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To the Graduate Council:

I am submitting herewith a thesis written by Timothy Pitts Campbell entitled "Variability of the Levels of P, K, Ca and Mg in Annual Lespedeza." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

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VARIABILITY OF THE LEVELS OF P, K, Ca
AND Mg IN ANNUAL LESPEDEZA

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

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ABSTRACT

Six cultivars and four breeding lines of annual lespedeza (Lespedeza striata, L. stipulacea L.) were grown during the summer of 1971 at Knoxville, Crossville, and Martin, Tennessee, to evaluate the possibility of inherited variability in accumulation of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). Whole-plant samples were harvested, in some cases for the three stages of growth approximating prebloom, full bloom, and early seed formation. Phosphorus, K, Ca, and Mg concentrations were determined from the nitric-perchloric acid digestate using the Technicon Autoanalyzer.

Concentrations of P, K, Ca, and Mg were found to be significantly different among genotypes revealing the existence of genetic mechanisms controlling accumulation levels of these elements in the annual lespedeza species. The type of results obtained led to speculation that the genetic control mechanisms, at least for P and Ca, are multigenic.

A significant location x genotype interaction occurred indicating that differences in concentrations among genotypes were altered by the effect of different environments upon the expression of the genetic mechanisms controlling concentration.

Generally, K, Ca, and Mg concentrations decreased as the plant matured, but P seemed to remain relatively constant.

Commercial Korean was relatively high in P, Ca, and Mg but medium in K. Ark 226-10 was low in relative concentration for all four elements. Kobe was low in relative concentration of P and K but high in Ca and Mg.

Generally, a positive relationship existed between P and K, and between Ca and Mg. Potassium was negatively correlated with Ca and with Mg.

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CHAPTER I

INTRODUCTION

A better understanding of plant nutrition, fertilization, and soil-plant relationships requires knowledge of the variability of chemical element accumulation within the plant. Many workers have reported on investigations of tissue concentrations of the macronutrient and many of the micronutrient elements in plants. From these investigations has come knowledge of factors which affect or control concentration levels in different plant species and within species. Variability with respect to chemical element concentration within the plant and with time over the life cycle of the plant, as well as the interaction of the two has become known to be important. The influence of genotype is also important.

One factor which has been recognized in recent years is the genetic control of element accumulation in plant tissues. Research has shown that genetic mechanisms exist and have a great influence upon the variability of accumulation levels in different crop species.

The major objective of this study was to evaluate the possibility of inherited variability in accumulation of chemical elements by annual lespedezas (Lespedeza striata, L. stipulacea L.). Several cultivars and breeding lines were selected to be analyzed for phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) to determine the variability in concentration of these elements, and to gain some insight into the genetics controlling this variability if it does exist.

The application of genetically controlled variability in accumulation of chemical elements would depend upon the relationship of this variability to yield. If plants which are high accumulators of particular elements produce more dry matter, high accumulation might be desirable. However, if the high accumulators do not yield better, greater efficiency would be obtained by using cultivars with low accumulation potential.

A related potential application of genetic control of concentrations is in developing cultivars which alleviate the problem of "luxury consumption" of elements. Another approach might involve the use of "luxury consumers" for soil improvement in areas where toxic levels of elements exist. Reduction of the levels of these elements through crop removal would perhaps create more favorable soil conditions for other crops.

Controlling element concentrations through genetic mechanisms would perhaps not only result in better utilization of added or existing fertility but at the same time result in better meeting the nutritional requirements of animals that consume the plants.

Once evidence of genetic mechanisms controlling tissue concentrations is established, further research to more clearly define these mechanisms in existing cultivars will enable the plant breeder to better utilize existing cultivars or further the development of improved cultivars.

CHAPTER II

LITERATURE REVIEW

Hensen and Cope (20) recognized that annual lespedezas are of major importance in the eastern half of the United States as forage crops, for midsummer and early fall grazing, for soil improvement, and for hay and seed production. From 1951 to 1960, 4.89 million acres were grown annually for forage and seed.

The quality of the food consumed is an important factor in the nutritional status of both man and animals. According to Beeson (5) nutritional diseases of animals which have been traced to soil characteristics may be divided into two classes. In the first class, which is geographically more important, are those due to a nutritional deficiency of one or more of the essential elements. These deficiencies may produce bone maladies, anemias, goiter, and related diseases. The second class includes those diseases resulting from excesses of certain minerals in soils. An example is selenium (Se) toxicity.

In 1946, Beeson (6) stated that the mineral composition of plants is a function of many factors such as differences in soils, use of soil amendments or fertilizers, and rainfall and other climatic influences. He stated that differences in these factors will operate to modify the mineral composition of the plant in different ways.

The effect of adding or varying fertility upon the growth and mineral composition of plants has been investigated by many workers. Working with Korean lespedeza on less fertile soils, Albrecht and

Klemme (1) suggested that it could not reach its highest efficiency without soil treatments. They suggested that limestone and phosphate used together may increase yield and protein content by mobilizing the P.

Foote and Jackobs (10) investigated the response of partridge pea (Cassia fasciculata Mich.) and Korean lespedeza to two rates of P fertilization on five soil types at four plant populations. Partridge pea was able to tolerate lower soil P levels, more adverse physical soil conditions, and extremely low and high plant populations with less adverse effect on yield. It responded more to added P on soils with adverse physical conditions and low natural levels of available P. Korean lespedeza responded more to added P on soil which had good physical condition and was naturally fertile.

Offutt, Hileman, and Stallcup (24) reported significant increases in hay yields of Korean lespedeza when P and K were used together or with lime but not when P or K was used alone without lime.

Contrary to the results of Offutt et al. and Foote and Jackobs, no yield differences of several annual lespedezas due to applications of P and K fertilizers were measured by Fribourg, Gray, and Overton (11) in experiments conducted over a three year period at Knoxville and Jackson, Tennessee. Differences in yield occurred among varieties.

Stitt (31) failed to obtain a significant response of Korean lespedeza to lime and fertilizer on a Cecil loam soil which had received a complete fertilizer the previous season. Kobe lespedeza responded slightly to lime. However, on a Cecil gravelly loam soil, which was too poor for normal growth without treatment, Kobe, Korean, and Common lespedeza responded to phosphate and lime.

These results indicate that higher lespedeza yields may be expected with the addition of P or K fertilizers and lime on soils of low fertility but not when P or K are used without lime.

There is not much information available on tissue composition of lespedeza as affected by added fertility, but Blaser, Volk, and Stokes (7) found that fertilizer added to a virgin Leon soil low in readily soluble P, Ca, and K significantly increased the P, K, Ca, and nitrogen (N) content of annual lespedeza sampled during early bloom stage. Phosphorus fertilizer increased the P content of the lespedeza. Increases in P were accompanied by significant increases in N content.

Stitt (31) reported that the addition of both lime and superphosphate increased the Ca and crude protein content of both Kobe and Korean lespedeza. Phosphorus content was increased by application of superphosphate.

Similar results were reported by Offutt et al. (24). Significant increases in the percent of crude protein in Korean lespedeza hay occurred with the addition of lime. The percentage of nitrogen-free extract was significantly decreased. Phosphorus applied at the rate of 80 pounds per acre significantly increased the percentage of P in the hay. Eighty pounds per acre of K significantly increased the percentage of K in the hay; it also significantly increased the percentage of P.

The effect of soil, season, and stage of growth on mineral and N content of lespedeza and other hay crops in Tennessee was investigated by Weathers (36). The varieties No. 76, Korean, and Kobe were grown at Knoxville, Columbia, Clarksville, and Crossville on soils classified as

good soils or poor soils. A decrease in the mineral and N content of the whole plant was noted as the plants matured. A gradual decrease was noted in the stems while the leaves tended to increase in earlier stages and decrease in later stages.

Hay from the good soils contained an average of 23 percent more ash, 23 percent more lime, 30 percent more magnesia, 6 percent more potash, 96 percent more iron oxide, 30 percent less manganese oxide, 114 percent more phosphoric acid, and 11 percent more N than hay from the poor soils.

Weathers noted that on Hartsells silt loam, where lime and phosphate were greatly deficient, moderate applications of lime and phosphate increased the hay yield, but with practically no improvement in hay quality.

The effect of soil pH on mineral composition of plants appears to be variable. Paden and Garman (25) investigating yield and composition of cotton (Gossypium hirsutum L.) and Kobe lespedeza grown at different soil pH levels, found lespedeza yields increased with increasing pH values of 5.0, 5.5, 6.0, and 6.5, but there was no uniform influence on the mineral composition of the lespedeza. Potassium was more variable than other elements. Amounts of Ca in the lespedeza did not increase with increasing pH but remained almost uniform. Sodium (Na) and Mg levels were constant for all soil reactions. With respect to the four mineral elements, P, Na, Ca, and Mg, lespedeza was more uniform than cotton.

In contrast to the results reported by Paden and Garman (25), Moser (22) reported significant differences in Ca composition of lespedeza at various pH levels. Calcium content increased from 0.88 to 1.28 percent when the pH was adjusted from 4.0 to 7.2 in pot cultures of lespedeza on

Cecil sandy loam. He stated that Ca content was influenced largely by available soil Ca. The percentages of P and K differed very little at various pH levels but were affected by double applications of fertilizer. The C : K ratio of lespedeza appeared to be low and remained low regardless of treatment. The Ca : P ratio was approximately four but increased slightly under less favorable (lower pH) growing conditions.

The effect of soil fertility on yield and composition has been reported in other plants. Hanway (18,19) reported that differences in soil fertility resulted in different rates of dry matter accumulation in corn (Zea mays L.) plants, but influenced all plant parts similarly. The chemical composition of the leaves was extremely variable but the yield of total dry matter and grain in plants from different soil fertility levels influenced amounts of N, P, and K taken up by corn plants, but did not markedly change the seasonal pattern of uptake and distribution of these elements in the plants. Large amounts of N and P and a small amount of K were translocated from other plant parts to the grain. Potassium was translocated from the leaves to the stalks late in the season. Phosphorus accumulated at a rapid rate early in the season, then the accumulation rate decreased but remained nearly linear for the remainder of the season. Nitrogen accumulated more rapidly than P early in the season, but the rate decreased later in the season and continued to decrease until maturity. Potassium uptake was rapid early in the season and much slower late in the season.

Broyer and Stout (8) recognized the need for complete and accurate knowledge of the mechanisms by which plants extract macronutrients from

soils. The need exists to know whether there is a difference between plant species in their abilities to extract nutrients. The idea that some species do extract more efficiently than others is one of popular acceptance. Phosphorus is one of the macronutrients most frequently referred to in this respect. He stated that clarification of these mechanisms would perhaps enable the plant breeder to arrange genetic material of crop plants in new hybrids capable of extracting adequate supplies of macronutrients from poorer soils.

Location and variety are additional factors influencing the mineral composition of plants. Watson et al. (35), working with five varieties of hard red winter wheat (Triticum aestivum L.), found highly significant differences among varieties for the concentration of total P and various P compounds in the flour. Large location effects were also noted.

The idea of genetic control of chemical element accumulation by plants is relatively new. Genetic control has been shown and verified in corn and other plant species such as wheat, barley (Hordeum vulgare L.), and red clover (Trifolium pratense L.). There is no reported evidence of genetic control in the lespedeza species.

Myers (23) reviewed the work to 1960 pertaining to genetically controlled within-species and between-species variation for element content and concentration. Some species, such as alfalfa (Medicago sativa L.), have been reported as high element accumulators, while others, such as timothy (Phleum pratense L.), have been reported to be low.

Information relative to genetically caused differences in element accumulation by corn has been reported in recent years by many researchers

(3,13,14,15,16,17,27). Gorsline, Thomas, and Baker (17) recently reported the establishment of inheritance of concentration of 11 chemical elements in the ear leaf of field corn.

Baker, Thomas, and Gorsline (2) investigated differential accumulation of strontium (Sr), Ca, and other elements by corn under greenhouse and field conditions. Five selected single-cross corn hybrids were grown under both conditions to determine the effect of gross changes in environment on differential accumulation of ions. From this work they concluded that hybrids which accumulate relatively large or small concentrations of one ion do not accumulate all ions in the same relative manner. Indications were that differential accumulation resulted from independent mechanisms which were under different genetic control.

Gorsline, Ragland, and Thomas (14) reported differential ear-leaf accumulation of Ca, Mg, and K existed for single crosses and inbreds of corn. This differential accumulation was highly inherited on essentially an additive basis for Ca and Mg but included nonadditive elements for K. No relationship between ear-leaf concentrations of Ca, Mg, and K was found. Location effects for Ca, Mg, and K existed that were related to the amounts of these elements in the soil.

Rasmusson et al. (26) investigated varietal differences in P, K, Ca, and Mg content in wheat and barley grown under greenhouse and field conditions. Varietal differences in P, K, Ca, and Mg in grain and leaves were large and of similar magnitude in wheat and barley. Estimates of heritability of P, K, Ca, and Mg content in grain were low in the F_2 generation. Estimates ranged from .31 to .80 in grain and .18 to .72 in

leaves of wheat for replicated families in the F_4 generations. Calcium appeared most amenable to selection, followed by P and K.

Rasmusson further reported that increase in yield and in P and K content occurred with addition of P and K in the field. Varietal differences in P or K content of leaves or grain were not associated with differences among varieties in grain and dry-matter yields. Variety differences in P content were not associated with variety differences in dry-matter yields obtained in nutrient solution. Varieties did not appear to differ in efficiency of converting P or K into yield in either species.

Seay and Henson (28) reported that dry-matter yield, total uptake of soil and fertilizer P, utilization of fertilizer P mixed through the soil or topdressed, and uptake of soil and fertilizer K varied much more among 30 clones of Kenland red clover than among replications of clones. They stated the plant yield or uptake of nutrients by clover or like plants can be measured more precisely by using clones, since the plants of a single clone should have identical genotypes.

Other factors which add to the problem of variation in mineral composition of plants are deficient or toxic levels of nutrients in solution and interrelationships of cations and anions. Evans, Lathwell, and Mederski (9) reported that Ca deficient nutrient solutions increased P, K, Mg, and boron (B) content of soybean (Glycine max L.) leaves. Potassium deficiency increased Ca and P. Phosphorus deficiency increased K content. Magnesium deficiency increased P content twofold, whereas toxic amounts of Mg caused both K and Ca to decrease to trace amounts. Magnesium

deficiency inhibited uptake and translocation of P and B. Boron deficiency repressed uptake of Mg and K but favored uptake of Ca and Mg.

Studying interrelationships of cations and anions, Bear (4) stated the substitution of one cation or anion for another cation or anion is important in determining the quality of a crop. This can have important effects on the animals that consume the plants. The ratio of K to Ca is of special interest. Quantitatively, Ca is a much more important element to animals than K. As more K is applied to a soil and taken up by crops, the consuming animal gets less Ca, perhaps less than it requires.

Wallace, Toth, and Bear (34) studied the cation and anion relationships in plants with special reference to seasonal variation in the mineral content of alfalfa. They concluded that plants with high cation summation values tend to have high anion summation values and vice versa. This relationship holds with plants of different species and also within species. They explained the variations in cation and anion summation values of successive harvests of forage crops to be due to the operation of a carbohydrate dilution factor. During periods of rapid formation of carbohydrates, the cation and anion content per unit weight of tissue is "diluted" resulting in a lower cation and anion summation value. Successive harvests of alfalfa had a constant cation-anion ratio. The second of three successive harvests from the field had the lowest cation and anion content. They believed this to be related to the carbohydrate dilution factor.

Relationships among the four elements, P, K, Ca, and Mg have been reported by many workers. Tucker and Smith (33) reported that applications of Mg to four Kansas soil types increased the Mg concentrations in

red clover plants but only when K was absent in the chemical treatment. Applications of K always increased the K content of the plants. The percent P in the plant material was positively correlated to the percent of Mg. Percent K and percent Mg were negatively correlated. Potassium was shown to have a strong influence over Mg. For each increase in K content of the plant material there was a corresponding decrease in Mg uptake. Magnesium additions to the soil did not increase Mg content of the plant material unless K was excluded from the chemical soil treatment.

Webb, Ohlrogge, and Barber (37) reported similar findings of Mg and P relationships. A positive relationship existed between Mg and P content of soybean seed, whereas a negative relationship existed between the two elements in the soybean leaflets. This negative relationship existed in the leaflets of plants throughout the growing season.

Truog et al. (32) reported a consistent increase in the P content of seed of peas (Pisum arvense L.) with increasing supplies of available Mg. Increasing supplies of Mg increased P content much more than increasing supplies of P.

It becomes evident in reviewing the literature that many factors are involved in determining the mineral concentrations present in plants. Concentrations may be a result of the action of one of these factors but are much more likely to be the result of interactions of a number of factors.

Establishing the existence of genetic mechanisms which influence chemical element concentrations could prove to be a valuable tool to the plant breeder by enabling him to develop new cultivars capable of better

utilization of added fertility or of the existing fertility of soil in a given area.

One aspect of genetically controlled tissue concentration to consider is its relationship to yield. Miller, Pesek, and Hanway (21) investigated the relationship between soybean yield and the P and K concentrations in plant parts resulting from addition of P and K fertilizers. Using multiple regression analysis, he showed that over 80 percent of the variation in soybean yield was accounted for by the variation in the P and K content of some plant parts. However, Barber, Thomas, and Baker (3) found no significant relationship between genetically controlled P accumulation differences and yield of corn.

CHAPTER III

METHODS AND PROCEDURES

General Information

The experiments reported in this study were conducted to evaluate the variability of concentrations of P, K, Ca, and Mg in different genotypes of annual lespedeza at three locations in Tennessee during the 1971 growing season. Experiments A, B, and C were conducted on an Etowah silt loam soil at the Knoxville Plant Science Farm near Knoxville. Experiment D was conducted on a Tilsit silt loam soil at the Plateau Experiment Station near Crossville. Experiment E was conducted on a Grenada silt loam soil at the Agricultural Experiment Station at Martin.

Soil samples were taken at the end of the growing season. Soil pH values and amounts of available P and K for each location are given in Table 1.

Lespedeza genotypes were compared for accumulation of P, K, Ca, and Mg by chemical analyses to determine whole-plant (aboveground) concentration of these elements. Genotypes were sampled by harvesting plants at 2.5 to 5.0 cm (about one to two inches) above the soil surface using lawn shears.

In all experiments reported in this study, the harvested samples were placed in labeled cloth bags and dried in forced-air ovens at 70° C. The dried samples were ground in a Wiley mill using a 40-mesh screen and stored in Whirl-pak plastic bags until analyzed.

TABLE 1
SOIL pH AND AVAILABLE P AND K AFTER THE EXPERIMENTS
OF THIS INVESTIGATION

Experiment	Soil Series	Location	pH	Lb/A	
				P ¹	K ²
A	Etowah silt loam	Knoxville	6.1	17 (M)	400 (H)
B	Etowah silt loam	Knoxville	5.9	17 (M)	500 (H)
C	Etowah silt loam	Knoxville	6.1	14 (L)	450 (H)
D	Tilsit silt loam	Crossville	6.0	10 (L)	270 (H)
E	Grenada silt loam	Martin	5.8	14 (L)	400 (H)

¹Low (L) = 0-15, medium (M) = 16-25, and high (H) = over 25.

²Low (L) = 0-110, medium (M) = 111-190, and high (H) = over 190.

Experiment A

Experiment A was conducted using six cultivars and four breeding lines of annual lespedeza. The six cultivars were Climax, Kobe, Commercial Korean, Rowan, Summit, and Yadkin. The four breeding lines were Arkansas 119-5, Arkansas 226-10, North Carolina 66-13, and North Carolina 66-36, hereafter referred to as Ark 119-5, Ark 226-10, NC 66-13, and NC 66-36.

These ten entries were planted in a sand-soil-peat moss mixture in the greenhouse at Knoxville on February 22. They were grown under an extended photoperiod to prevent early flowering and to promote vegetative growth. On May 6, single plants were selected from each entry. Stem cuttings were made from each plant, dipped in a commercial rooting hormone, "Rootone," and placed in a greenhouse flat filled with vermiculite. The flats were placed in a mist chamber to promote root development.

Eighty cuttings (eight from one clone of each cultivar and breeding line) were transplanted to the Knoxville Plant Science Farm in a randomized complete block design with eight replications on July 2. Each replication was a row containing ten plots. The plots were single plants spaced 61 cm (two ft) apart. There was also 61 cm between replications. The plot area had been plowed, disked and surface smoothed to provide a very fine seedbed.

This experiment was conducted to determine the variability of the levels of P, K, Ca and Mg among the ten genotypes represented. It was assumed that plants of a clone had the same genetic makeup, as they were propagated from a single plant.

Weeds were controlled by hand hoeing and pulling during the growing season. The individual clones were harvested by hand September 13. Lawn shears were used. The clones were cut 2.5 to 5.0 cm (about one to two in) above the soil surface, placed in labeled cloth bags, dried and stored for later chemical analyses.

Experiment B

The same ten entries used in Experiment A were planted in Experiment B at Knoxville on April 29. A Planet, Jr. planter was used to plant 305 cm (ten ft) rows in a randomized complete block design with three replications. The replications were spaced 61 cm (two ft) apart with the rows spaced 45.7 cm (1.5 ft) apart in each replication. Border rows were planted in each replication. The rows were planted to give a uniform distribution of seed over the entire length of row. The same seeding rate was used for all cultivars and breeding lines. The plot area had been plowed, disked and surface smoothed to provide a very fine seedbed.

Weeds were controlled by hand hoeing and pulling. On September 8, ten plants were selected from each entry by taking plants at 30.5 cm (one ft) intervals in each row. The aboveground portion of the plant was harvested by hand at a height of 2.5 to 5.0 cm (about one to two in) above the soil surface using lawn shears. Chemical analyses were performed on these individual plants. The objective was to evaluate variation among and within cultivars.

Experiment C

Experiment C was conducted at Knoxville using five cultivars at three seeding rates in a factorial design with three replications. The five cultivars were Kobe, Commercial Korean, Rowan, Summit, and Yadkin. The seeding rates were 28.0 kg/ha (25 lb/acre), the recommended rate for Korean types; 44.8 kg/ha (40 lb/acre), the recommended rate for Kobe; and 61.6 kg/ha (55 lb/acre). This design permitted comparison of levels of element accumulation of these five cultivars at different population densities.

The plot area had been plowed, disked, and surface smoothed to provide a very fine seedbed. Weeds were not controlled in the area and a heavy infestation of crabgrass (Digitaria spp.) occurred.

Each plot was 152 x 1067 cm (5 x 35 ft). The seed was broadcast by hand on April 29. A bulked whole-plant sample was harvested from a 3716 cm² (two ft²) area in each plot. Two harvests were made. The first was on August 11 and 12 (full bloom) and the second was on September 13 and 14 (early seed formation).

Experiments D and E

The six cultivars, Climax, Commercial Korean, Kobe, Rowan, Summit, and Yadkin were planted in Experiments D and E at Crossville and Martin, respectively. The cultivars were seeded at the recommended rates of 44.8 kg/ha (40 lb/acre) for Kobe and 28.0 kg/ha (25 lb/acre) for the Korean types. The plot areas had been plowed, disked and surface smoothed to provide a very fine seedbed.

The experimental design was a split-plot factorial with cultivars as the main-plot treatments and cutting dates as the split-plot treatments. The cultivars were arranged in a randomized complete block design with three replications.

At each location the plots were 91 x 152 cm (3 x 5 ft) with a 61 cm (two ft) alley between replications and between plots within replications. The seed was broadcast by hand on May 3 at Martin and May 4 at Crossville.

Weeds were controlled at Martin by hand hoeing and pulling. On June 24 at Crossville a herbicide, Dalapon, was sprayed at the rate of 1.7 kg/ha (1.5 lb/acre) active ingredient to control an infestation of common lambsquarters (Chenopodium album L.) and crabgrass (Digitaria spp. L.). On July 22, approximately four weeks after chemical treatment, some stunting of growth and burning of lespedeza plant tops was noted. The lambsquarters and crabgrass were killed. The lespedeza plants remained stunted for the entire growing season, but put forth new axillary stem growth giving a dense ground coverage and bushy appearance.

Bulked whole-plant samples were harvested by hand 2.5 to 5.0 cm (about one to two in) above the soil surface, using lawn shears, from a 3716 cm² (two ft²) area at each location. Harvests were taken July 31, August 30, and September 25 at Martin and August 4, September 3, and October 1 at Crossville to test for variation of chemical element concentrations among cultivars and stages of growth.

Chemical Analyses

A modification of the wet oxidation procedure of Gieseking, Snider, and Getz (12) was used to digest all tissue samples for P, K, Ca, and Mg determinations. This procedure is outlined in Appendix A.

Phosphorus, K, Ca, and Mg concentrations were determined on the digestate using the Technicon Autoanalyzer. This procedure, as modified from that of Steckel and Flannery (29), is outlined in Appendix B. Potassium and Ca were determined by flame, while P and Mg were determined colorimetrically. The data obtained were converted to percentage content for each element.

CHAPTER IV

RESULTS AND DISCUSSION

I. EXPERIMENT A

Variance analyses of experiment A revealed highly significant differences in the concentration of each of the chemical elements P, K, Ca, and Mg in the aboveground portion of different genotypes of annual lespedeza. The genotypic F ratios, shown in Table 2, were very highly significant for P, K, and Mg, and highly significant for Ca.

Highly significant differences in K and Mg concentrations in the different replications probably indicate that K and Mg vary with the microclimate, soil variation, or both. Since the replication F ratios for P and Ca were nonsignificant, accumulation of these two elements may be affected less by small variations in the microclimate; but much greater evaluation of microclimatic variables would be necessary before this conclusion could be justified.

Significant differences among genotypes indicate that some genotypes have higher concentrations of P, K, Ca, or Mg than others. There are two plausible explanations for these differences. One is that some genotypes take up more P, K, Ca, or Mg than others. Another possible explanation is that all genotypes take up similar amounts of P, K, Ca, or Mg; but some genotypes are capable of accumulating more dry matter without extracting more of a particular element from the soil, so that the concentration of that element is diluted.

TABLE 2

F RATIOS AND COEFFICIENTS OF VARIATION FOR CONCENTRATIONS OF P, K, Ca, AND Mg IN ANNUAL
LESPEDEZAS GROWN IN EXPERIMENT A AT KNOXVILLE, TENNESSEE, IN 1971

Element	Replication		Genotype		CV (Percent)
	F Ratio	Probability > F ¹	F Ratio	Probability > F	
P	1.21	0.3085	4.61***	0.0002	10.68
K	3.10**	0.0072	8.81***	0.0001	7.91
Ca	0.78	0.6030	3.06**	0.0044	13.75
Mg	11.83***	0.0001	13.72***	0.0001	16.54

¹The probability reported is the probability of obtaining an F ratio this large or larger if there is no difference among treatments (reps or genotypes).

** Significant at the .01 level of probability.

***Significant at the .001 level of probability.

Duncan's New Multiple Range Test was used to compare mean concentrations of each element of the genotypes included in this study. The results of these tests are shown in Table 3. Phosphorus concentrations ranged from 0.19 to 0.25 percent among the ten genotypes. The genotype from Kobe was significantly lower in P concentration than any other genotype. The genotypes from Rowan and NC 66-36 had significantly more P than those from NC 66-13, Ark 226-10, and Kobe. The Commercial Korean genotype was not significantly lower in P than the ones from Rowan or NC 66-36, and was significantly higher than the Ark 226-10 and Kobe genotypes.

Potassium concentration ranged from 0.98 to 1.25 percent. The Kobe genotype was the lowest in this element also, though not significantly lower than the genotypes from Rowan or Yadkin. The four genotypes from NC 66-36, Summit, Ark 119-5, and Climax were significantly higher in K than the other six genotypes, including the one from Commercial Korean, and not significantly different from each other.

Calcium concentrations ranged from 0.65 to 0.84 percent. Calcium concentration was highest in the Commercial Korean genotype, but those of Climax and Rowan were not significantly less. The eight genotypes with the lowest Ca concentrations were not significantly different from each other; these eight genotypes included the one from Rowan, which was not significantly different from any genotype in this experiment.

Magnesium concentrations ranged from 0.28 to 0.50 percent, so there was almost a twofold difference between the lowest and highest Mg concentrations. Summit, Climax, Ark 226-10, and Ark 119-5 genotypes

TABLE 3

MEAN CONCENTRATIONS OF P, K, Ca, AND Mg FOR TEN GENOTYPES OF ANNUAL LESPEDEZA
GROWN IN EXPERIMENT A AT KNOXVILLE, TENNESSEE, IN 1971

Genotype	Percent P ¹	Genotype	Percent K	Genotype	Percent Ca	Genotype	Percent Mg
Rowan	.254 ^a	NC 66-36	1.252 ^a	C. Korean	.840 ^a	NC 66-13	.501 ^a
NC 66-36	.248 ^a	Summit	1.244 ^a	Climax	.792 ^{ab}	Yadkin	.478 ^{ab}
C. Korean	.244 ^{ab}	Ark 119-5	1.235 ^a	Rowan	.741 ^{abc}	NC 66-36	.471 ^{ab}
Ark 119-5	.238 ^{abc}	Climax	1.215 ^a	Summit	.715 ^{bc}	Kobe	.444 ^{ab}
Summit	.236 ^{abc}	NC 66-13	1.122 ^b	Ark 119-5	.710 ^{bc}	C. Korean	.436 ^{ab}
Yadkin	.232 ^{abc}	Ark 226-10	1.108 ^b	NC 66-36	.708 ^{bc}	Rowan	.426 ^b
Climax	.232 ^{abc}	C. Korean	1.084 ^b	Ark 226-10	.671 ^c	Summit	.324 ^c
NC 66-13	.220 ^{bc}	Rowan	1.066 ^{bc}	Kobe	.665 ^c	Climax	.302 ^c
Ark 226-10	.212 ^c	Yadkin	1.056 ^{bc}	Yadkin	.665 ^c	Ark 226-10	.292 ^c
Kobe	.190 ^d	Kobe	0.981 ^c	NC 66-13	.650 ^c	Ark 119-5	.280 ^c

¹Means within columns followed by the same letter are not significantly different at the .05 level of probability.

were significantly lower than the other six, including those from Kobe and Commercial Korean. The NC 66-13 genotype was also higher than the Rowan genotype.

Kobe, the genotype with the lowest concentration of P, K, and Ca, was among the highest in Mg concentration. The Ark 226-10 genotype was among the lowest genotypes in concentration of Ca and Mg, and had significantly less P than three genotypes and significantly less K than four genotypes; only the Kobe genotype had significantly less P or K than the Ark 226-10 genotype.

II. EXPERIMENT B

Experiment B was conducted to compare ten genotypes and to evaluate variation from plant to plant within each genotype. Failure of Kobe to emerge resulted in comparison of only nine genotypes. Variance analyses of Experiment B revealed no significant differences in the concentrations of P, K, Ca, or Mg in the aboveground portion of the nine different genotypes of annual lespedeza, although P concentration differences approached significance ($\alpha = .075$).

Highly significant differences in the K and Mg concentrations in different replications probably indicates that the microclimate or soil variation or both causes K or Mg to vary from replication to replication. These are the same two elements for which such variation was noted in Experiment A.

Variances shown in Table 4 were computed for each genotype in order to gain some idea of the variation of concentrations of the elements,

TABLE 4

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCES AMONG FIVE GENOTYPES
AND FOUR BREEDING LINES OF ANNUAL LESPEDEZA GROWN IN
EXPERIMENT B AT KNOXVILLE, TENNESSEE, IN 1971

Genotype	Variances			
	P	K	Ca	Mg
Ark 119-5	.00028	.04633	.01188	.00190
Ark 226-10	.00025	.01774	.00847	.00180
Climax	.00037	.03393	.02460	.00442
C. Korean	.00100	.03075	.01328	.00324
Rowan	.00085	.02230	.00971	.00268
Summit	.00040	.02645	.10639	.00246
Yadkin	.00018	.02514	.00276	.00450
NC 66-13	.00066	.01634	.00774	.00191
NC 66-36	.00096	.03361	.01206	.00310
X^2 from Bartlett's ¹	39.73	12.42	124.93	13.12

¹For eight degrees of freedom, the chi-square value with a probability of .05 is 15.507.

P, K, Ca, and Mg in individual genotypes. The variances were tested for homogeneity using Bartlett's test (30).

Because the chi-square values from Bartlett's were nonsignificant, the variances of the concentrations of K and Mg in the genotypes tested were concluded to be homogeneous. Variances for P and Mg concentrations proved not to be homogeneous. A series of Bartlett's Test for homogeneity were computed, dropping the high or low variance cultivar from each consecutive test, until the chi-square value was nonsignificant. From these tests it was learned that P concentrations in Commercial Korean were more variable than concentrations in Ark 119-5, Ark 226-10, Climax, Summit, and Yadkin. Concentrations of Ca in Summit were more variable (.10639) than in any other genotype. Yadkin had less variability (.00276) in Ca concentration than any other genotype.

If the concentration of a particular element were controlled by a single gene or by a relatively simple system of inheritance, the differences in variability that were found for Ca would not be expected. Even the possibility that there is a single controlling locus where one allele restricts uptake to a low level and an alternate allele allows uptake to vary with the local soil conditions seems negated by the diversity of variability in Ca concentrations. Therefore, it would appear that for Ca, and possibly for P, genetic control of accumulation is multigenic.

III. EXPERIMENT C

Differences in P, K, Ca, and Mg concentrations in the aboveground portion of five annual lespedeza genotypes were compared in Experiment C.

These five genotypes were compared at different plant populations so that element concentration differences due to genotype, plant population or a genotype x population interaction could be detected. A genotype x population interaction would indicate that genotypes having high concentrations at low plant populations had low concentrations at high plant populations and vice versa.

In addition, differences in P, K, Ca, and Mg concentrations were compared at two stages of growth. These stages approximated "full bloom" and "early seed formation."

Results shown in Table 5 indicate that P concentrations were significantly affected by microclimate or soil variation or both, perhaps due to a difference in the amount of available soil P over the test plot area. No significant differences in P concentration were found among genotypes or plant populations or between the two growth stages. However, differences between the two growth stages approached significance ($\alpha = .094$). The mean P concentration was 0.164 percent in mid-August and 0.156 percent in mid-September.

Highly significant variation of K concentration occurred among replications, indicating the effect of microclimate or soil variation or both. No significant differences in K concentration were observed among genotypes, plant populations, or growth stages. However, differences in genotypes approached significance ($\alpha = .074$).

No significant differences were observed in Ca concentrations for replications, genotypes, plant populations, or stages of growth. However, differences among genotypes approached significance ($\alpha = .065$).

TABLE 5

F RATIOS OF P, K, Ca, AND Mg CONCENTRATIONS IN FIVE GENOTYPES
OF ANNUAL LESPEDEZA GROWN AT THREE SEEDING RATES AT
KNOXVILLE AND HARVESTED AT TWO GROWTH
STAGES IN 1971

Source of Variation	F Ratios			
	P	K	Ca	Mg
Replications	8.68**	9.42***	1.71	3.74*
Genotypes	1.86	2.38	2.49	5.99**
Seeding rate	1.96	1.50	0.87	2.65
Growth stage	2.92	1.90	0.002	0.64

* Significant at the .05 level of probability.

** Significant at the .01 level of probability.

***Significant at the .001 level of probability.

The replication F ratio was significant, so concentrations of Mg were affected by changes in the microclimate or soil variation or both. No significant differences were observed among plant populations or between growth stages. Therefore, neither plant population nor stage of growth significantly affected accumulation of any of the four elements evaluated.

Genotype differences were highly significant for Mg concentrations and approached significance for the other elements so Duncan's New Multiple Range Tests were computed for each element. The results of these tests are shown in Table 6.

Commercial Korean had a significantly higher P concentration than the improved Korean genotype, Rowan. Rowan was also lower in K concentration than Commercial Korean, Summit, or Yadkin. In Ca concentration, Rowan was lower than any other genotype. Rowan also had less Mg than Kobe, as did all the other Korean genotypes.

IV. EXPERIMENTS D AND E

Experiments D and E were conducted to compare the variability of P, K, Ca, and Mg concentrations in six genotypes of annual lespedeza under two environments. The locations were Crossville and Martin, respectively. The objectives were to determine if a significant location x genotype interaction occurred, to see if P, K, Ca, and Mg concentrations changed as the plant matured, and to see if a significant genotype x stage of growth interaction existed.

Results reported in Table 7 indicate a tremendous difference in P concentrations due to location. This very highly significant difference

TABLE 6

DUNCAN'S NEW MULTIPLE RANGE COMPARISON OF MEAN P, K, Ca, AND Mg CONCENTRATIONS
IN FIVE GENOTYPES OF ANNUAL LESPEDEZA GROWN IN EXPERIMENT C
AT KNOXVILLE, TENNESSEE, IN 1971

Genotype	Percent P ¹	Genotype	Percent K	Genotype	Percent Ca	Genotype	Percent Mg
C. Korean	.168 ^a	Summit	1.049 ^a	Kobe	.827 ^a	Kobe	.320 ^a
Kobe	.165 ^{ab}	Yadkin	1.032 ^a	Yadkin	.823 ^a	Yadkin	.269 ^b
Yadkin	.159 ^{ab}	C. Korean	1.020 ^a	Summit	.818 ^a	Rowan	.255 ^b
Summit	.158 ^{ab}	Kobe	0.982 ^{ab}	C. Korean	.814 ^a	C. Korean	.252 ^b
Rowan	.154 ^b	Rowan	0.888 ^b	Rowan	.738 ^b	Summit	.252 ^b

¹Means within columns followed by the same letter are not significantly different at the .05 level of probability.

TABLE 7

F RATIOS FOR CONCENTRATIONS OF P, K, Ca, AND Mg in SIX GENOTYPES
OF ANNUAL LESPEDEZA GROWN AT TWO LOCATIONS IN
TENNESSEE AND HARVESTED AT THREE
STAGES OF GROWTH IN 1971

Source of Variance	F Ratios			
	P	K	Ca	Mg
Location (L)	952.26***	19.06*	62.98**	0.84
Genotype (G)	7.15***	2.60	11.74***	8.28***
L x G	2.09	6.00**	4.70**	4.58**
Growth Stage (S)	14.20***	29.03***	47.15***	75.18***
L x S	11.05***	16.40***	23.35***	0.22
G x S	5.24***	2.62*	1.76	1.76
L x G x S	1.33	1.36	0.36	0.87

* Significant at the .05 level of probability.

** Significant at the .01 level of probability.

***Significant at the .001 level of probability.

can perhaps be explained by the difference in amounts of available soil P at the two locations. Perhaps another factor was the difference in the altitude of the two locations with Crossville having an elevation of approximately 1880 feet above sea level and Martin approximately 400. This difference influences the mean temperature and length of growing season. The lower altitude of Martin would favor higher mean temperatures and a longer growing season, so there may have been a longer period of uptake of the elements.

Very highly significant differences occurred among genotypes providing further evidence that P concentration is genetically controlled. The location x genotype interaction for P was nonsignificant indicating that the ranking of genotypes for P concentration was not significantly different for the two locations. Very highly significant differences were observed for growth stages indicating that differences occurred from prebloom to early seed formation. The location x stage interaction was very highly significant indicating that the accumulation pattern or the vegetative growth pattern was different at the two locations. The genotype x stage interaction was very highly significant indicating that the genotypes had different accumulation or vegetative growth patterns.

The overall mean P concentrations for the two locations, .340 percent for Crossville and .217 percent for Martin (Table 8), reflect the very highly significant location effect. The higher concentration at Crossville may be due to less vegetative growth resulting in a higher concentration per unit of dry matter.

TABLE 8

MEAN P CONCENTRATIONS OF SIX GENOTYPES OF ANNUAL
LESPEDeza AT THREE STAGES OF GROWTH IN 1971

Experiment D - Crossville				
Genotype	Percent P Concentration			Mean P Percent
	Aug. 4	Sept. 3	Oct. 1	
Kobe	.330 ¹	.276	.326	.310
C. Korean	.330	.350	.380	.353
Climax	.371	.313	.313	.332
Rowan	.383	.350	.373	.368
Summit	.333	.306	.353	.330
Yadkin	.350	.323	.370	.348
Mean	.350 ²	.320	.352	.340

Experiment E - Martin				
Genotype	Percent P Concentration			Mean P Percent
	July 31	Aug. 30	Sept. 25	
Kobe	.220 ³	.206	.223	.216
C. Korean	.186	.236	.236	.220
Climax	.213	.206	.216	.212
Rowan	.223	.243	.243	.237
Summit	.210	.196	.200	.202
Yadkin	.206	.210	.230	.215
Mean	.210 ⁴	.216	.224	.217

¹LSD for comparison of genotype means at different growth stages at Crossville = .032.

²LSD for comparison of stage means at Crossville = .026.

³LSD for comparison of genotype means at different growth stages at Martin = .022.

⁴LSD for comparison of stage means at Martin = .017.

Phosphorus concentration remained relatively constant at Martin as the plant matured, although there was a slight increase from prebloom (.210 percent), to full bloom (.216 percent), to early seed formation (.224 percent). When the Martin experiment was analyzed alone, the F ratio for growth stage differences was nonsignificant so it was concluded that concentration did not change from prebloom to early seed formation. Phosphorus concentrations at Crossville generally decreased from prebloom (.350 percent), to full bloom (.320 percent), and then increased at the early seed formation stage (.352 percent). This deviation was statistically significant. It may be, however, that the lower concentrations at the full bloom stage resulted from the stunting of plant growth observed after chemical weed control treatment. Thereafter, much initiation of new secondary growth was noted. This spurt in vegetative growth may have caused a dilution of the plants' total P at the second harvest. At both locations, there was no consistent pattern of variation within the different genotypes. Therefore, it seems most likely that P concentration remains relatively constant through the growth stages evaluated in this experiment.

A significant difference in K concentration due to location occurred. The F ratio for differences among genotypes was nonsignificant, but approached significance ($\alpha = .056$). A highly significant location x genotype interaction occurred, indicating that the ranking of genotypes was significantly different for the two locations. When the experiments were analyzed separately, the F ratio for differences among genotypes in Experiment E (Martin) was nonsignificant ($\alpha = .14$) but there were very

highly significant differences among genotypes in Experiment D (Crossville).

Very highly significant differences due to growth stage were found. In general, K concentration decreased as the plant matured (Table 9). The location x stage interaction was very highly significant indicating that the accumulation pattern at Crossville was different from the one at Martin. The mean K concentrations at each stage and location are also shown in Table 9. In separate analyses of variance, it was found that there was no significant difference among stages at Crossville, but that the F ratio for K at different growth stages at Martin was very highly significant.

The significant genotype x stage interaction indicates that the ranking of genotypes was significantly different from the prebloom to early seed formation stage. This is illustrated by Kobe, which was the highest K genotype in the first two harvests at Crossville, but the lowest at the third harvest.

Table 9 shows that K concentrations at Crossville were higher than at Martin, just as P concentrations had been. This is perhaps the result of a difference in the amount of vegetative growth and dry matter accumulation at the two locations.

All genotypes decreased in K concentration at Martin as the plant matured. At Crossville, the differences among the mean K concentrations at the three growth stages were nonsignificant. The actual K concentration measured at prebloom (1.435 percent) was less than the K concentration at full bloom (1.492 percent). This is perhaps a result of stunting

TABLE 9

MEAN K CONCENTRATIONS OF SIX GENOTYPES OF ANNUAL
LESPEDEZA AT THREE STAGES OF GROWTH IN 1971

Experiment D - Crossville				
Genotype	Percent K Concentration			Mean K Percent
	Aug. 4	Sept. 3	Oct. 1	
Kobe	1.716 ¹	1.663	1.283	1.554
C. Korean	1.233	1.316	1.340	1.296
Climax	1.423	1.550	1.380	1.451
Rowan	1.426	1.390	1.303	1.373
Summit	1.533	1.550	1.416	1.499
Yadkin	1.280	1.483	1.330	1.364
Mean	1.435 ²	1.492	1.342	1.422

Experiment E - Martin				
Genotype	Percent K Concentration			Mean K Percent
	July 31	Aug. 30	Sept. 25	
Kobe	1.186 ³	1.023	.860	1.023
C. Korean	1.256	1.046	.993	1.098
Climax	1.366	1.133	1.210	1.236
Rowan	1.323	1.130	1.070	1.174
Summit	1.216	.993	.910	1.039
Yadkin	1.233	1.066	1.050	1.116
Mean	1.263 ⁴	1.065	1.016	1.114

¹LSD for comparison of genotype means at different growth stages at Crossville = .186.

²LSD for comparison of stage means at Crossville = .132.

³LSD for comparison of genotype means at different growth stages at Martin = .138.

⁴LSD for comparison of stage means at Martin = .057.

observed after chemical weed control treatment. If K accumulation continued when dry matter was not increasing, the K concentration would increase.

Highly significant differences in Ca concentration occurred between the two locations. The very highly significant differences observed among genotypes further indicate that Ca concentration is an inherited trait.

A highly significant location x genotype interaction occurred indicating that relative performance of genotypes was different for the two locations.

Very highly significant differences were observed among growth stages. Table 10 shows that Ca decreased as the plants advanced from the prebloom to the early seed formation stage. At both locations, each genotype followed this pattern. The location x stage interaction was very highly significant, apparently because the reduction in Ca concentration from the earliest to the last stage sampled was much greater at Crossville. In fact, at Crossville the percent Ca at the growth stage approximating early seed formation was barely one-half of the amount present at the prebloom stage.

The overall mean concentrations of Ca were 1.55 percent at Crossville and 0.91 percent at Martin. The difference between them explains the highly significant location effect. Differences in temperature, length of growing season, and amount of accumulated dry matter and vegetative growth may have caused this difference in concentration.

No significant effect on Mg concentrations was observed due to locations. The overall means shown in Table 11 at Crossville (.356

TABLE 10

MEAN Ca CONCENTRATIONS OF SIX GENOTYPES OF ANNUAL
LESPEDeza AT THREE STAGES OF GROWTH IN 1971

Experiment D - Crossville				
Genotype	Percent Ca Concentration			Mean Ca Percent
	Aug. 4	Sept. 3	Oct. 1	
Kobe	1.383 ¹	1.096	1.066	1.182
C. Korean	2.480	1.836	1.256	1.857
Climax	2.146	1.433	1.160	1.579
Rowan	2.210	1.516	1.366	1.697
Summit	1.670	1.163	1.046	1.293
Yadkin	2.210	1.740	1.073	1.674
Mean	2.016 ²	1.464	1.161	1.547

Experiment E - Martin				
Genotype	Percent Ca Concentration			Mean Ca Percent
	July 31	Aug. 30	Sept. 25	
Kobe	.966 ³	.846	.803	.872
C. Korean	1.026	.890	.810	.909
Climax	1.056	.890	.836	.927
Rowan	1.046	1.006	.726	.850
Summit	.996	.830	.726	.850
Yadkin	1.016	.906	.836	.919
Mean	1.018 ⁴	.894	.814	.908

¹LSD for comparison of genotype means at different growth stages at Crossville = .478.

²LSD for comparison of stage means at Crossville = .240.

³LSD for comparison of genotype means at different growth stages at Martin = .229.

⁴LSD for comparison of stage means at Martin = .106.

TABLE 11

MEAN Mg CONCENTRATIONS OF SIX GENOTYPES OF ANNUAL
LESPEDA AT THREE STAGES OF GROWTH IN 1971

Experiment D - Crossville				
Genotype	Percent Mg Concentration			Mean Mg Percent
	Aug. 4	Sept. 3	Oct. 1	
Kobe	.336 ¹	.253	.280	.289
C. Korean	.500	.453	.356	.436
Climax	.433	.350	.286	.356
Rowan	.453	.363	.373	.396
Summit	.350	.276	.243	.289
Yadkin	.453	.396	.266	.372
Mean	.420 ²	.348	.300	.356

Experiment E - Martin				
Genotype	Percent Mg Concentration			Mean Mg Percent
	July 31	Aug. 30	Sept. 25	
Kobe	.466 ³	.350	.350	.388
C. Korean	.443	.333	.290	.355
Climax	.356	.266	.273	.298
Rowan	.436	.360	.296	.365
Summit	.326	.233	.180	.246
Yadkin	.353	.330	.213	.298
Mean	.396 ⁴	.312	.267	.325

¹LSD for comparison of genotype means at different growth stages at Crossville = .078.

²LSD for comparison of stage means at Crossville = .042.

³LSD for comparison of genotype means at different growth stages at Martin = .069.

⁴LSD for comparison of stage means at Martin = .032.

percent) and Martin (.325 percent) were similar. Apparently the differences in environmental factors sampled at these two locations in 1971 had no significant effect on Mg concentration.

The very highly significant difference among genotypes supports the previous conclusion that Mg concentration is influenced by some genetic factor or factors. The location x genotype interaction was highly significant indicating that ranking of genotypes for Mg concentration was different for the two locations.

Magnesium concentration differences occurred from prebloom to early seed formation as indicated by a very highly significant growth stage effect. Magnesium concentrations tended to decrease as the plant matured, although in Kobe this tendency was less pronounced.

Significant differences were observed among genotypes for P, Ca, and Mg concentrations and the F ratio for K concentration approached significance ($\alpha = .056$). Therefore, Duncan's New Multiple Range Test was computed to separate the mean differences of genotypes. Phosphorus accumulation of the different genotypes was compared by Duncan's analysis of the overall means. However, because the genotype x location interaction was highly significant for the other three elements, K, Ca, and Mg concentrations were evaluated by Duncan's comparison of the mean values observed within the separate experiments.

Rowan (Table 12) was found to be higher in P concentration than any of the other genotypes with the possible exception of Commercial Korean. Kobe was lower in P concentration than Rowan, Commercial Korean, or Yadkin.

TABLE 12

DUNCAN'S NEW MULTIPLE RANGE OF P, K, Ca, AND Mg CONCENTRATIONS IN SIX GENOTYPES OF ANNUAL LESPEDEZA GROWN AT TWO LOCATIONS IN TENNESSEE AND HARVESTED AT THREE STAGES OF GROWTH IN 1971

Percent P				Percent K			
Genotype	Exp. D	Exp. E	Mean ¹	Genotype	Exp. D	Exp. E ²	Mean
Rowan	.368	.237	.302 ^a	Climax	1.451 ^{bc}	1.236	1.344
C. Korean	.353	.220	.286 ^{ab}	Kobe	1.554 ^a	1.023	1.288
Yadkin	.348	.215	.282 ^{bc}	Rowan	1.373 ^c	1.174	1.279
Climax	.332	.212	.272 ^{bcd}	Summit	1.499 ^{ab}	1.039	1.270
Summit	.330	.202	.266 ^{cd}	Yadkin	1.364 ^{cd}	1.116	1.240
Kobe	.310	.216	.264 ^d	C. Korean	1.296 ^d	1.098	1.196

Percent Ca				Percent Mg			
Genotype	Exp. D	Exp. E ²	Mean	Genotype	Exp. D	Exp. E	Mean
C. Korean	1.857 ^a	.909	1.383	C. Korean	.436 ^a	.355 ^a	.396
Rowan	1.697 ^a	.850	1.336	Rowan	.396 ^a	.365 ^a	.378
Yadkin	1.674 ^a	.919	1.297	Kobe	.289 ^b	.388 ^a	.339
Climax	1.579 ^{ab}	.927	1.254	Yadkin	.372 ^a	.298 ^a	.336
Summit	1.293 ^{bc}	.850	1.072	Climax	.356 ^{ab}	.298 ^a	.328
Kobe	1.182 ^c	.872	.976	Summit	.289 ^b	.246 ^b	.268

¹Means within columns followed by the same letter are not significantly different at the .05 level of probability.

²Means of K and Ca were not significantly different in Experiment E.

Comparison of the mean K concentrations of genotypes in Experiment D with Duncan's New Multiple Range revealed that Kobe was significantly higher in K concentration than Climax, Rowan, Yadkin, and Commercial Korean. Commercial Korean was significantly lower in concentration than all other genotypes except Yadkin.

Significant differences in genotype Ca means occurred at Crossville only. Kobe was lowest in Ca concentration and Commercial Korean was among the highest.

Of the genotypes in these experiments, Kobe was lowest in Mg at Crossville, but highest at Martin. Commercial Korean was among the highest at both locations. Summit was the lowest at both locations.

V. EVALUATION OF RELATIVE CONCENTRATION OF P, K, Ca, AND Mg AND CORRELATIONS AMONG THESE ELEMENTS

Using results of Duncan's Test in Experiments A, C, D, and E (Tables 3, 6, and 12, pages 24, 31, and 42, respectively), the summary of relative element concentrations shown in Table 13 was derived. Letters of the Duncan's Test were arbitrarily assigned a rating as follows: a = 1, b = 2, c = 3, d = 4, ab = 1.5, bc = 2.5, and cd = 3.5. For each element, averages of these ratings were computed; then each genotype was designated as high (H) if its average was less than 1.5, medium (M) if its average approximated two, and low (L) if its average approximated three.

TABLE 13

SUMMARY OF RELATIVE P, K, Ca, AND Mg CONCENTRATION OF TEN
GENOTYPES OF ANNUAL LESPEDEZA GROWN IN EXPERIMENTS
A, C, D, AND E IN 1971

Genotype	Relative Element Concentration			
	P	K	Ca	Mg
C. Korean	H ¹	M	H	H
NC 66-36	H	H	M	H
Rowan	H	L	M	M
Climax	M	H	H	L
Summit	M	H	M	L
Yadkin	M	M	M	M
NC 66-13	M	L	L	H
Ark 119-5	M	H	M	L
Kobe	L	L	M	M
Ark 226-10	L	L	L	L

¹Ratings of 1, 2, and 3 were arbitrarily assigned to designate concentrations of high (H), medium (M), and low (L), respectively.

From these averages, relative element concentration levels were determined for each genotype classifying them as genotypes generally expected to have high, medium, or low concentrations with respect to the elements, P, K, Ca, or Mg. Such a classification would perhaps enable the plant breeder, agronomist, or other researchers to choose the genotype which best suits the requirements desired.

Summary results indicate Commercial Korean to be high in P, Ca, and Mg concentrations and medium in K, whereas NC 66-36 was high in P, K, and Mg and medium in Ca concentration. Yadkin had medium concentrations for all four elements. Kobe had low concentrations of P and K and medium concentrations of Ca and Mg, whereas Ark 226-10 had low concentrations of all four elements.

Correlations between pairs of the elements, P, K, Ca, and Mg in annual lespedezas were computed for Experiments A, D, and E. These results are presented in Table 14.

A highly significant positive relationship existed between P and K concentrations at Knoxville. A negative P and K relationship existed at Crossville for all stages of growth with a very highly significant relationship existing at the full bloom stage. At Martin the correlations were positive, but nonsignificant.

The percent P and Ca were generally positively correlated and at each location there was one significant relationship. At both Martin and Crossville, the significant P-Ca relationship occurred at the full bloom stage. Phosphorus and Mg were generally positively correlated, but only the correlation at the second harvest at Crossville was significant.

TABLE 14

CORRELATION COEFFICIENTS OF P, K, Ca, AND Mg IN ANNUAL
LESPEDEZAS GROWN AT KNOXVILLE, CROSSVILLE, AND
MARTIN, TENNESSEE, IN 1971

Element	A - Knoxville	D - Crossville			E - Martin		
		Growth Stage ¹			Growth Stage		
		1	2	3	1	2	3
-----Phosphorus-----							
K	+.36**	-.20	-.74***	-.07	+.40	+.36	+.09
Ca	+.49***	+.41	+.64**	+.39	-.14	+.48*	+.32
Mg	+.04	+.44	+.60**	+.31	-.14	+.45	+.40
-----Potassium-----							
Ca	+.06	-.92***	-.84***	-.34	+.40	+.12	+.32
Mg	-.36**	-.80***	-.85***	-.40	-.37	+.04	-.15
-----Calcium-----							
Mg	+.13	+.89***	+.88***	+.80***	-.40	+.35	+.32

¹One, two, and three are growth stages prebloom, full bloom, and early seed formation, respectively.

* Significant at the .05 level of probability.

** Significant at the .01 level of probability.

***Significant at the .001 level of probability.

A negative relationship existed between K and Ca and between K and Mg at all growth stages at Crossville with very highly significant correlations occurring at the prebloom and full bloom stages. A highly significant negative correlation existed between K and Mg at Knoxville.

Calcium content and Mg content were positively correlated at all locations and stages of growth, except that a nonsignificant negative relationship existed at the prebloom stage at Martin. All correlations at Crossville were very highly significant while all correlations at the other two locations were nonsignificant.

A possible explanation for the very high magnitude of many of the correlations at Crossville is some effect of the herbicide applied there. It is possible that artificial relationships were created through differential stunting and injury or through some unexplained alteration in the plant's physiology. Although some of the relationships observed, such as P-Ca and K-Mg, were rather consistent, there was no clearcut pattern of correlations that would strongly suggest common genetic mechanisms. It is possible that many positive correlations were obtained as a result of a dry matter dilution effect. Positive correlations might also result from the influence of differences in environment caused by such factors as soil variation and climate. Of course, a single gene or group of genes could be operating to induce higher concentrations of each of these elements in a certain balance, but it would seem more likely that there are separate and independent genetic factors affecting the control of accumulation of each element, as has been found to be the case in corn (3).

CHAPTER V

SUMMARY

The investigation was conducted during the growing season of 1971 at the Knoxville, Crossville, and Martin, Tennessee, experiment stations. Six cultivars and four breeding lines of annual lespedeza were used to evaluate the possibility of inherited variability in accumulation of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg).

In Experiment A clones were used to determine the variability of P, K, Ca, and Mg among ten genotypes. Experiment B consisted of rows of each of nine genotypes, and individual plants were harvested from the rows to evaluate variation among and within genotypes. Experiment C compared five cultivars at three seeding rates; harvests were taken at growth stages approximating full bloom and early seed formation. The experiment design for Experiments D and E compared genotypes as main-plot treatments and cutting dates as split-plot treatments. This design permitted comparison of element concentrations among genotypes at two locations and three stages of growth. For each experiment, aboveground whole-plant samples were harvested, dried, and analyzed on the Technicon Autoanalyzer for their concentration of P, K, Ca, and Mg.

Significant differences in concentrations of P, K, Ca, and Mg in genotypes were detected indicating the existence of genetic mechanisms which control concentration. Significant differences in concentration were detected due to replications and locations indicating the effects of soil microclimate and differences in environment upon availability of

chemical elements. No significant differences occurred at Knoxville with varying plant populations or different growth stages. However, differences in concentrations occurred from prebloom to early seed formation at both Crossville and Martin. A highly significant location x genotype interaction occurred for K, Ca, and Mg indicating that rankings of genotypes differed at Crossville and Martin. Significant location x stage and genotype x stage interactions occurred indicating differences in accumulation or vegetative growth patterns at the two locations and different rankings of genotypes at different stages of growth.

Phosphorus concentration remained relatively constant at Martin as the plant matured, whereas concentrations at Crossville generally decreased from prebloom to full bloom and increased at early seed formation. Generally, K concentrations decreased at both locations as the plant matured. Calcium concentrations decreased at both locations as the plant matured; each genotype followed this pattern. Magnesium concentrations tended to decrease at both locations although Kobe appeared to be an exception to this tendency. Phosphorus, K, Ca, and Mg concentrations were generally higher at Crossville than at Martin.

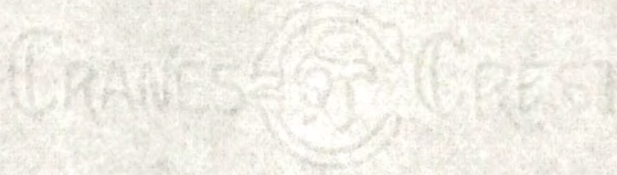
Among the genotypes evaluated, Commercial Korean was classified as high in relative concentration of P, Ca, and Mg and medium in K. Yadkin was medium in relative concentration for all four elements, whereas Ark 226-10 was low in relative concentration of P, K, Ca, and Mg. Kobe was low in relative concentration of P and K but high in Ca and Mg.

Generally, P was positively correlated with Ca and Mg. Potassium was negatively correlated with Ca at Crossville and with Mg at Crossville

and Knoxville. Generally, a positive relationship existed between Ca and Mg at all locations.

Results of this study led to speculation that genetic control of Ca, and possibly P concentration, is multigenic. However, further research to more clearly define these mechanisms would be necessary before this conclusion could be justified. It was judged that there was ample evidence to conclude that relative accumulation of each of the chemical elements, P, K, Ca, and Mg is genetically controlled.





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APPENDICES

APPENDIX A

Procedure for the wet ashing of P, K, Ca, and Mg by the aluminum heating block method.

1. Dry plant tissue at 70°C , grind into fine particles and store in air-tight bottles.
2. Weigh a 0.5000 gram portion of each sample and place into a 50 ml test tube.
3. Add two or three small glass beads and three ml of concentrated nitric acid to each sample. Place a small funnel in the mouth of each tube to act as a condenser.
4. Place tubes into the aluminum heating block and let the sample digest at room temperature overnight.
5. Place block on hotplate and raise the temperature to 150°C . Digest at this temperature for one hour.
6. Add two ml of 60 to 70 percent perchloric acid to each tube and digest at 235°C for two hours. The liquid in each sample should be clear at this point. If not, continue digestion until clear.
7. Cool the block to room temperature and add one ml of concentrated hydrochloric acid. Digest at 150°C for one hour.
8. Transfer cooled samples into 100 ml volumetric flasks and bring to volume with distilled water.
9. Shake the flask thoroughly and let stand overnight.
10. At this point the samples are ready for analyses on the Technicon Autoanalyzer.

APPENDIX B

Simultaneous determination of P, K, Ca, and Mg concentrations of wet-digested tissue samples by Autoanalysis.

1. The instrumentation for the Technicon Autoanalyzer employed here consisted of 14 separate modules as follows: small automatic sampler equipped with a liquid wash system; three positive displacement proportioning pumps; two colorimeters equipped with continuous tubular flow cells; two two-pen recorders; two voltage stabilizers; one time-delay coil; and two range expanders.

2. Potassium and calcium determinations were made by using the Technicon III dual-channel flame photometer. Lithium nitrate (0.525 g in one liter of water) is used as an internal standard and lanthanum chloride (five g in one liter of water) is used to increase the calcium flame response.

3. Phosphorus determinations were made using a colorimeter. The sample is first diluted with .05 N HCl. The diluted sample stream is then joined by a stream of ammonium vanadate (25 g ammonium molybdate in 400 ml of H_2O mixed with an equal volume of a solution of 1.25 g ammonium metavanadate in 300 ml of water, a few drops of concentrated NH_4OH , and 250 ml of concentrated HNO_3). The stream then passes through the colorimeter, equipped with a 420 m μ filter, where phosphorus is determined as PO_4^{-3} .

4. Magnesium concentrations were determined in a colorimeter by a modified lake procedure in which $Mg(OH)_2$ is precipitated in an alkaline

solution and Magnesium Blue dye (.02 percent) is adsorbed on the $\text{Mg}(\text{OH})_2$ in the presence of a detergent (.05 percent Brij 35 in water) and a suspending material (two g EGTA and two g polyvinyl alcohol in one liter H_2O). The colorimeter used in this procedure was equipped with a 630 m μ filter.



VITA

Timothy Pitts Campbell was born in Weakley County, Martin, Tennessee, on January 4, 1948. He attended elementary and high school there and graduated from Central High School, Shelbyville, Tennessee, in 1966. The following September he entered the University of Tennessee at Martin, and in June, 1970, he received a Bachelor of Science degree in General Agriculture. Also at that time he received his commission of 2nd Lieutenant in the Quartermaster Corps, United States Army.

With the aid of a research assistantship in the Department of Plant and Soil Science he began study toward a Master's degree. He received this degree in August, 1972.

He is a member of the American Society of Agronomy and the Crop Science Society of America. He is a member of the National Society of Scabbard and Blade and the Phi Sigma Society.

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