 80° F. the RGR of Viking, Tana and Leo was similar but all were greater than Empire. RGR differences between Empire and the other 3 varieties was not due to NAR but was due to differences in LAR.

The order of variety rank for germination, elongation and emergence speed in the heterotrophic stage of development was the same order of variety rank for RGR and plant weight at 4 weeks of age in the autotrophic stage of development. The physiological mechanisms responsible for the expression of vigor in the heterotrophic and the autotrophic stages of growth may not be the same in both stages.

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A STUDY OF THE EARLY GROWTH CHARACTERISTICS OF BIRDSFOOT TREFOIL (_Lotus corniculatus_ L.) AS THEY RELATE TO SEEDLING VIGOR

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ABSTRACT

Seedling vigor of 4 varieties of birdsfoot trefoil was studied during the heterotrophic and autotrophic stages of development at various temperatures.

In the heterotrophic stage the variety Leo had a greater germination, elongation and emergence speed than the varieties Tana, Viking and Empire. This characteristic of Leo was not due to differences in respiration rates nor was it due to a more rapid water absorption rate. Respiration rates were the same for all varieties at each of 6 different ages. Respiration rates of all varieties increased significantly with increasing temperature levels of 60, 70 and 80 F.

In the autotrophic stage Leo had a greater relative growth rate (RGR) than the other varieties at 70 F. When compared to Viking and Tana the greater RGR of Leo was a result of Leo having a greater leaf area to plant weight ratio (LAR). Net assimilation rates were similar for these three varieties. The greater RGR of Leo as compared to Empire, however, was due to Leo having a greater net assimilation rate (NAR). Differences which occurred in LAR were due to changes in the leaf area to leaf weight ratio (LALW) since the ratio of leaf weight to plant weight (LWR) was similar for all varieties. At 80 F. the RGR of Viking, Tana and Leo was similar but all were greater than Empire. RGR differences between Empire and the other 3 varieties was not due to NAR but was due to differences in LAR.

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INTRODUCTION

Birdsfoot trefoil (Lotus corniculatus L.) was first utilized as a forage crop in Europe and introduced to the United States in the late 1800's. Today, birdsfoot trefoil is a recommended forage legume for most of the northern U.S.A. Although birdsfoot trefoil has many desirable characteristics (17), its wide acceptance as a forage crop has been seriously suppressed by its poor seedling vigor.

Seedling vigor is difficult to define but in general when a plant has good seedling vigor it means that in the seedling stage of development the plant can successfully combat the immediate environmental forces and survive. Likewise, poor seedling vigor would be described as the failure of a plant, in the seedling stage of development, to combat the immediate environmental forces and survive.

Research in this area is needed to determine what factors account for the poor seedling vigor and which stages of growth are most critical in relation to the survival of the seedling. The purpose of this investigation was to examine some of the early vegetative growth characteristics of birdsfoot trefoil as they relate to seedling vigor.

REVIEW OF LITERATURE

Birdsfoot trefoil has been characterized as to its advantages and weaknesses as a forage crop by Hughes et al. (17).

> "Advantages: Deep-rooted perennial, heat and drought resistant ---wide soil tolerance, both as to fertility and acidity---produces seed abundantly, reseeding even when closely 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".

The advantages show that birdsfoot trefoil has all of the characteristics of a high value forage crop. Of the weaknesses, slow establishment is probably trefoil's worst problem which is the result of poor seedling vigor. Seedling vigor of various forage plants has received considerable attention but only recently has seedling vigor of birdsfoot trefoil been studied.

Whalley et al. (30) have characterized seedling growth and development into three distinct stages: the heterotrophic, transition, and autotrophic stages. The heterotrophic stage is the phase between imbibition of water by the seed and the emergence of the first leaves above the soil with the commencement of photosynthesis. The transition stage is the phase following commencement of photosynthesis but before the exhaustion of the seed food reserves. During this phase the seedling is obtaining organic compounds both from photosynthesis and from the remainder of the stored reserves. The autotrophic stage is the phase following the exhaustion of the stored reserves. In this phase the seedling obtains its organic compounds from the products of photosynthesis and is a true autotroph. A developing seedling, therefore, must pass through each of the above stages to become established. Factors which affect vigor of a seedling can be studied in each of these three stages.

Heterotrophic Stage

The effect of seed age on seedling vigor of soybeans was studied by Ching and Abu-Shakra (7). Oxygen consumption was measured in relation to the amount of phosphorus fixed (P/O ratio) in seedlings from three-year and three-month-old seeds. They found that O_2 consumption was similar for both ages but that the young seeds fixed more phosphorus per unit of O_2 consumed than the old. Also the number of mitochondria per seed decreased with age and those that remained were highly inefficient in their ability to fix phosphorus into adenosine triphosphate (ATP).

The effects of seed size on seedling vigor have been studied rather extensively. Beveridge and Wilsie (2) reported results with alfalfa indicating that plants produced from larger seed show more vigorous growth. Henson and Tayman (15) found that differences in seed size significantly affected plant height, dry weight production and root weight of birdsfoot trefoil seedlings. He suggested that an increase in seed size would improve the vigor of birdsfoot trefoil considerably. The effect of seed size and planting depth on early growth of subterranean clover has been studied by Black (3). Decreasing seed size and increasing planting depth both reduced the weight of the cotyledons at emergence. Cotyledon area remained constant for a given seed size regardless of planting depth. He stated that "the importance of embryo size is to be found not so much in the weight of the tissue, but in the area of the cotyledons contained therein, and that the weight of a plant at any one time during the early vegetative stage is a direct result of cotyledon area". He also pointed out that once emergence had taken place, cotyledonary food reserves were of no further significance in the growth of the plant, even though the effects of seed size extended throughout the entire growing season. On the other hand, Smith (27) found that the effect of seed size had diminished one month after planting alfalfa and red clover.

Williams (33) studied seedling growth of wooly pod vetch, a legume with hypogeal germination, in relation to seed weight. Increasing seed weight resulted in increased seedling dry weight and increased leaf area in the autotrophic stage but did not affect the relative growth rate. Varying the depth of planting did not affect seedling dry weight or leaf area. He concluded that the positioning of the cotyledons in either the aerial or the subterranean environment has no marked effect on the growth in dry weight or leaf area of forage legumes. The important role of cotyledon area on subsequent plant growth has also been observed by Aexeman (1). He removed 50 and 25% of the cotyledon area of cucumbers and soybeans at emergence and found a proportional reduction in dry weight of the seedlings at 30 to 40 days of age. Henry (14), working with birdsfoot trefoil, found a linear relationship between remaining cotyledon and plant dry weight six weeks after emergence.

Elongation and germination speed in the non-photosynthetic stage in range grasses was studied by Whalley _et al_. (30). They stated that "germination speed largely accounts for the good seedling vigor of some grass species". Larger seed produced seedlings with a higher daily elongation rate than small seed of the same variety. They also stated that "not only did seed weight affect the growth rate but seedlings from large seeds kept growing longer than did seedlings from small seeds". In general, if a seedling had a rapid growth rate immediately following germination its growth rate usually declined sooner than that of a slower growing seedling over the same period of time.

Both elongation speed and germination speed are indices of rapid growth and should result in a more rapid emergence. Kittock and Patterson (19) as well as Kneebone and Cremer (20) have reported that seedling emergence is favored by larger seeds in grasses. Emergence force, defined as the amount of force a seedling can exert, was studied by Williams (32). Emergence force was measured by the ability of seedlings to lift weighted glass rods. Criteria were gathered by measuring the median emergence force which was defined as the force which 50% of the population was too weak to exert. He found a significant correlation between seed size and emergence force. He stated that "the major differences in emergence force between varieties lies in unidentified factors".

Seedling growth from germination to emergence of any species involves the mobilization and utilization of seed reserves. Differences in mobilization and utilization of reserves should show up as differences in respiration. Woodstock (34) measured growth and respiration rates in germinating corn seedlings. He found that respiration rates during the first 3 to 4 hours of germination were correlated at the 5% level of significance with root and shoot elongation at 2 to 3 days of age. The significance of this is that seedling vigor could be detected simply by measuring respiration rates of germinating seedlings.

Transition Stage

Lin (21) studied the growth and development of cotyledons of birdsfoot trefoil up to 14 days after emergence. He found that the growth in area of cotyledons takes place at a rapid rate the first week after emergence but slows down after the appearance of the first leaves. He stated that "if cotyledon area is independent of environmental effects such as planting depths, as mentioned by Black (3), the relationship between cotyledon area and seed size must be constant with time or stage". Lin's data show a decrease in seedling total dry weight during the first week after planting which corresponds to the rapid increase in cotyledon area. Small seed giving rise to small cotyledons which, in turn, are slow in expansion of area is thought to be the chief reason for the poor seedling vigor in birdsfoot trefoil.

If a seedling is to benefit from its cotyledon area it must be able to conduct photosynthesis at a substantial rate and utilize the remaining seed reserves efficiently. Shibles and MacDonald (26) in their work with birdsfoot trefoil have reported that no differences in maximum photosynthetic rate exists between cotyledons and leaves of equal area.

Autotrophic Stage

Black (3,4) has shown that differential strain growth of subterranean clover could be traced to differential seed size, i.e., at a common seed size all strains possessed similar cotyledon and leaf areas per plant at any given time and grew at the same rate. On the other hand, Whitney (31) has found that while pre-emergence growth in Viking and Empire birdsfoot trefoil was correlated with seed size, subsequent post emergence growth diverged so that at the end of a two-week period Viking showed considerable superiority in dry weight per plant over Empire seedlings from seeds of the same size and weight. Therefore, differential varietal growth in birdsfoot trefoil, in contrast to subterranean clover, could not be explained satisfactorily on the basis of seed size.

A thorough study of seedling growth using Empire and Viking birdsfoot trefoil has been reported by Shibles and MacDonald (26). They showed that at emergence, seeds of similar size produce seedlings with similar dry weights and cotyledon areas, independent of variety. After emergence, however, seedling dry weights diverged rapidly with Viking having a greater relative growth rate, resulting in more dry weight per seedling, than Empire. Both varieties were found to possess a similar net photosynthetic rate per unit leaf area. They (26) have concluded that divergence in growth between the two varieties at a common seed size can be ascribed to differential rate of production of photosynthetic area.

Contrary to the findings of Shibles and MacDonald are the data of Lin (21). Lin, using seeds of similar size of Empire and Viking birdsfoot trefoil, found that Viking had a greater relative growth rate than Empire at three light intensities. The net result is the same as that found in the Shibles and MacDonald study; that is, Viking produced more dry weight than Empire. Lin, however, found that net photosynthetic rates were different between the two varieties. He concluded that the difference in dry matter production between Empire and Viking was due to differences in net assimilation rate rather than a differential dry matter partition to roots, stems or leaves.

Partitioning of dry matter by the plant is easily modified by external conditions, especially by light. Rhykerd, Langston and Mott (25) found that an increase in light intensity decreased the leafiness of alfalfa and red clover but increased the leafiness of birdsfoot trefoil. McKee (23), also working with birdsfoot trefoil, reported a reduction in leaf area with increasing shading intensity.

Growth rates of alfalfa and birdsfoot trefoil under 51, 76, and 92% shade was studied by Cooper (8). He found that birdsfoot trefoil had a greater relative growth rate than alfalfa under all shading intensities. He concluded that birdsfoot trefoil is as tolerant to shading as alfalfa but, because of its poor seedling vigor and slow recovery following grazing, is more likely to become shaded than alfalfa.

MATERIALS AND METHODS

In the experiments to be reported, varieties of Tana, Viking, Empire and Leo birdsfoot trefoil were studied. Prior to initiation of the experiments every effort was made to obtain seed of uniform size, age and weight so that differences in seedling vigor obtained would not be a result of these factors. Steps taken to insure constancy of seed size, age and weight were as follows:

Age of Seed

Seeds of Tana, Viking, Empire and Leo birdsfoot trefoil, which were harvested in the fall of 1965, were obtained. Differences in the environmental conditions of the parent plants, however, were not controlled. Leo was grown in Canada, Empire in Wisconsin, Viking in New York and Tana in Montana.

Seed Size And Weight

To insure uniform seed size and weight among the four test varieties the initial seed lots were first passed through a Dakota Blower. This operation separates seed by weight and volume. The seeds used in these experiments were obtained by adjusting the blower to the same setting as each variety was passed through. Small seeds and extremely large seeds of each variety were discarded and this left a seed lot fairly uniform as to weight and volume. Seed lots were further sorted to a uniform size by screening. Seeds used for these tests were those which passed through a 1/18 inch sieve but failed to pass through a 1/20 inch sieve. To assume at this point that all seed lots were uniform in weight would be incorrect since seed density must be considered. The density of the seeds of all four varieties was determined by the pycnometer method (11). Differences in density among the four varieties were not significant at the 5% level of probability. Seed densities for Viking, Tana, Leo and Empire were 1.3162, 1.3120, 1.2984 and 1.2922, respectively. Investigations to determine differences in seedling vigor between the four test varieties were conducted in the heterotrophic and autotrophic stages of growth.

Heterotrophic Stage

Speed of Germination

One hundred seeds of each variety were treated for seed diseases with Arasan and placed in 5 x 5 inch plastic germination dishes. Seeds were allowed to germinate at 60, 70 and 80 F. in the dark. Each day the germinated seedlings were counted and removed from the dish. Each variety was grown in four replications at each temperature. The test was terminated in 7 days. Speed of germination was determined by the method of Maguire (22). The formula is given below:

$$\text{Germination Speed} = \frac{\text{No. Germinated}}{\text{Days to First Count}} \ldots + \ldots\ldots + \ldots \frac{\text{No. Germinated}}{\text{Days to Final Count}}$$

Analysis of variance of germination speed was as follows:

Source	Degrees of Freedom
Varieties (V)	3
Temperatures (T)	2
T x V	6
Error	36
Total	47

Imbibition Rate Tests

Four seed lots of each variety of approximately 300 seed per seed lot were weighed dry and placed in water for 12, 24 and 36 hours. At the end of this period seeds were removed from the water, blotted and weighed to the nearest 0.1 mg. Moisture content of the four varieties was determined for the three time intervals.

Speed of Elongation

Twelve seed lots of 13 seed of each variety were weighed to the nearest 0.1 mg. While the seed were being weighed large and small seed were removed by hand. Thus, each lot was adjusted to + or - 0.5 mg between each variety. Seed were then placed in slanted germination dishes with the seed held in place by a strip of tissue paper. Seedlings were allowed to elongate in dark germination chambers at 60, 70 and 80 F. At daily intervals up to day 12, each dish was opened, the length of each seedling was recorded, the dish was re-covered and the seedlings were allowed to continue growth. Speed of elongation was calculated by the method of Carleton and Cooper (6), which is a modification of Maguire's formula (22). The formula is given below.

$$\text{Elongation Speed} = \frac{\text{Seedling Length}}{\text{Days to First Count}} + \ldots + \frac{\text{Seedling Length}}{\text{Days to Final Count}}$$

Analysis of variance of elongation speed was the same as for germination speed.

Respiration Tests

Twenty-four seed lots of 40 seeds of each variety were placed in germination dishes in each of 3 germinators set at 60, 70 and 80 F. Twenty ml of water was added initially to blotters in each germination dish and extra water added to maintain the proper moisture throughout the growth period. Seed were treated with Arasan prior to placement in the dishes. Tests were conducted previously to determine the effect of Arasan on respiration and no affect was observed.

At two-day intervals for 12 days three lots of each variety were removed from the germinator. Oxygen consumption was measured on a Gilson Differential Respirometer at the same temperature at which the seeds were germinated. Standard manometric techniques were employed (29). Oxygen consumption was measured at 5 minute intervals for approximately 1 hour. The tissue was then removed from the flasks, dried and weighed to the nearest 0.1 mg. Regression coefficients were then determined for each flask to obtain the best measure of O_2 consumption per unit of time. Data were then converted to $\mu l \cdot O_2$/mg/hour. Analysis of variance of respiration data was as follows:

Source	Degrees of Freedom
Varieties (V)	3
Temperatures (T)	2
Ages (A)	5
V x T	6
V x A	15
T x A	10
V x T x A	25
Error	149
Total	215

Autotrophic Stage

Growth Analysis Study

Fifteen seeds of each variety were weighed on an electric balance to the nearest 0.1 mg. Differences in weight among seed lots allowed for grouping into replications. For example: replication 1 was made up of 15 of the heaviest seeds of each variety while replication 10 was made up of 15 of the lightest seeds of each variety. Differences in seed lot weight among varieties within a replication did not exceed + or - 0.3 mg. Seeds of each variety were planted ½ inch deep in 4 x 4 inch plastic pots filled with plaster grade vermiculite. Twenty pots of each variety were placed in a growth chamber with a day length of 16 hours, a light intensity of 2000 ft-c and a temperature of 70 F. Seedlings were watered throughout the experiment with Hoagland's Nutrient Solution (15). Stock solutions of 1 molar KH_3PO_4, KNO_3, $Ca(NO_3)_2$, and $MgSO_4$ were prepared in separate containers and added to distilled water prior to watering. Micronutrients and iron were also contained separately in stock solutions and also added to the mixture of stock solution and distilled water prior to watering.

Plants were thinned to the five most uniform plants per pot one week after emergence. Pots were rotated within each block and blocks within the chamber daily. The experimental design employed was a randomized complete block with paired samples. At time 1 (16 days after planting) ten pots of each variety were removed from the growth chamber and the plants separated into roots, stems and leaves. Leaf area was determined by placing the leaves on an overhead projector that magnified the image

25 times onto a piece of paper. The image was traced, cut out, and the area measured on an air flow planimeter (18). The actual leaf area was then determined by dividing the area measurement by 25. Cotyledon area was determined separately by the same method. All roots, stems, cotyledons and leaves were then oven dried and weighed to the nearest 0.1 mg. At time 2 (12 days later) the remaining plants were sampled in the same manner as at time 1. The entire experiment was repeated at 80 F. All procedures were the same except only 8 replications were used and the number of days from time 1 to time 2 was 15 instead of 12.

From the data obtained relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf area-leaf weight ratio (LALW) and leaf weight to plant weight ratio (LWR) were calculated.

RGR and LAR were computed from formulas devised by Thorne (28).

$$RGR = (\log_e W_2 - \log_e W_1)/ (t_2 - t_1)$$

$$LAR = \frac{\left[(A_2 - A_1) \quad (\log_e W_2 - \log_e W_1)\right]}{\left[(\log_e A_2 - \log_e A_1) \quad (W_2 - W_1)\right]}$$

where W_1, A_1, W_2, and A_2 represent dry weights and leaf areas at times 1 and 2 (T_1 and T_2).

NAR was computed on a leaf basis following Gregory (11).

$$NAR = \left[(W_2 - W_1)(\log_e A_2 - \log_e A_1)\right] \;/\; \left[(A_2 - A_1)\,(t_2 - t_1)\right]$$

where W_1 and A_1 are the dry weights and leaf areas at time 1, and W_2 and A_2 are the dry weights and leaf areas at time 2.

LALW and LWR were computed from formulas following Cooper (7).

$$\text{LALW} = \frac{\left[(A_2 - A_1)\ (\log_e LW_2 - \log_e LW_1)\right]}{\left[(\log_e A_2 - \log_e A_1)\ (LW_2 - LW_1)\right]}$$

where LW_2, A_2 and LW_1, A_1 equal leaf weight and leaf area at times t_2 and t_1 (note, same equation as 3 , only leaf weights substituted for total plant weight).

$$\text{LWR} = \frac{\left[(LW_2 - LW_1)\ (\log_e W_2 - \log_e W_1)\right]}{\left[(\log_e LW_2 - \log_e LW_1)\ (W_2 - W_1)\right]}$$

when LW_2 and LW_1 equal leaf weights and W_2 and W_1 plant weights at times t_2 and t_1.

Values calculated were analyzed statistically by the analysis of variance. Sources of variance and degrees of freedom were as follows:

Source	Degrees of Freedom
Replications within temperatures	16
Varieties (V)	3
Temperatures (T)	1
T x V	3
Error	48
Total	71

RESULTS AND DISCUSSION

Heterotrophic Stage

<u>Speed of Germination</u>

Speed of germination of variety Leo was significantly greater than the other three varieties at all temperatures (Table I). The germination speed of all varieties was higher at 70 than at 60 F. At 80 F. only the germination speed of Leo responded significantly, the other varieties remained unaffected. Even though the germination speed of Empire, Viking and Tana was not statistically different a trend is evident; Leo is highest with Tana, Viking and Empire following in order. Species which can germinate quickly have a definite competitive advantage over species which cannot. The major advantage afforded a seedling with rapid germination is that of a more rapid emergence which, in turn, places the seedling in a good competitive position with weeds and other crop plants near by.

<u>Imbibition Rates</u>

The more rapid germination of Leo was not due to a more rapid water absorption rate. Imbibition rates were the same for all varieties at 3 different time intervals. The percentage of moisture absorbed within 12 hours of soaking seed lots of Tana, Viking, Empire and Leo was 61.9, 57.1, 61.1 and 60.0%, after 24 hours of soaking it was 68.3, 68.0, 70.3 and 69.8%, and after 36 hours of soaking it was 70.6, 71.3, 73.5 and 74.7% respectively.

<u>Speed of Elongation and Emergence</u>

Leo elongated at a faster rate than the other varieties at all temperatures (Table II). As temperatures increased, elongation rates for