The effect of seed weight on photosynthetic area development and weight of the sainfoin (Onebrychis spp. Scop.) seedling
by Stephen Carl Fransen

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
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Abstract:

The hypothesis that cotyledon area to seed weight ratio might vary among sainfoin accessions was tested by comparing regression coefficients of area on weight. In addition, effects of seed weight upon areas and weights of cotyledons, first and second leaves and total seedlings were studied.

Ten accessions of sainfoin representing three different species were studied. After 17 days of growth in a growth chamber, seedlings of all accessions were sampled for the characteristics to be studied.

The ratios of cotyledon area to seed weight were similar for most accessions. Seedlings from heavier seeds emerged and developed more rapidly than seedlings from lighter seeds. Embryo axis length and width and leaf primordia length were all highly correlated with seed weight.

Areas and weights of cotyledons, first and second leaves were correlated with seed weight for most accessions. Total seedling area and weight, at 17 days of age, were also correlated with initial seed weight.

Trifoliolate seedlings had greater first leaf area but less second leaf area than unifoliolate seedlings. As a result, trifoliolate seedlings had only 3.5 percent more total leaf area than unifoliolate seedlings at 17 days.

These results help explain some of the variability among previous tests comparing unifoliolate and trifoliolate seedlings for seedling vigor.

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A thesis submitted in partial fulfillment
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ABSTRACT

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INTRODUCTION

Few investigations have been made of the cotyledon contribution to the forage legume seedling. In earlier work Cooper and Fransen (17) found that the sainfoin cotyledon was a major assimilatory organ during the growth of the young seedling. In other species, epigeal cotyledons vary in size and shape and in how long they remain functional. The question arose as to whether or not sainfoin accessions might vary in cotyledon area to seed weight ratio. If this were the case, seedling vigor may be enhanced because of a greater cotyledon area expansion.

The objectives of the present study were 1) to determine whether or not sainfoin accessions vary in their ability to distribute seed weight into cotyledon area; and 2) to determine the effect of seed weight on photosynthetic area development and seedling vigor.

LITERATURE REVIEW

Role of the Cotyledon

Cotyledons are the primary storage organs of most dicotyledonous seeds. Lipids and proteins, but primarily starch provides the stored energy used for germination and seedling growth.

Cotyledons of angiosperms occur either: 1) monocotyledons (one cotyledon) or 2) dicotyledons (two cotyledons). Dicotyledons are further divided into hypogeal and epigeal cotyledon types. The cotyledons of the hypogeal type, such as the runner bean (*Phaselous multiflorus* Lam.) and peas (*Pisum sativum* L.), remain below ground. The stored food reserves in the cotyledons provide energy for seedling growth while the leaves develop into photosynthetic organs. The cotyledons of epigeal types are above ground. Within the last 10 years the cotyledons of epigeal types have been recognized as photosynthetic organs (1). Lovell and Moore (31) found that cotyledons vary in size, shape, and duration of life. Longer lived epigeal cotyledons have higher area, higher net photosynthetic rate, and are more leaf-like in appearance. Plants with this type of cotyledons are cucumbers (*Cucumis sativa* L.) (32), soybeans (*Glycine max* L.) (46), and white mustard (*Sinapsis alba* L.) (31).

The cotyledons of some epigeal types such as the french bean (*Phaseolus vulgaris* L.) (35) do not expand and are short lived, which makes these cotyledons less important as photosynthetic organs. Sainfoin and most forage legumes have epigeal cotyledons which are longer lived than the french bean.

Cooper and Fransen (17), working with sainfoin, found that cotyledons provided 100% of the total seedling photosynthesis at seven days when cotyledons were first unfolded; 54% of the photosynthesis at 11 days when first leaves were unfolded; and 18% at 19 days when second leaves were unfolded. When cotyledon photosynthesis was compared to true leaf photosynthesis, on an area basis, true leaf photosynthesis was much greater after 9 days than cotyledon photosynthesis. Cotyledon photosynthesis declined with time and was attributed to cotyledon senescence. Sainfoin cotyledons of dark grown seedlings did not contain enough stored reserves to form a fully developed first leaf.

Sasaki and Kozlowski (41) working with pine seedlings, a gymnosperm, found that when cotyledon photosynthesis was impaired, primary needles failed to expand.

Hence, cotyledon photosynthesis for both gymnosperm and leguminous plants is imperative to provide for normal development of primary photosynthetic tissue.

Evidence exists that cotyledons produce substances that are transferred to the seedling which effects plant growth.

Moore (34) found that pea seedlings with excised (removed) cotyledons had a marked reduction in growth. Normal growth rate of these seedlings did not resume for 2 to 3 weeks. He also found that plant height of the first flowering node was reduced and anthesis delayed for 2 weeks.

Reduction in height of flowering node and in time of flowering are common phenomenon when cotyledons are excised (15, 24, 44). However, there is a conflict of opinions whether this phenomenon is an inhibitor or promoter effect. Barber and associates (3, 4, 43) have developed the theory that later flowering and reduced plant height of peas is due to the presence of an inhibitor which must be destroyed before flowering can take place. Through a series of experiments, Barber and associates showed that the inhibitor, called colysanthin, was formed in the cotyledons. When they grafted a late pea scion on an early stock, flowering was earlier than normal; but when an early scion was grafted on a late stock, the reverse was true. From these results, they postulated that colysanthin produced in the cotyledon affects flowering time. When cotyledons were excised from early and late varieties, lower flowering nodes were only found in late maturing varieties. This suggested that only late maturing varieties contained the inhibitor. Sprent (44) also found that early excision of cotyledons reduced flowering time for later varieties. These results confirm those of Johnston and Crowden (24) who showed that the inhibitor begins translocating from cotyledons about the 4th day of germination and continues to the 9th day. Sprent and Barber (43) completely removed the inhibitor within 14 days after germination by leaching. Thus, it is thought that the inhibitor is translocated from the cotyledons to the shoot apex and inhibits flowering.

Collins and Wilson (15) present another theory. They found that early cotyledon excision delayed flowering in both early and late pea varieties which was in contrast to the results of Barber *et al*. They postulated that the substance in the cotyledon which influences flowering is a promoter rather than an inhibitor. Cotyledon excision also decreased the number of leaves and leaf area expansion which could reduce plant growth. They feel that the interpretation of other research supporting the colysanthin theory can be reversed to support their promoter theory.

Structure and Senescence of Cotyledons

Scientists have studied the gross and fine structure of many different cotyledons from the initiation of germination to senescence (13, 46). However, investigations of cotyledon structure in forage legumes, including sainfoin, have not been reported.

Öpik (38) found large granules of stored reserve nutrients in the cytoplasm of mesophyll cells within non-meristematic tissue and in meristematic tissue within cotyledons of dry seeds. Because of these large granules, organelles were often obscured. These reserve nutrients are utilized quickly and in some species such as beans (37) cotyledons start shriveling at 5 days and the majority of cells are dead at 7 days after germination. In other species such as cucumbers, cotyledons may live for 35 to 50 days before senescing (13).

Cotyledons in seedlings of different species have many types of cells in common. Generally, all cotyledons have small epidermal cells which surround a layer of bulky parenchyma cells (37, 38), palisade cells (13, 46), mesophyll cells (13) and plastids (46).

Treffry *et al.* (46) found that cotyledon greening initially occurred in the adaxial palisade cells followed by cells adjacent to the abaxial epidermis and lastly in mesophyll cells. Mesophyll cells initially contain a cytoplasm filled with large deposits of stored material and no vacuoles (13). Plastids, chloroplasts and thylakoids develop and expand with time (46). Mitochondria numbers and activity increase as cristae become swollen (23, 36). Rough endoplasmic reticulum is abundant and Golgi bodies are present in some species (37) but not in others (49). Large vascular bundles are tightly packed between parenchyma and mesophyll cells (13, 37). Large numbers of protein and lipid bodies present (21) provide utilizable energy for seedlings of some crop species.

Cotyledons with a high fat content rapidly convert lipids to carbohydrates through the glyoxylate cycle (21,27). In these cotyledons, isocitratase activity is greater when seedlings are grown in the dark than in the light (1) and there is good evidence that isocitratase (20) and malate synthetase (30) may occur de nova. In watermelon seeds (*Citrullus vulgaris*, Schrad.), glyoxylate enzyme activity peaked at 4 to 5 days after germination and then declined as photosynthesis increased (25).

The digestion of stored food begins as soon as germination starts. Digestion first begins in the outer mesophyll cells and progresses towards the interior vascular bundles (37).

Cotyledon senescence begins as food reserves are utilized. At this time cotyledon water content has maximized and cotyledon dry weight and respiration decline dramatically (35, 37). Vacuoles appear and swell (13) and the mitochondrial matrix darkens (37). Finally, the tonoplast, plasma membrane, ribosomes, and chloroplasts break down leaving the nucleus with an irregular membrane and a few smaller cristae within the mitochondria (13).

Cotyledons turn different hues of yellow due to the breakdown of chlorophyll within chloroplasts and the buildup of carotenoids. Two predominant patterns of cotyledon yellowing during senescence have been found (33). "Marginal senescence" usually begins at the edge of one or both cotyledon lobes and progresses towards the base. "Basal senescense" usually begins close to the petiole and blade and spreads into the center of each lobe. An abscission layer forms between the cotyledon and the hypocotyl which allows cotyledons to shed.

Influence of Seed Weight on Seedling Vigor

The effect of seed weight on seedling vigor and stand establishment has been well documented. Kidd and West (26) in 1919 state that, "larger seeds give rise to more vigorous plants and a better yield." Seed size effects are most pronounced in small seeded forage species like birdsfoot trefoil (*Lotus corniculatus* L.). Henson and Tayman (22) and Strickler and Wassom (45) have shown a positive relationship between seed weight and seedling vigor of birdsfoot trefoil. Lin (29) also found similar results which he attributed to a greater amount of cotyledon area from larger seed. Carleton and Cooper (14) found that seed size of birdsfoot trefoil, alfalfa and sainfoin was highly correlated with seedling performance. Twanley (47, 48) suggests that seed size of birdsfoot trefoil can be increased through breeding. The larger seed size should allow this small seeded species to compete better during stand establishment. Shibles and MacDonald (42) and Cooper and Qualls (16) have shown differences in growth of seedlings grown from seeds of cultivars processed to obtain the same seed size. They attribute these differences to more efficient metabolism and conversion of photosynthate into leaf area. Thus, factors other than seed size influence seedling and plant growth. Ashby (2) found that larger embryos and not relative growth rates were responsible for differences in corn heterosis. This result substantiated Blackman's (12) beliefs that plant weight depends on "initial capital" (seed size) since "rate of interest" (growth rate) is the same. Pedersen and Barnes (40) working with alfalfa, believe size of hybrid seed may be due to varying degrees of heterosis.

The effect of seed size and depth of seeding on emergence have been thoroughly investigated (6, 7, 8, 9, 10). Erickson (19) found that alfalfa plants from small seed would catch up in growth with plants from larger seed after a four-month period. Beveridge and Wilsie (5) however state that, "stands from large seed would have a better chance of survival under adverse growing conditions or management."

Black (6, 7, 8, 9, 10, 11), through a series of classical experiments, has demonstrated the importance of seed weight in relation to emergence and seedling vigor of subterranean clover. He found that seed weight influenced the growth of the young seedling until the 8th leaf. By decreasing seed size and increasing depth of planting, cotyledon weight was reduced at emergence. Seed from the smallest seed size would not emerge from a depth of 2 inches, whereas, large seed would. He states that cotyledon area and not weight is the important factor at emergence and that cotyledon reserves remaining at emergence do not affect subsequent growth. Cooper and Fransen (17), however, found that sainfoin grown in the dark did not have adequate reserves to produce a first leaf. In their work maximum cotyledon photosynthetic rate did not occur until cotyledon reserves were utilized. Black (8) also reports that while cotyledon area is affected by seed size, it is not affected by depth of planting.

The effect of seed size is important in terms of seedling competition. Black (10) grew equal numbers of seedlings from large and small legume seed in a mixed sward. From this data he calculated that at a 20 cm plant height, 75 percent of the leaf area was from seedlings with large seed and only 25 percent from small seeded plants. He states that if 100 units of light energy are measured at 20 cm and 20 units at 18 cm, then large seeded plants absorbed 60 units and small seeded plants 20 units. He attributes the greater competitive ability of large seeded plants to their shading of small seeded plants.

From the foregoing discussion, it is apparent that seed size is important to total germination, emergence, and development of the seedling and its competitive ability. Seed size is a major factor in forage seedling establishment.

MATERIALS AND METHODS

Ten accessions of *Onobrychis species* were chosen to represent ranges of seed weight, origin and species (Table 1). Seed from each accession was screened into four distinct seed size groups ranging from small to large. From each of the forty groups (10 accessions x 4 seed sizes), two lots of 50 seeds were randomly selected and weighed. Seed lots were then treated with the seed fungicide, Ceresan, and planted 1.27 cm deep in greenhouse flats filled with plaster grade vermiculite. A completely random design with two replications was used. The plants were grown in a growth chamber at 20± 1 C with a 16-hour photoperiod. Seedlings were watered every other day with a complete nutrient solution and tap water was added as needed. On the fourth, fifth, sixth and seventh days after emergence, seedlings were counted and their stage of development recorded. After 17 days, seedlings were removed from the flats and the vermiculite washed free from the roots. Seedlings were separated into two groups, one with unifoliolate first leaves and another with trifoliolate first leaves. From each group the cotyledons, and first, second and third leaves were removed and their area (cm^2) measured with a Hashi-Denkyo leaf area meter. The photosynthetic components and the axis

Table 1. Accession numbers, weight per 100 seeds, range of seed weights, geographical origin and sainfoin species studied[a].

Species	Lethbridge Accession	g/100 Seeds	Range of Seed Weight mg/seed	Origin
O. viciaefolia	L-1904	2.50	12.1 - 25.8	Poland
O. viciaefolia	L-1875	2.48	10.0 - 21.0	Greece
O. viciaefolia	L-1799	2.58	12.2 - 23.9	Turkey
O. viciaefolia	L-1800	2.13	9.7 - 18.4	Turkey
O. viciaefolia	L-1919	2.62	15.3 - 24.5	Hungary
O. transcaucasia	L-1770	2.75	12.7 - 25.0	U.S.S.R.
O. transcaucasia	L-1805	2.71	10.8 - 23.8	U.S.S.R.
O. transcaucasia	L-1834	2.70	13.6 - 23.0	U.S.S.R.
O. arenaria	L-1842	2.05	8.9 - 18.2	U.S.S.R.
O. arenaria	L-1762	1.78	9.0 - 18.3	U.S.S.R.

[a] Seed harvested at Lethbridge, Alberta, in 1971.

(roots and petiole) were bagged separately, oven dried for 48 hours at 70 C, and weighed to the nearest 0.1 mg.

Values obtained from each seed lot were divided by the number within them to reduce data to an individual seedling basis. Simple correlations and regressions of areas and weights of cotyledons, first and second leaves, and total seedling leaf area and weight were estimated for each accession. In addition, correlations and regressions were estimated for these traits for unifoliolate and trifoliolate first leaf seedlings pooled over seed size classes, accessions and replications. All correlation coefficients were tested for statistical significance using an approximate T test based on N-2 degrees of freedom. Where T was significant, b values are also assumed to be significant. Estimates of standard errors of b values were used to compare pairs of b values. Additionally, a paired T test was employed to compare the two leaf types summed over seed size classes, accessions and replications.

Eighty-one individual seeds from L-1762 were selected to represent a range in seed weights of 2.7 to 24.6 mg. Seeds were oven dried for 24 hours at 40 C to insure uniform moisture. After drying, each seed was individually weighed to the nearest 0.1 mg. Seeds were placed in distilled water to allow the seed coat to soften. Seed coats were surgically removed and cotyledons separated to expose the embryo axis. The embryo axis was removed from the cotyledons and the length and width of the embryo and length of the leaf primordia measured ocularly in microns at 20 X.

Simple correlation and regression coefficients were used to determine relationships of embryo axis measurements on seed weight. Standard analysis of variance methods and the F test were employed to analyze emergence data.

RESULTS AND DISCUSSION

Seedlings from heavier seeds emerged, and developed first leaves more rapidly than seedlings from lighter seeds (Table 2). On the 4th day after seeding percent plants emerged from the lightest and heaviest seeds were 22.4 and 54.0, respectively. Greater seed weights gave greater plant emergence through the 5th and 6th days. First leaf buds began to appear on the 6th day and their development was proportional to seed weight. On the 7th day, seedlings from the heaviest seed had the lowest percent of folded first leaves. Black (8) also observed more rapid development of seedlings from heavier seed in subterranean clover.

The more rapid development of seedlings from the heavier seed may be due to 1) greater amounts of stored food reserves, 2) larger embryos, 3) more physiologically mature embryos, or 4) a combination of those. Embryo axis length and width and leaf primordia length were all highly correlated with seed weight (Figures 1, 2, 3). However, embryo axis appeared to be immature when seeds weighed less than 5 mg. Embryo axis length and width increased 5.27 and 1.70 microns, respectively, for each mg increase in seed weight. Leaf primordia length increased 1.86 microns for each mg increase in seed weight. These results were surprising since one might expect embryo length to maximize rapidly and that

Table 2. Phenological stage of development of sainfoin seedlings[a] during early growth.

		Days From Beginning Germination					
		4	5	6		7	
Seed Size Class[b]	Weight (mg)[c]	% Plants Emerged	% Plants Emerged	% Plants Emerged	% First Leaf Buds Showing	% First Leaves Folded	% First Leaves Unfolded
1	11.8	22.4	45.6	61.8	16.8	45.6	6.8
2	15.2	28.8	56.2	73.8	28.8	51.2	15.2
3	18.4	41.0	67.4	75.6	37.6	54.8	19.2
4	22.0	54.0	71.0	79.8	52.4	37.0	32.4

[a] Values in the table represent averages of all ten accessions.
[b] Seed size ranked from small to large.
[c] Mean seed weight for all ten accessions for each seed size class.

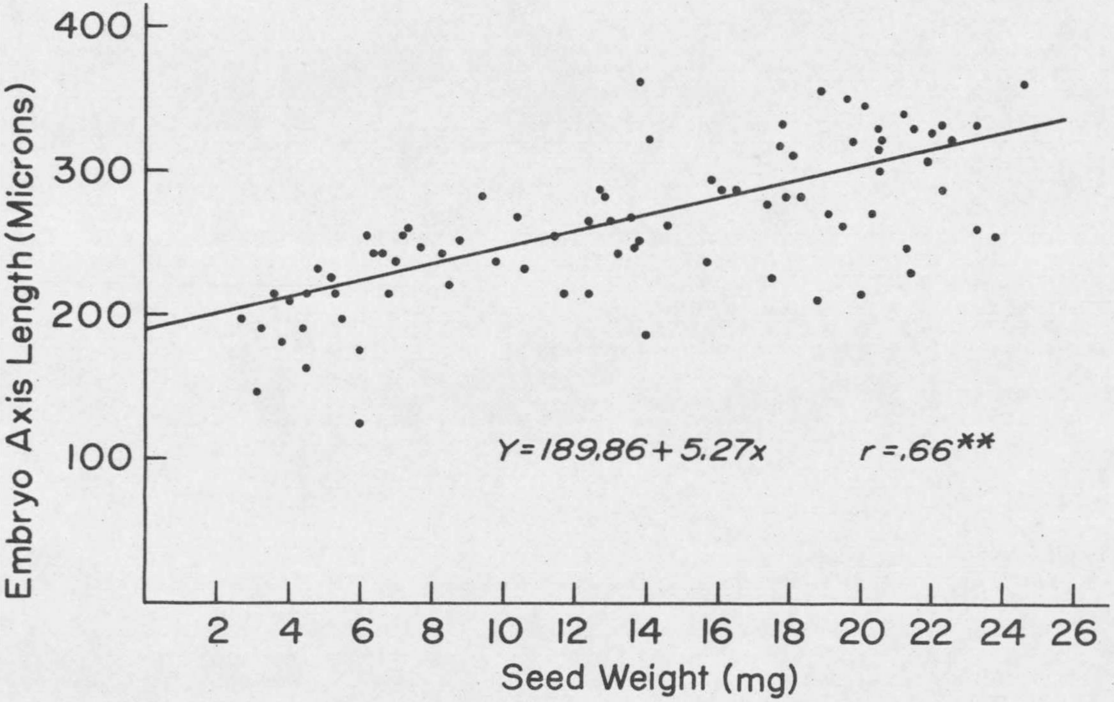

Figure 1. Relationship of embryo axis length to seed weight in sainfoin.

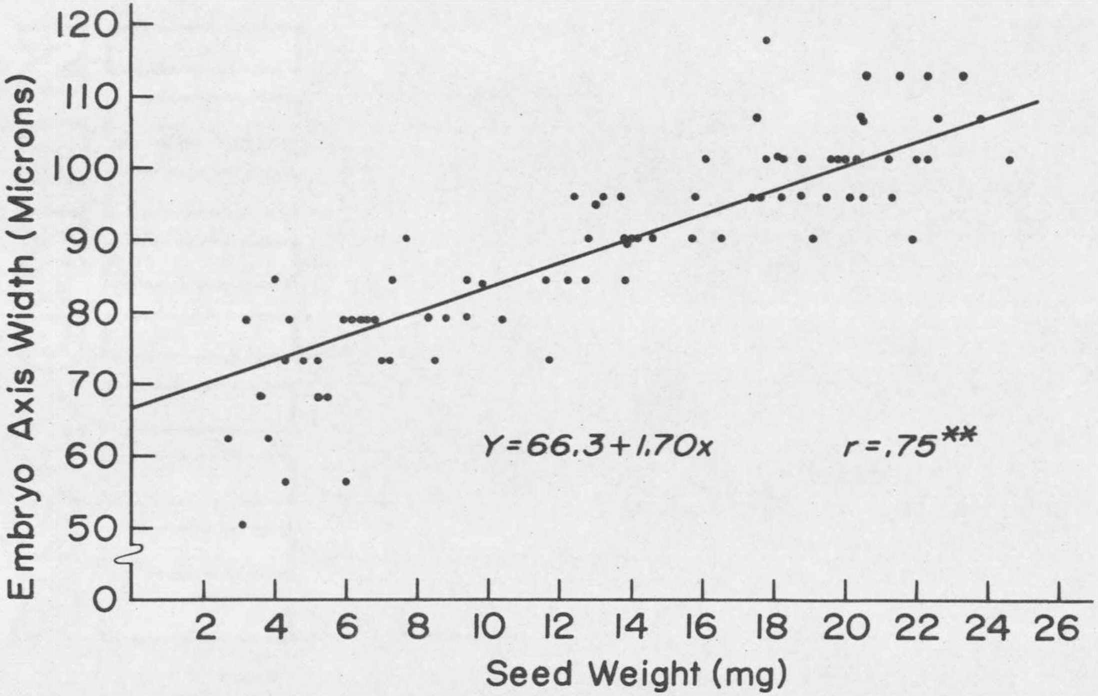

Figure 2. Relationship of embryo axis width to seed weight in sainfoin.

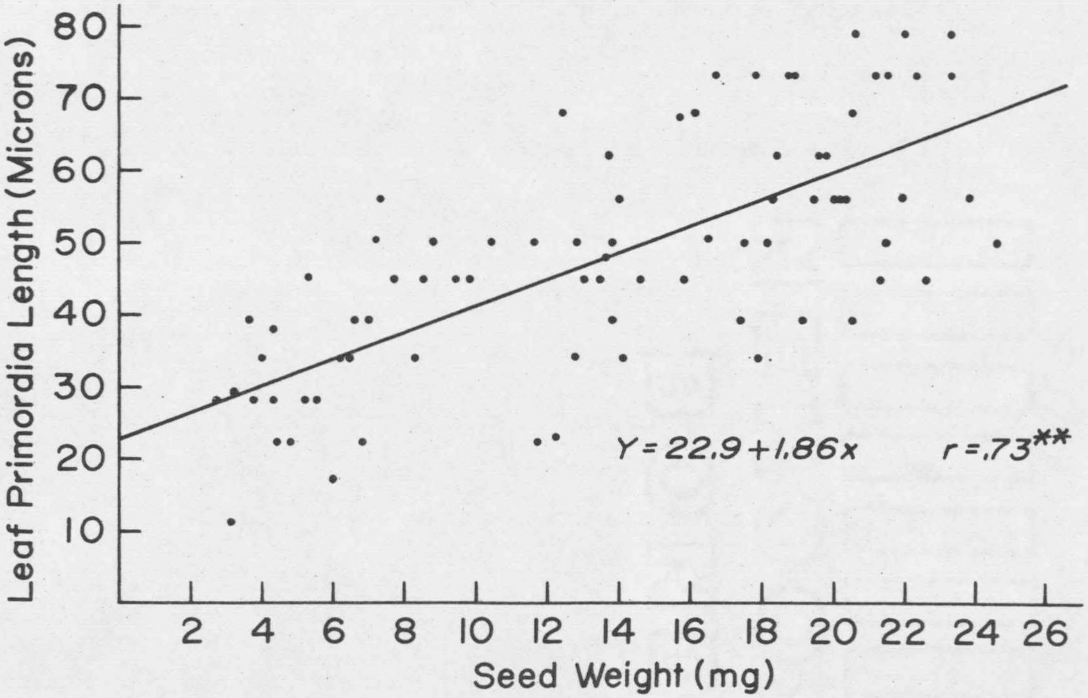

Figure 3. Relationship of leaf primordia length to seed weight in sainfoin.

additional seed weight increase would be due to greater food reserve storage in the cotyledons. These data suggest a linear relationship between the size of embryo parts and seed size. The greater embryo size appears to contribute to more rapid emergence and development. Photographs of the embryo axis alone and attached to one cotyledon are shown in Plates 1, 2, 3 and 4.

The importance of cotyledon photosynthesis on seedling vigor of sainfoin was shown by Cooper and Fransen (17). Black (9) stated the major advantage of large seed is the greater photosynthetic area of the cotyledons. In this study, cotyledon area was correlated with seed weight in all accessions (Table 3). A major hypothesis of this study was that accessions may differ in their relationship of seed weight to cotyledon area. If so, it would then be possible to determine which cotyledons were more efficient (on an area basis) and have a greater amount of net photosynthesis. The regression coefficients of cotyledon area on seed weight differed only between two accessions (Table 3). Poor germination and emergence of L-1800 resulted in a very small sample size, thus the reliability of the data obtained from this accession may be questionable. Black (7) working with

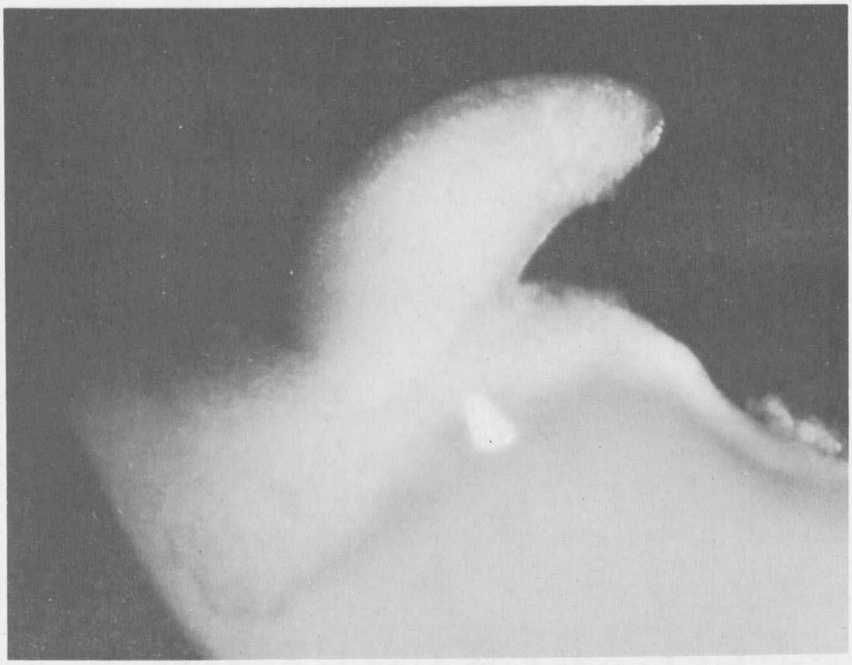

Plate 1. Primordia of a unifoliolate leaf of sainfoin.

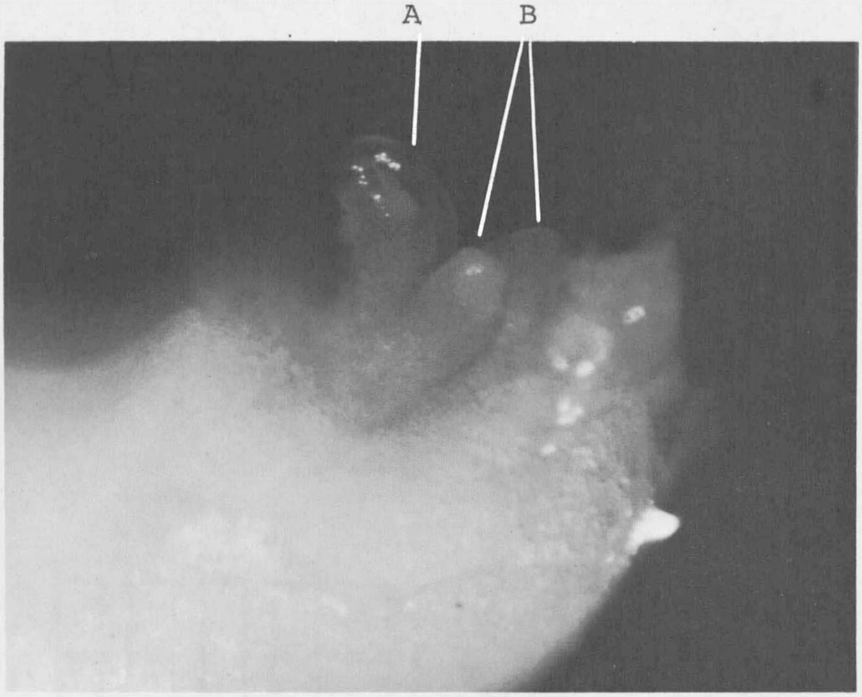

Plate 2. Primordia of a trifoliolate leaf of sainfoin showing (A) central leaflet (B) lateral leaflets.

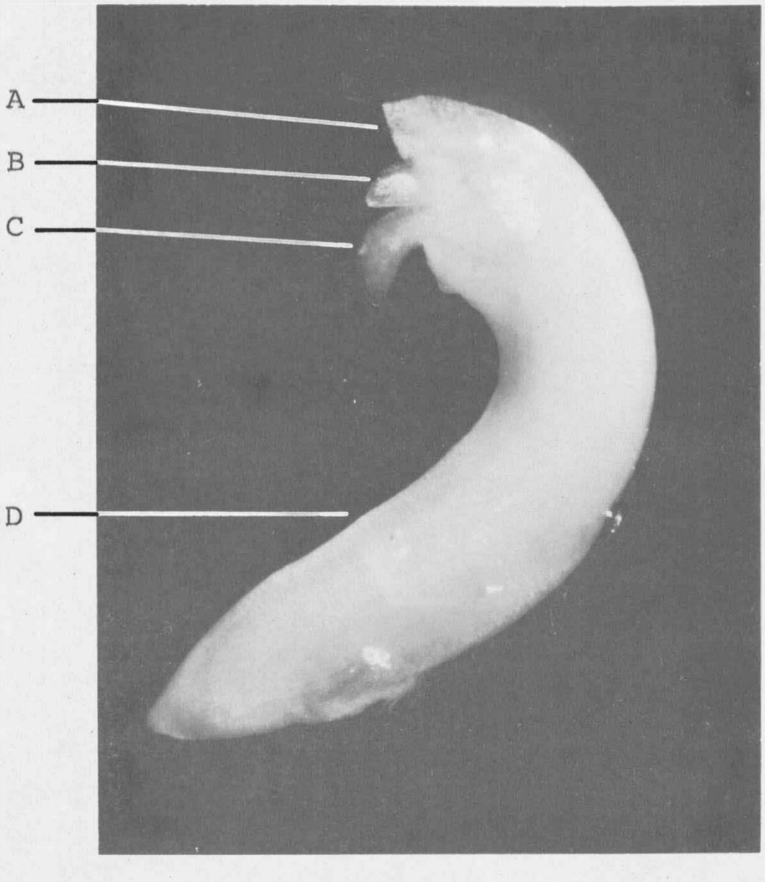

Plate 3. Excised embryo axis of sainfoin showing (A) cotyledon scar (B) lateral leaflets (C) central leaflet (D) radical.

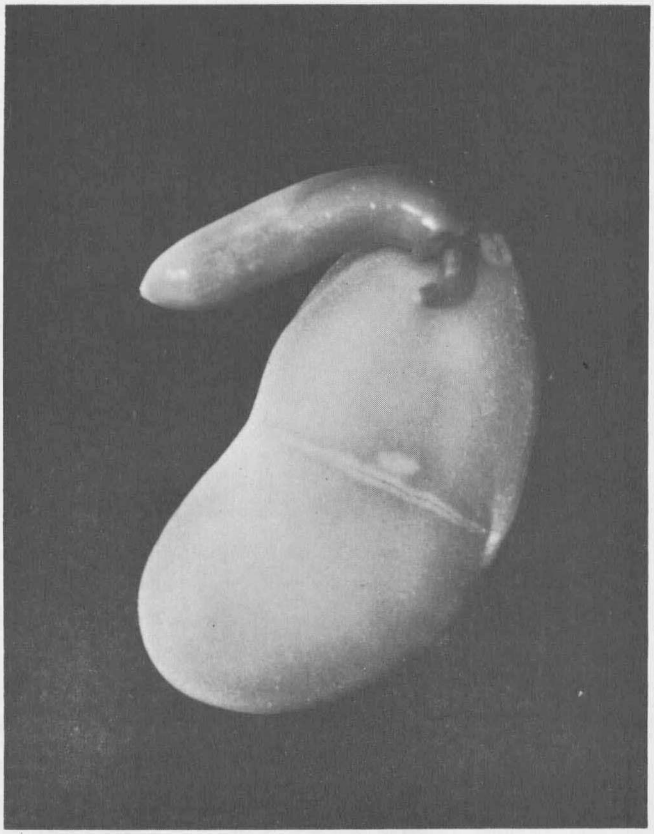

Plate 4. Embryo axis and cotyledon after staining for 30 minutes with a 1% aqueous solution of tetrazolium chloride.

Table 3. Coefficients of determination (r^2)[a] and regression coefficients (b)[b] of cotyledon area, weight and specific cotyledon weight (SCW) on seed weight for seedlings at 17 days of age.

Species	Accession Number	Cotyledon Measurements					
		Area (cm^2)		Weight (mg)		SCW (mg/cm^2)	
		r^2	b	r^2	b	r^2	b
O. viciaefolia	L-1904	.88 **	.031	.92 **	.201	.48	.050
O. viciaefolia	L-1875	.67 *	.029	.79 **	.264	.51 *	.119
O. viciaefolia	L-1799	.90 **	.034	.86 **	.195	.64 *	.041
O. viciaefolia	L-1800	.59 *	.017 *	.70 **	.132	.42	.048
O. viciaefolia	L-1919	.68 *	.039	.85 **	.220	.43	.042
O. transcaucasia	L-1770	.90 **	.039	.40	.162	.00	-.004
O. transcaucasia	L-1805	.77 **	.032	.36	.126	.00	.003
O. transcaucasia	L-1834	.66 *	.041	.85 **	.233	.68 *	.050
O. arenaria	L-1842	.72 **	.032	.81 **	.204	.69 **	.073
O. arenaria	L-1762	.92 **	.049	.90 **	.224	.35	.040

a. Coefficients of determination (r^2) followed by a single asterisk had correlation coefficients which were significant at the 5% level of probability and those followed by a double asterisk had correlation coefficients which were significant at the 1% level of probability.

b. Regression coefficients (b) followed by an asterisk are significantly different from the highest b value within a column (underlined) at the 5% level of probability.

subterranean clover and Lin (29) working with birdsfoot trefoil found a constant cotyledon area was produced for each unit of seed weight.

Cotyledon weight at 17 days of age was proportional to seed weight in all but two accessions (Table 3). Both accessions were *O. transcaucasia* species. Since cotyledon reserves are exhausted at nine days of age (17), weights of cotyledons at 17 days represent the skeleton of the cotyledon, and do not reflect differences which may have originally occurred in stored material. The regression coefficients of cotyledon weight on seed weight did not differ among accessions.

Specific cotyledon weight (SCW, mg/cm^2) was correlated with seed size for four accessions (Table 3). The regression coefficients of SCW on seed weight did not differ among accessions. Thus, no differences in cotyledon thickness among accessions were found.

First leaf weights and areas were correlated with seed weight for six accessions (Table 4). The significant correlation of leaf area with seed weight was mostly due to the more rapid development of seedlings from heavier seed. Leaves in this study had not become fully expanded by the 17th day. However, one would expect that seedlings from

Table 4. Coefficients of determination (r^2)[a] and regression coefficients (b)[b] of first leaf area, weight and specific leaf weight (SLW) on seed weight for seedlings at 17 days of age.

		First Leaf Measurements					
	Accession	Area (cm^2)		Weight (mg)		SLW (mg/cm^2)	
Species	Number	r^2	b	r^2	b	r^2	b
O. viciaefolia	L-1904	.27	.014 *	.06	.023 *	.04	-.011
O. viciaefolia	L-1875	.59 *	.050	.67 *	.130	.00	-.003
O. viciaefolia	L-1799	.51 *	.048	.32	.067 *	.08	-.036
O. viciaefolia	L-1800	.20	.030 *	.62 *	.162	.12	.042
O. viciaefolia	L-1919	.70 **	.053	.64 *	.195	.22	.021
O. transcaucasia	L-1770	.53 *	.052	.76 **	.212	.14	.031
O. transcaucasia	L-1805	.35	.032 *	.09	.085 *	.00	.003
O. transcaucasia	L-1834	.00	.001 *	.32	.026 *	.22	.114
O. arenaria	L-1842	.74 **	.082	.81 **	.260	.06	.021
O. arenaria	L-1762	.81 **	.110	.74 **	.342	.01	.008

a. Coefficients of determination (r^2) followed by a single asterisk had correlation coefficients which were significant at the 5% level of probability, and those followed by a double asterisk had correlation coefficients which were significant at the 1% level of probability.

b. Regression coefficients (b) followed by an asterisk are significantly different from the highest b valuewithin a column (underlined) at the highest level of probability.

heavier seed would produce larger leaves. Black (8) has shown that seed weight has a positive effect on leaf area through the 8th leaf in subterranean clover.

Specific leaf weight (SLW, mg/cm^2) of first leaves was not correlated with seed weight for any accession. Regression coefficients of specific first leaf weights on seed weight did not differ among accessions (Table 4).

Second leaf areas and weights were correlated with seed weight for most accessions (Table 5), but only two regression coefficients of second leaf weight on seed weight were significant. Second leaf specific weights were not significantly correlated and not significantly regressed upon seed weight (Table 5).

Total leaf areas and total leaf weights were each significantly correlated with seed weight for all but one accession at 17 days of age (Table 6). Regression coefficients of total leaf area or total leaf weight on seed weight did not differ among accessions (Table 6).

Areas and weights of cotyledons and leaves were correlated with seed weight for most accessions, but regression coefficients among accessions differed in only a few cases. For a more precise estimate of relationships (cotyledon, first, second and total leaf area) of growth of a sainfoin

Table 5. Coefficients of determination (r^2)[a] and regression coefficients (b)[b] of second leaf area, weight and specific leaf weight (SLW) on seed weight for seedlings at 17 days of age.

		Second Leaf Measurements					
	Accession	Area (cm^2)		Weight (mg)		SLW (mg/cm^2)	
Species	Number	r^2	b	r^2	b	r^2	b
O. viciaefolia	L-1904	.63 *	.075	.68 *	.209 *	.01	.003
O. viciaefolia	L-1875	.69 **	.084	.68 *	.266	.15	.022
O. viciaefolia	L-1799	.61 *	.154	.86 **	.401	.02	-.011
O. viciaefolia	L-1800	.00	.006	.04	.059 *	.04	.018
O. viciaefolia	L-1919	.72 **	.127	.77 **	.402	.11	.019
O. transcaucasia	L-1770	.45	.055	.72 **	.362	.47	.071
O. transcaucasia	L-1805	.88 **	.107	.83 **	.337	.04	.014
O. transcaucasia	L-1834	.88 **	.149	.85 **	.467	.08	.018
O. arenaria	L-1842	.65 *	.124	.81 **	.396	.27	.031
O. arenaria	L-1762	.47	.143	.55 *	.425	.01	.010

a. Coefficients of determination (r^2) followed by a single asterisk had correlation coefficients which were significant at the 5% level of probability and those followed by a double asterisk had correlation coefficients which were significant at the 1% level of probability.

b. Regression coefficients (b) followed by an asterisk are significantly different from the highest b value within a column (underlined) at the 5% level of probability.

Table 6. Coefficients of determination $(r^2)^a$ and regression coefficients $(b)^b$ of total leaf area and total leaf weight on seed weight for seedlings at 17 days of age.

		Total Leaf Measurements			
		Area (cm^2)		Weight (mg)	
Species	Accession Number	r^2	b	r^2	b
O. viciaefolia	L-1904	.84 **	.305	.80 **	1.769
O. viciaefolia	L-1875	.83 **	.248	.71 **	2.086
O. viciaefolia	L-1799	.85 **	.442	.78 **	2.524
O. viciaefolia	L-1800	.05	.055 *	.21	1.233
O. viciaefolia	L-1919	.74 **	.414	.51 *	2.545
O. transcaucasia	L-1770	.72 **	.272	.67 *	2.230
O. transcaucasia	L-1805	.83 **	.289	.50 **	1.822
O. transcaucasia	L-1834	.79 **	.368	.59 *	2.348
O. arenaria	L-1842	.67 *	.281	.66 *	2.228
O. arenaria	L-1762	.72 **	.372	.80 **	2.656

[a] Coefficients of determination (r^2) followed by a single asterisk had correlation coefficients which were significant at the 5% level of probability, and those followed by a double asterisk had correlation coefficients which were significant at the 1% level of probability.

[b] Regression coefficients (b) followed by an asterisk are significantly different from the highest b value within a column (underlined) at the 5% level of probability.

seedlings to seed weight, data was pooled across accessions (Figure 4). These relationships were separated for seedlings with unifoliolate and trifoliolate first leaves.

The regression of unifoliolate and trifoliolate cotyledon area, first and second leaf area on seed weight were all positive and the correlation coefficients were highly significant (Figure 4). Slopes of regression lines for each component did not differ between leaf types. Average cotyledon areas were 1.31 and 1.34 cm^2 for unifoliolate and trifoliolate seedlings, respectively. For each mg increase in seed weight cotyledon area increased .03 cm^2. Trifoliolate seedlings had significantly larger first leaf area (2.27 cm^2) than unifoliolate seedlings (1.54 cm^2). First leaf area of trifoliolate and unifoliolate seedlings increased .066 cm^2 and .033 cm^2, respectively, for each mg increase of seed weight. Unifoliolate seedlings had larger second leaf area (2.83 cm^2) than second leaves from trifoliolate seedlings (2.53 cm^2). Trifoliolate seedlings had significantly more (3.5 percent) total leaf area than unifoliolate seedlings. Total seedling weights among unifoliolate and trifoliolate seedlings did not differ at 17 days of age. Frequency of unifoliolate or trifoliolate first leaf seedlings was not affected by seed size.

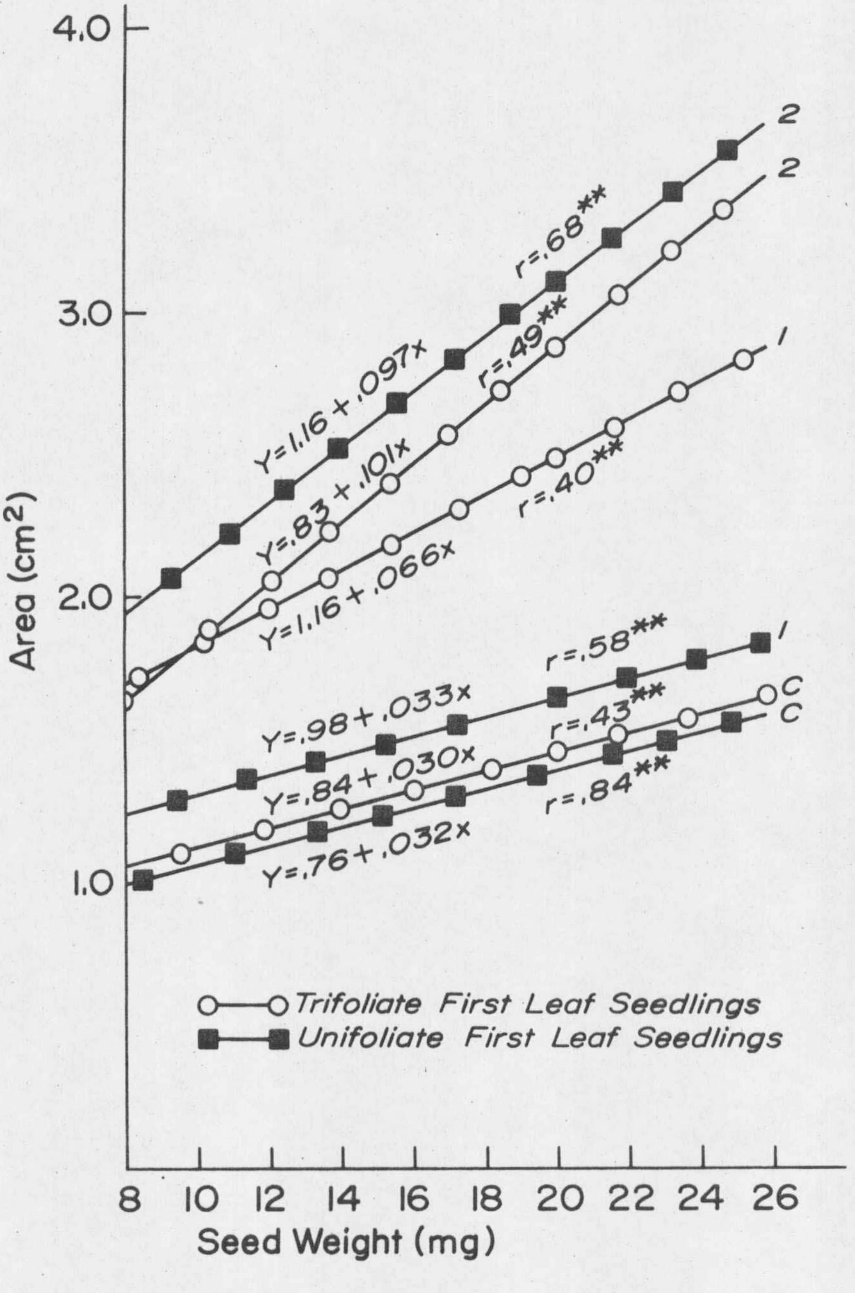

Figure 4. The effect of seed weight upon area of cotyledons (c), first (1) and second (2) leaves of sainfoin seedlings with unifoliolate and trifoliolate first leaves.

Variable results have been obtained in previous studies concerning the effect of unifoliolate and trifoliolate first leaves on seedling vigor (17, 18). These results have been perplexing since one would expect the larger first leaf area of trifoliolate seedlings to always result in more vigor. The data presented here help explain some of this variability. Since second leaves of unifoliolate seedlings are larger than second leaves of trifoliolate seedlings, it appears that the larger second leaves of unifoliolate seedlings tend to nullify the advantage of the larger first leaf of the trifoliolate seedling.

The primary hypothesis in this study was that the cotyledon area to seed weight ratio might vary among sainfoin accessions. If this were the case, one might select for accessions with greater cotyledon expansion and thus enhance seedling vigor. These data suggest, however, that large variation among accession tested does not exist. However had the world collection been tested, some accessions may have been found with greater cotyledon area expansion. Similar results were found for birdsfoot trefoil by Lin (29) and subterranean clover by Black (8). Thus, it would appear that within a species the ratio of cotyledon area to seed weight is nearly constant. However, this ratio varies immensely among species. Lin (29) regressed cotyledon area (mm^2) on seed weight (mg) and obtained regression coefficients of 14.0, 10.1 and 3.5 for alfalfa, birdsfoot trefoil and sainfoin, respectively. In the present study the regression coefficients of cotyledon area (mm^2) on seed weight (mg) of 3.2 and 3.0 were found for seedlings with unifoliolate and trifoliolate first leaves, respectively. These results are similar to those regression coefficients of Lin's for sainfoin. An estimate of the regression of cotyledon area on seed weight from Black's work with subterranean clover (8) would appear to be 1.5 mm^2/mg. It would appear that regression coefficients for cotyledon area on seed weight are smaller for larger seeded species. Thus, seed weight should be of greatest importance for small seeded species.

In this study, seed weight had a major effect upon development and size of seedlings. The size of the embryo axis and leaf primordia of dry seeds and of fully expanded cotyledons was highly correlated with seed weight. Black (8) has stated that the primary advantage of large seed is the increased photosynthesis from the larger cotyledons. Ashby (2) concluded that "in certain strains of corn, size heterosis operates through an increase in the biological capital in the embryo and not through a greater relative growth rate." Pedersen and Barnes (40) believe that the size of hybrid seed on a given alfalfa plant may be due to varying degrees of heterosis and suggest that the relationship between specific combining ability for seed weight and forage yield should be investigated. Cooper and Qualls (16) and Shibles and MacDonald (43) have shown marked differences of trefoil seedlings grown from cultivars processed to obtain the same size. These differences seemed to be related to more efficient metabolism and to greater ability to partition photosynthate into leaf area (16). Carleton and Cooper (14) state that differences in size among plants may be correlated with seedling performance in some species but not in others. Differences in seed weight within plants are highly correlated with seedling performance.

This study has not shown that the primary factor affecting seedling growth is not a more physiologically mature embryo or greater stored material. Investigations of growth from different sized embryos with cotyledons excised and with energy supplied from nutrient media might help to explain whether embryo size and maturity or quantity of food reserves is the main factor in early seedling growth.

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