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
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Montana State University
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Abstract:
Components of seedling vigor were investigated} using 120 randomly selected clones of Tana bidsfoot 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 tb 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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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 dryland 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		Net Assimilation Rate
		Leaf Area	Dry Weight	
		*	*	*
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

Table II. The average variation and standard error of several measurements made on randomly selected clones of Tana birdsfoot trefoil (Lotus corniculatus L.) plants

Measurement	Unit of Measure	Average	Variation		Standard Error	N
1963 hand picked seed	gm/100	0.1281	0.1956	0.0858	0.00147	137
1963 bulk seed	gm/100	0.1288	0.1954	0.0847	0.00146	137
1963 seed yield/plant	gm	6.2396	22.03	0.410	0.67340	133
1962 sieved seed	gm/100	0.1458	0.1907	0.1177	0.00137	115
1962 bulk seed	gm/100	0.1193	0.1721	0.0810	0.00132	137
1962 seed yield/plant	gm	14.4687	97.27	0.440	0.14870	137
initial cotyledon area	mm^2	6.5400	6.549	6.539	0.00051	137
R.G.R. cotyledon area	$mm^2/mm^2/day$	0.0610	0.0855	0.0367	0.00065	137
final cotyledon area	mm^2	41.90	85.16	19.70	0.08044	137
R.G.R. leaf area	$mm^2/mm^2/day$	0.2151	0.2444	0.1860	0.00122	137
final leaf area	mm^2	4561.76	9980.9	1749.6	174.73	137
R.G.R. photosynthetic area	$mm^2/mm^2/day$	0.2155	0.2444	0.1870	0.00121	137
final photosynthetic area	mm^2	4603.66	10027.1	1785.8	174.66	137
N.A.R.	$mg/mm^2/day$	0.00170	0.002942	0.0004061	0.00004845	137
R.G.R. (dry weight)	mg/mg/day	0.3368	0.3728	0.3062	0.00141	137
final dry weight	mg	35.08	110.87	10.60	1.6054	137
fall stand	plants/2 ft.	67.5	93.0	34.0	1.023	115
spring stand	plants/2 ft.	47.9	84.0	12.0	1.159	115
winter survival	percentage	76.98	100.0	28.9	1.225	115

Table III. Correlation coefficients of seed weight measurements and seed yields obtained from randomly selected clones of Tana birdsfoot trefoil (Lotus corniculatus L.) plants.

	N	1962 bulk seed weight	1962 sieved seed weight	1963 total yield per plant	1963 bulk seed weight	1963 hand-picked seed weight
1962 total yield per plant	120	0.282**	0.246**	N.S.	N.S.	N.S.
1962 bulk seed weight	120		0.745**	N.S.	0.218**	N.S.
1962 sieved seed weight	119			N.S.	0.270**	N.S.
1963 total yield per plant	109				0.165**	0.236**
1963 bulk seed weight	109					0.997**
1963 hand-picked seed weight	109					

* significant at 0.05 probability level; ** significant at 0.01 probability level

weeks nor did it affect leaf area or photosynthetic area at four weeks.

Initial cotyledon area or seed weight had a highly significant negative correlation coefficient with relative growth rate (dry weight). Initial cotyledon area was correlated with percentage spring survival.

The relative growth rate of cotyledon area was highly influential in determining final 7/ cotyledon area (Table IV). Relative growth rate of cotyledon area accounted for 96 percent of the variation in final cotyledon area. The remaining 4 percent of variation may be due to random variation and/or differences in initial cotyledon area.

Cotyledon area and the rate of expansion of cotyledon area did not influence the relative growth rate of leaf area or the final leaf area. Both the relative growth rate of cotyledon area and final cotyledon area affected fall stand significantly, yet they did not affect spring stand or percentage spring survival.

The cotyledon area at four weeks was correlated with net assimilation rate but did not affect two of the factors used to calculate net assimilation rate, namely final photosynthetic area and final dry weight.

Leaf Area

Table V shows the correlation coefficients obtained among leaf area data and other growth measurements.

7/ final - as used in this paper with cotyledon area, leaf area, photosynthetic area, or dry weight is the area or weight at four weeks after emergence.

Table IV. Cotyledon area measurement interrelationships and affects of subsequent growth measurements.

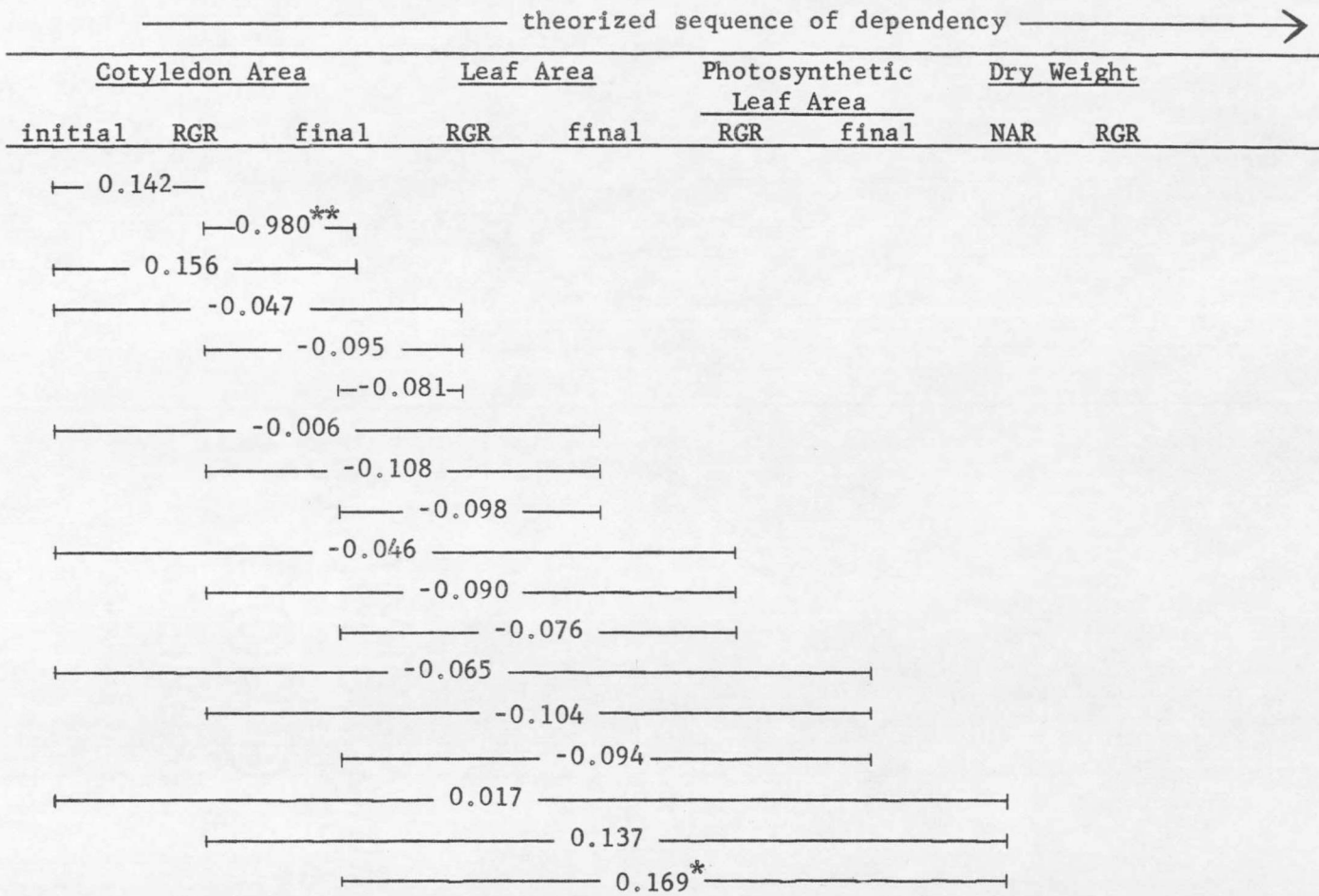
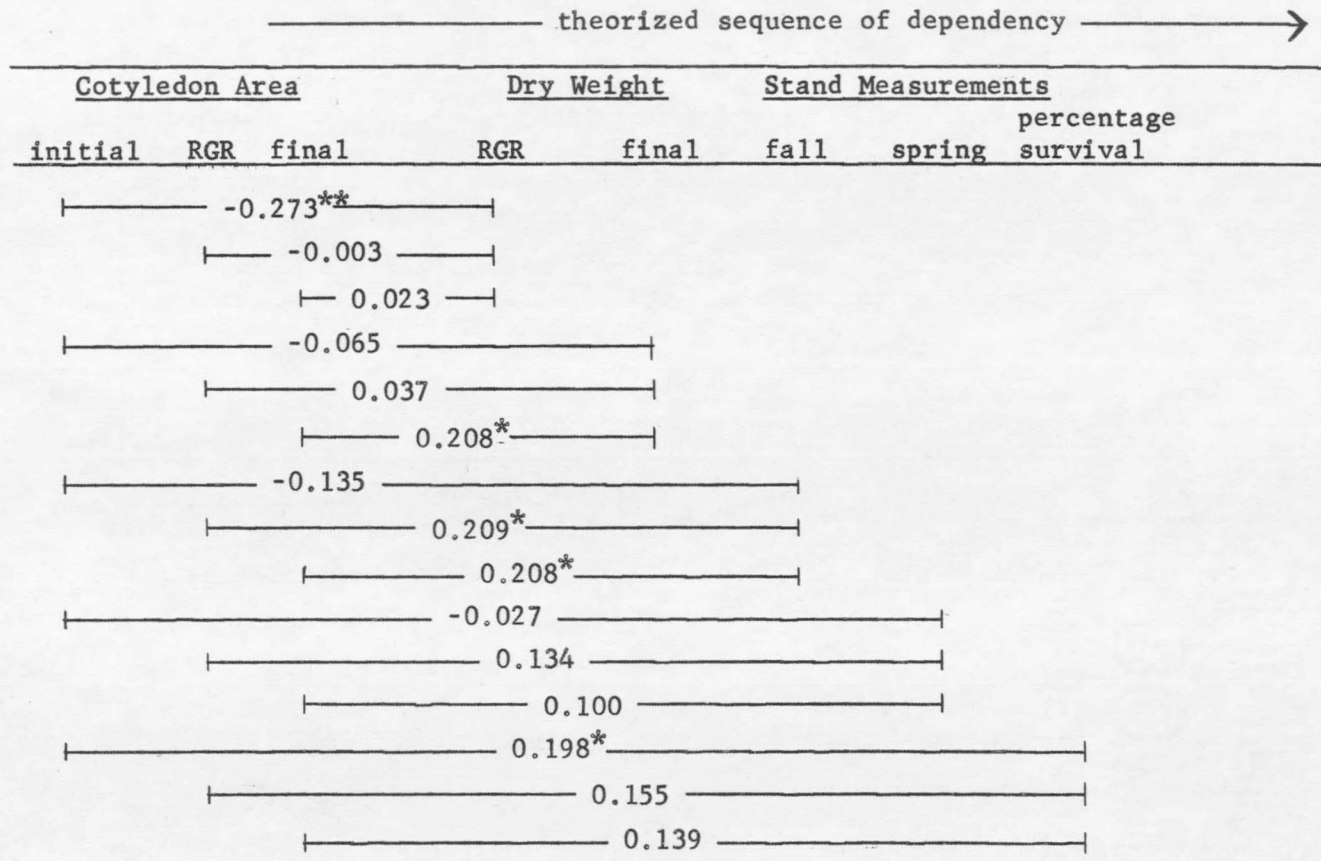
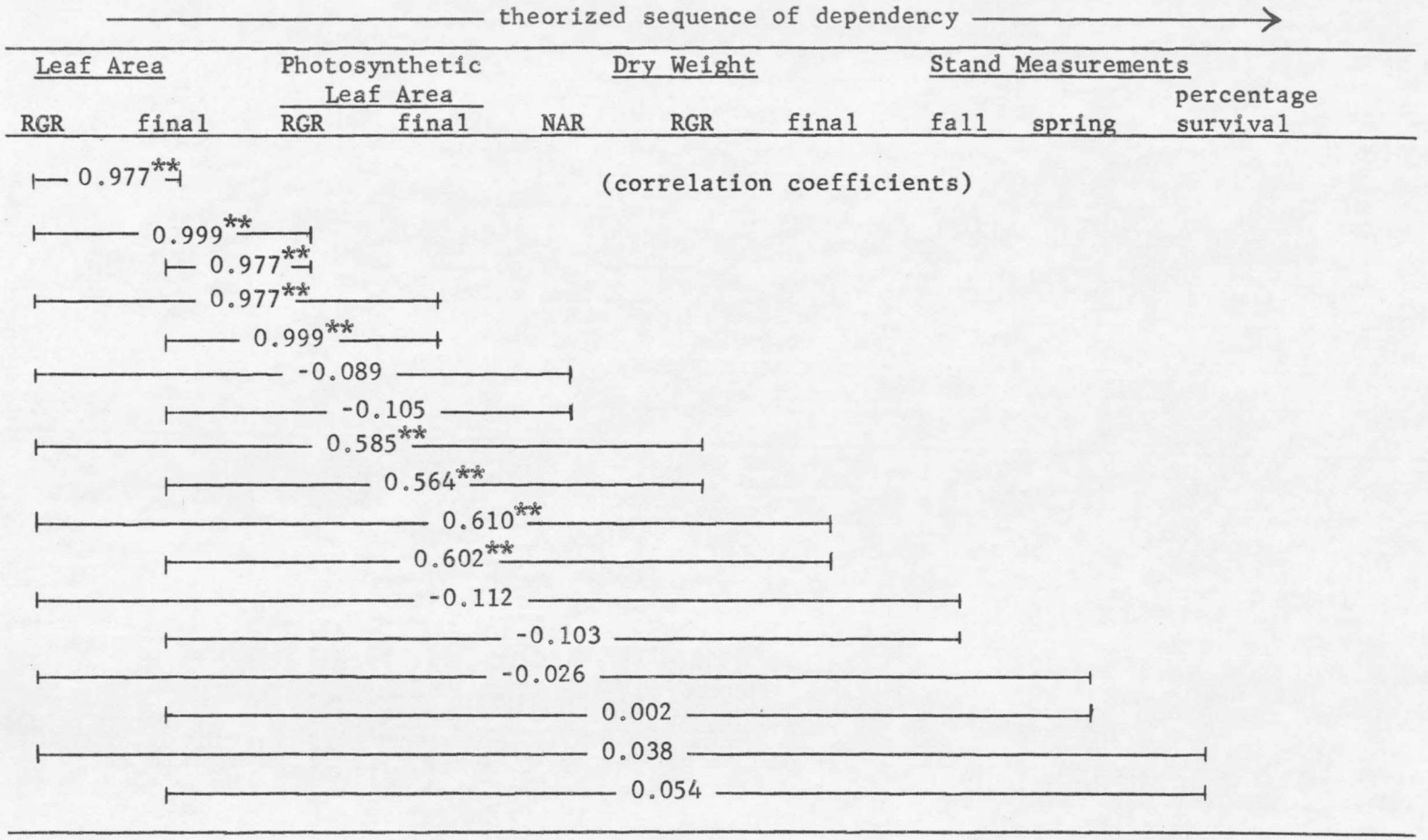


Table IV. continued



* significant at 0.05 probability level; ** significant at 0.01 probability level.

Table V. Leaf area measurement interrelationships, and effects on subsequent growth measurements.



* significant at 0.05 probability level; ** significant at 0.01 probability level.

The relative growth rate of leaf area accounted for 95.4 percent of the variation in final leaf area. The relative growth rate of leaf area and final leaf area accounted for 99.8 percent of the variation in relative growth rate of photosynthetic area and final photosynthetic area, respectively.

The relative growth rate of leaf area and final leaf area did not affect net assimilation rate; however, they affected the relative growth rate (dry weight) and final dry weight.

There were no significant correlation coefficients among leaf area measurements and field stand measurements.

Photosynthetic Area

The relationship of photosynthetic area measurements to all other growth data were the same as for the leaf area measurements (Table VI). This was to be expected due to the high degree of relationship between leaf area determinations and photosynthetic area measurements.

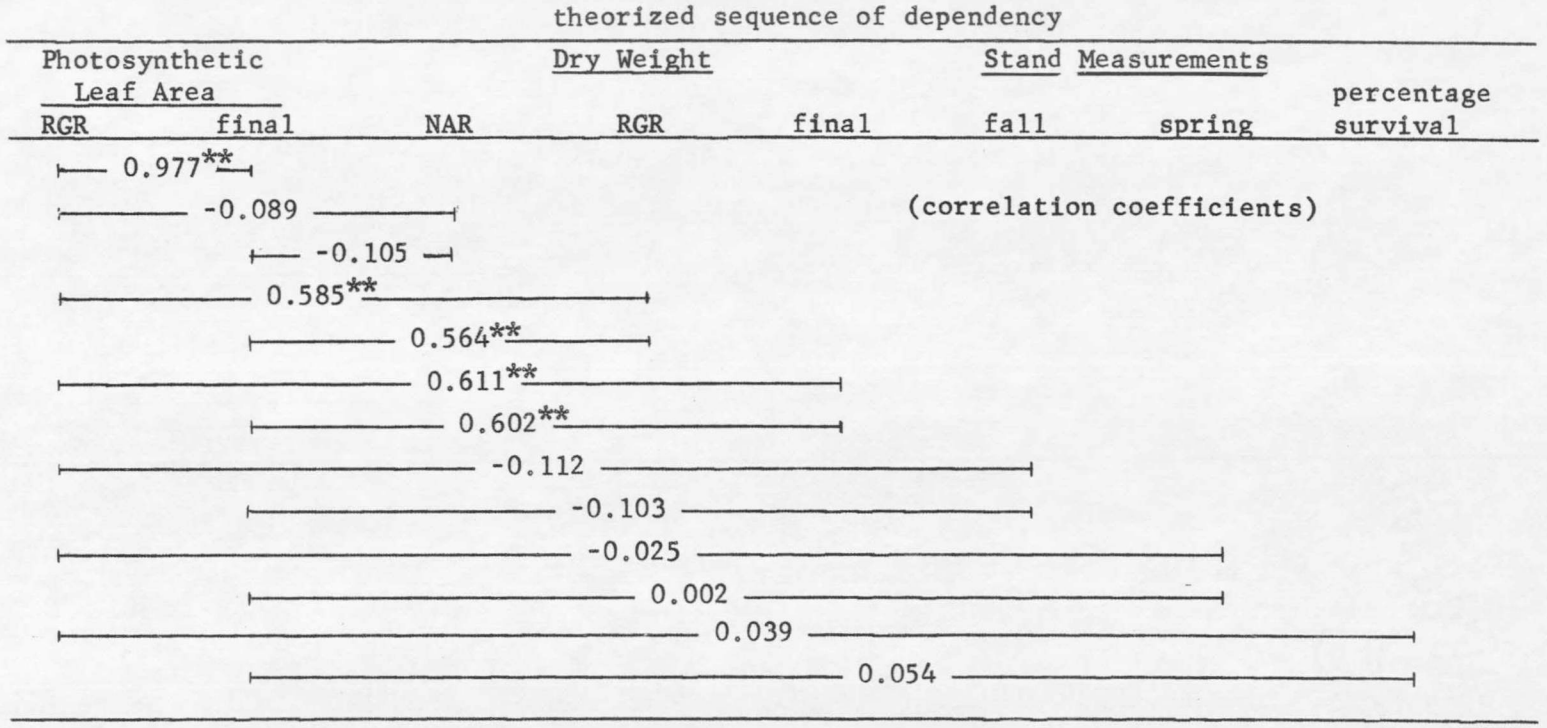
Dry Weight

Net assimilation rate significantly influenced the relative growth rate (dry weight) and final dry weight (Table VII). Relative growth rate (dry weight) accounted for 88.4 percent of the variation in final dry weight. Dry weight measurements were weakly related to stand measurements as shown in Table VII.

Standard Measurements

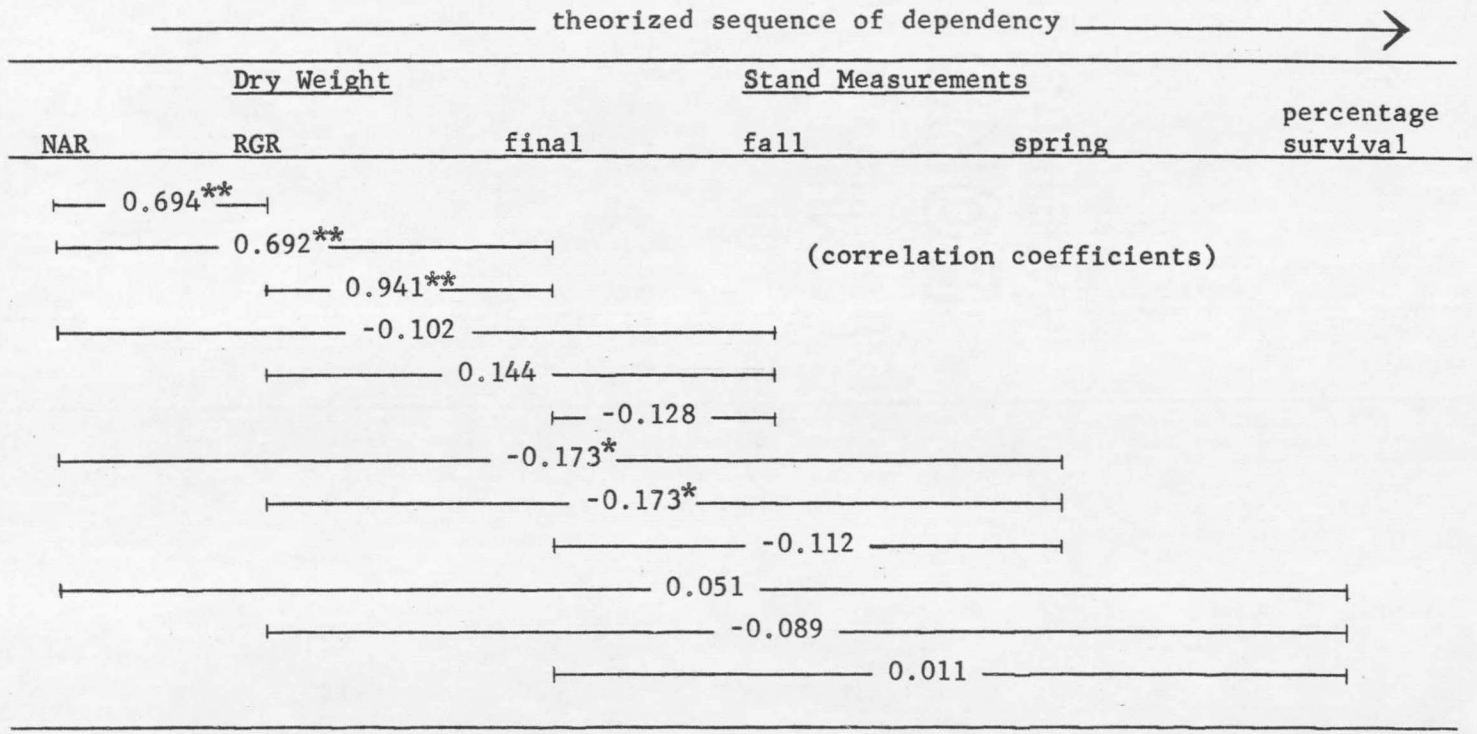
Fall stands accounted for 50.4 percent of the variation in spring stands as shown in Table VIII. The remaining variation was attributed to winter killing.

Table VI. Photosynthetic area measurement interrelationships, and effects on subsequent growth measurements



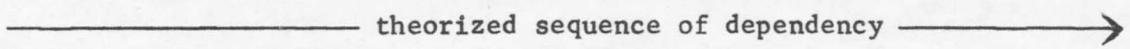
* significant at 0.05 probability level; ** significant at 0.01 probability level

Table VII. Dry weight measurement interrelationships, and affects on subsequent growth measurements.

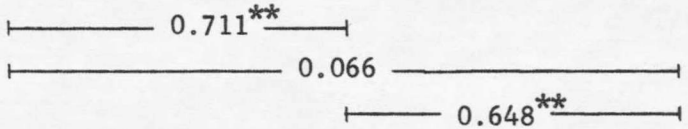


* significant at 0.05 probability level; ** significant at 0.01 probability level.

Table VIII. Stand measurement interrelationships.



Stand Measurements		
fall	spring	percentage survival



(correlation coefficients)

* significant at 0.05 probability level

** significant at 0.01 probability level

DISCUSSION

A definition of seedling vigor briefly stated is: the ability of a seedling to establish itself by overcoming or being insensitive to environmental factors that tend to suppress it. A problem arises in trying to define a seedling, especially with respect to the upper age limit. In this thesis a seedling has been thought of as the re-distributed growth and subsequent new growth produced during the first growing season of birdsfoot trefoil. An assumption was made that seedlings capable of accumulating a greater amount of dry matter were more vigorous in that they apparently were able to grow well under the existing environmental conditions.

Variation of the 100-seed-weights and growth measurements were considered desirable for the reason that the analysis was one of correlation. Of special interest were the range and standard error for net assimilation rate. Shibles and MacDonald (28) and Black (4) have reported that net assimilation rates were the same among varieties of birdsfoot trefoil and subterranean clover respectively (Table I). Cooper 6/ and Lin 3/ found that net assimilation rates differed as shown in Table I. If the data on net assimilation rate presented in Table II are put into the form used in Table I, it agrees with Lin's 3/ results. The data concerning relative growth rate of leaf area and relative growth rate (dry weight) also agree with Lin 3/.

Seed Weight

One-hundred-seed weight was a heritable trait as shown by the significant correlation coefficient of 100-seed-weights obtained in 1962 and 1963. Selection for 100-seed-weight is possible. Selection for seed yield

per plant may not be effective because of the non-significant correlation coefficient between seed yields in different years. Peacock and Wilsie (25) found that two cycles of selection produced no apparent gain in the amount of seed produced per plant. However, yield is not a simple characteristic and yield per se may not be heritable, yet breeders have been able to select for seed yield or components of seed yield with success.

Seed weight has been shown to be highly correlated with embryo size (4). The larger or heavier seed has been reported to produce a more vigorous seedling with respect to height (10, 15), cotyledon area (4), leaf area (5, 23, 28) and dry weight (5, 15, 30). The length of time that a large seed contributes to a greater photosynthetic area and dry weight accumulation in birdsfoot trefoil is apparently less than four weeks (Table IV). The author will agree that seed weight affected initial cotyledon area but the initial photosynthetic area advantage must be short-lived as seed weight was not affecting final cotyledon area or subsequently developed leaf area. A larger initial cotyledon area may be advantageous when light is limiting growth. In this experiment competition for light was considered negligible because only 10 seedlings were present in a 16 square inch area.

The highly significant negative correlation coefficient between 100-seed-weight and relative growth rate (dry weight) was not expected. The weight of one seed was used as the initial dry weight in the calculation of relative growth rate (dry weight) per plant. An examination of the

$\log_e$ of numbers in the range of 0.001965 to 0.00085 shows these numbers have large negative values and thus are very influential in determining the relative growth rate (dry weight). The possible overweighting of the initial weights by use of this standard formula could possibly lead to this unexpected result.

Spring stand and 100-seed-weight were also statistically related. The stand data must be used with caution due to the procedures used in obtaining the preliminary data. One limitation of the stand trial was that it was not replicated. Non-uniform seeding rates may have resulted in intra-row competition confounding the results. The seeding rate was not based on 8 pounds of pure live seed per acre but rather on 8 pounds of seed per acre.

Cotyledon Area

The relative growth rate of cotyledon area was a more potent determinant of final cotyledon area than was the initial cotyledon area. The effect of seed size may not extend beyond initial cotyledon area development. Black (4) stated that seed size determines initial cotyledon area. However, he found that the initial cotyledon area was correlated with subsequent leaf area expansion and dry weight accumulation in subterranean clover for a period of several months. Apparently in birdsfoot trefoil the initial cotyledon area does not influence subsequent growth of leaf area and the initial photosynthetic area advantage, if present, is obscured or absent at the end of four weeks.

On the basis that the three measurements determined on cotyledons can be related to field measurements, it may be concluded that a relationship

exists. Final cotyledon area and relative growth rate of cotyledon area were related to fall stand and initial cotyledon area was related to winter survival. Survival was a function of spring stand which in turn was a function of fall stand. Although the exact relationships were not well established in this study, it would be unreasonable to ascribe the reported relationships to chance alone. The field measurements were of a preliminary nature and perhaps more refined trials utilizing replications, precise seeding rates and stand counts might better reveal the extent and nature of the relationships involved.

Leaf Area And Total Photosynthetic Leaf Area

Since the relative growth rate of leaf area and relative growth rate of photosynthetic leaf area were highly correlated (Table V), the relative growth rate of cotyledon area was not contributing appreciably to total photosynthetic area. Hereafter only relative growth rate of photosynthetic leaf area and final photosynthetic area need be considered.

Growth Rate and Dry Weight Meaurements

The relative growth rate of photosynthetic area accounted for 34 percent of the variation in relative growth rate (dry weight). The remaining variation should be accounted for by net assimilation rate but only an additional 48 percent of the variation in relative growth rate (dry weight) can be ascribed to net assimilation rate. This leaves 18 percent of the variation in relative growth rate (dry weight) unaccounted for by these two measurements. More complex correlation analyses using multiple and partial correlation analyses may increase the practical value of these two measurements in predicting relative growth rate (dry weight). Final leaf

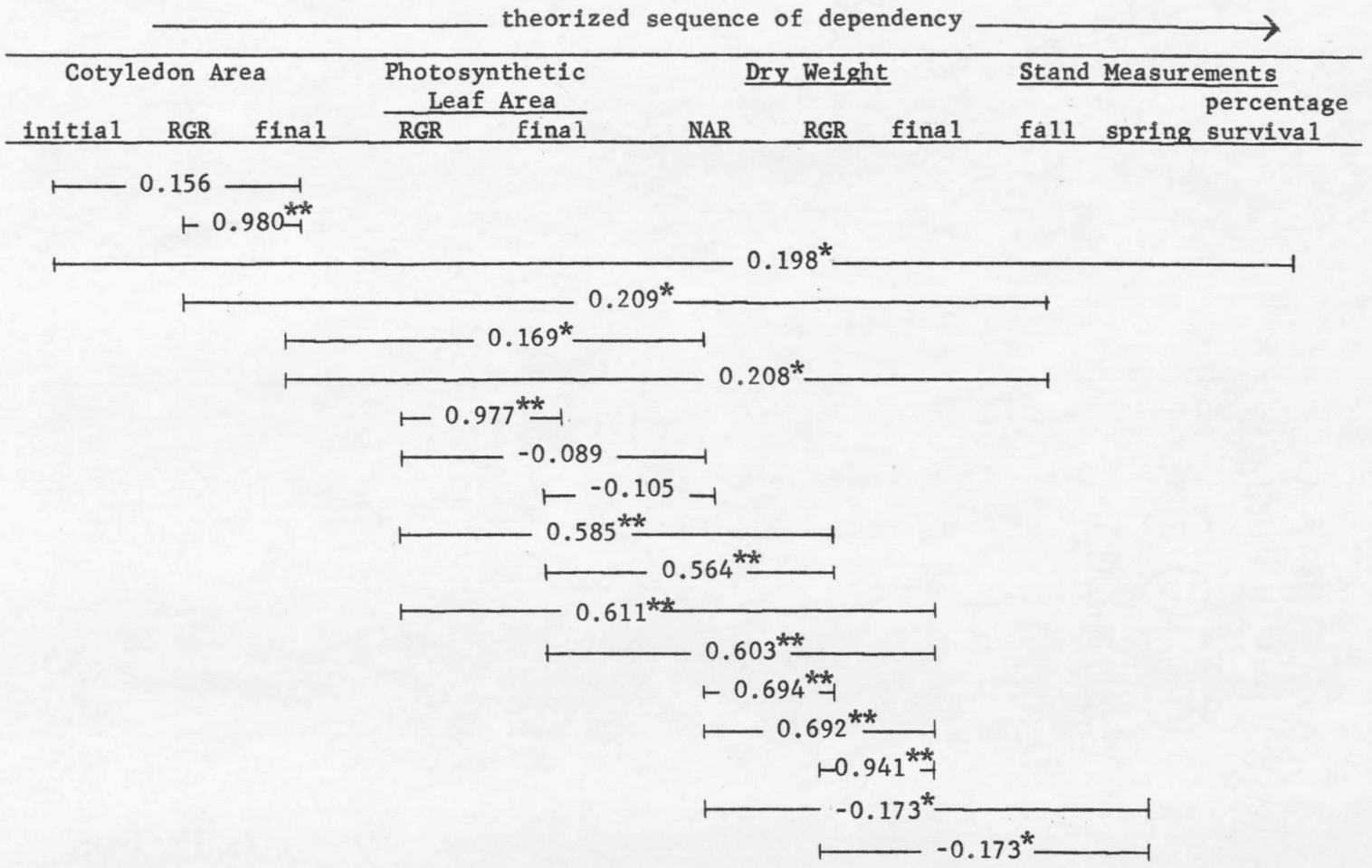
area (36.2 percent), net assimilation rate (47.9 percent) and final cotyledon area (4.3 percent) accounted for 88.4 percent of the variation in final dry weight using only simple correlation analyses.

Beinhart (2) has reported that leaf area was a more potent determinant of dry weight than net assimilation rate in white clover (Trifolium repens L.). The preceding results with birdsfoot trefoil do not confirm Beinhart's conclusions with white clover. In birdsfoot trefoil net assimilation rate was at least as potent a determinant of dry weight as leaf area.

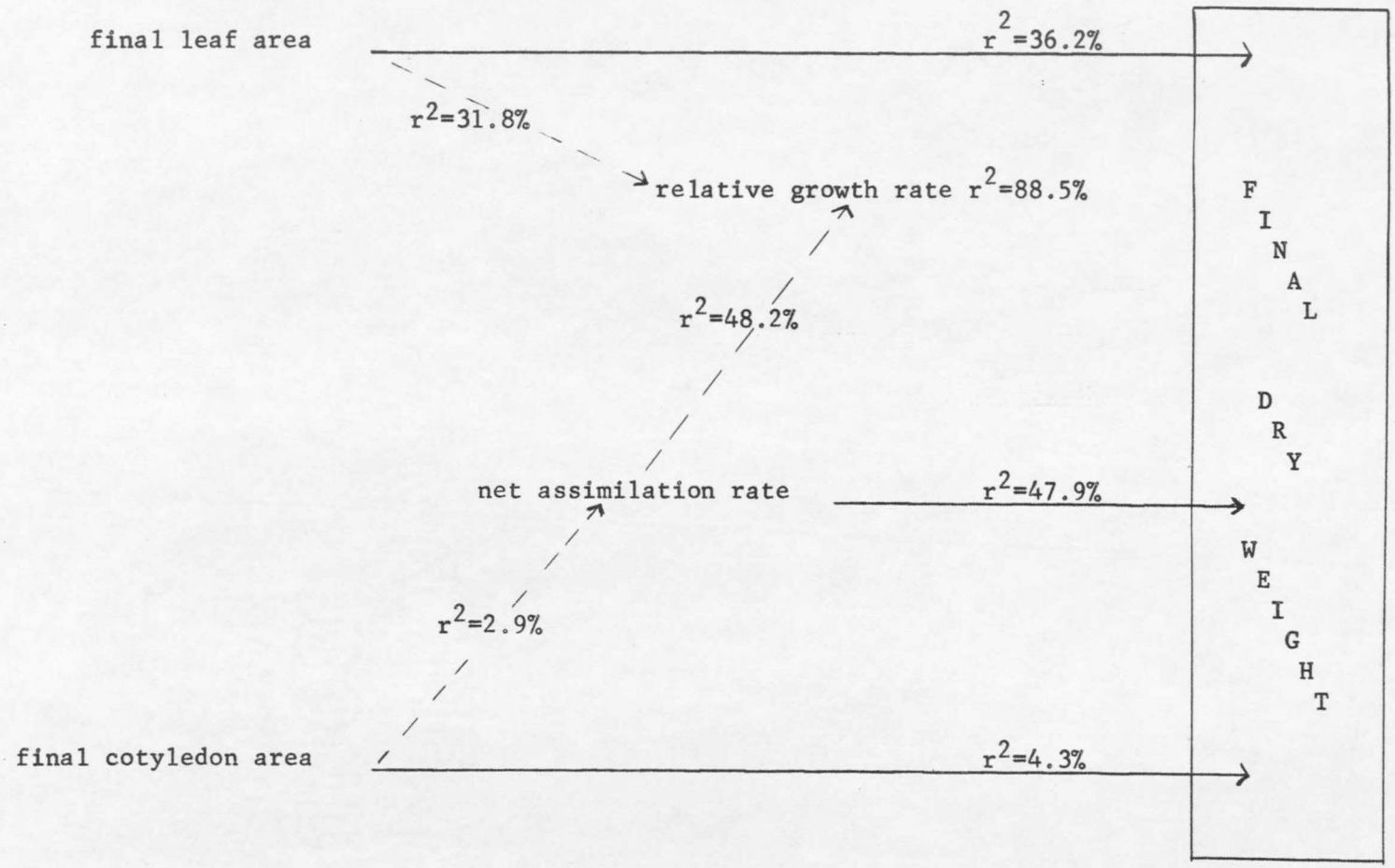
The ultimate goal, assuming that dry weight is an estimation of seedling vigor, was to determine those factors that contribute to dry matter accumulation in seedlings. The foregoing results and discussion have pointed out these factors. Table IX can be utilized to develop a sequence of events that will laad to greater seedling vigor. Figure 1 represents this growth sequence as a flow diagram. The coefficients of determination (r^2) are presented to give the reader an indication of the relative effect of one factor on the variation of the succeeding growth component and the combined effects of the several growth components.

Greater seed weight will produce a larger initial cotyledon area which in turn is affected by relative growth rate of cotyledon area to produce a larger final cotyledon area 3/. Cotyledon area did not affect leaf area in this study. An increased relative growth rate of leaf area results in a greater final leaf area. Although not a determinant of net assimilation rate, leaf area affects the relative growth rate (dry weight) and thereby contributes to seedling vigor. Superimposed upon the greater leaf area is net assimilation which influences the relative growth rate (dry weight). The

Table IX. A summary of relationships existing among various growth measurements in birdsfoot trefoil (Lotus corniculatus L.)



* significant at 0.05 probability level; ** significant at 0.01 probability level.



(coefficients of determination are for significant correlations only)

Figure 1. A schematic presentation of growth components contributing to seedling vigor in birdsfoot trefoil (Lotus corniculatus L.).

relative growth rate (dry weight) is the final determinant of seedling weight or vigor.

To obtain seedlings with maximum vigor, selection should be in such a manner as to combine maximum cotyledon area and maximum leaf area with maximum net assimilation rate. Selection for these three components of seedling growth could be more effective in attaining a vigorous birds-foot trefoil variety than selection for dry weight alone.

SUMMARY

Seeds of 120 randomly selected clones of Tana birdsfoot trefoil (Lotus corniculatus L.) were used to study some of the early vegetative growth traits. The measurements stressed were those which were expected to contribute to greater dry matter accumulation.

Ten seedlings were grown in a plastic pot for four weeks after emergence. They were harvested and roots were washed free of sand. Cotyledon and leaf areas were determined by enlarging with a projector, tracing and counting the squares occupied by the images. Entire seedlings were then oven dried and weighed.

Data on the following variables were analyzed by simple correlation:

1. one-hundred-seed weight of seeds produced in 1962 and 1963 and total seed yield per plant.
2. area of initial and final cotyledon, final leaf, total leaf.
3. final dry weight of seedlings.
4. relative growth rate of cotyledon area, leaf area, total leaf area, and dry weight.
5. net assimilation rate.
6. field stand in the fall and spring and percentage winter survival.

The cotyledon area accounted for 4.3 percent of the variation in dry weight. Leaf area accounted for 36.2 percent of the variation in dry weight, and net assimilation rate accounted for 47.9 percent of the variation in dry weight.

Leaf area was independent of cotyledon area and net assimilation rate was independent of leaf area. Leaf area and net assimilation rate accounted

for 31.8 and 48.2 percent, respectively, of the variation in relative growth rate (dry weight).

The effect of seed weight was obscured at four weeks after emergence.

It was concluded that selection for maximum cotyledon and leaf area, and net assimilation rate would be useful in developing greater seedling vigor in birdsfoot trefoil.

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