STUDIES OF INHERITANCE, PHOTOPERIODIC RESPONSE, AND DETERMINATION OF TANNIN CONTENT OF LESPEDEZA CUNEATA Don

by

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INTRODUCTION

Lespedeza cuneata Don, commonly known as sericea lespedeza or sericea, is adapted to the Southeastern United States and thrives well on soils where more desirable perennial legumes are not adapted. It makes excellent growth and provides abundant forage during the summer months. It grows well on infertile soils of this region and has been referred to by many growers as "poor land alfalfa". If grazed or cut at an early stage of growth, it provides very leafy forage with a high protein percentage.

Even though this legume appears to have great promise in the Southeastern United States as a hay, pasture and silage crop; there have been conflicting reports of its value. While some farmers and research workers report very good results from sericea, the majority of reports have indicated poor consumption of this plant by livestock.

The poor palatability of Lespedeza cuneata has generally been related to its relatively high tannin content. Some plants have been found to contain as high as 22 percent tannin in their leaves during July as determined by the formaldehyde-hydrochloric acid precipitate method of analysis. Even though tannin content is known to very with season of year, light intensity, soil type, and soil fertility; previous investigations indicate that there are differences in ranges of variability among strains.

Since previous studies (25) have indicated that tannin content is inherited, breeding programs are being initiated at several locations in an effort to produce low tannin strains of this species. Such breeding programs might well be accelerated by some knowledge of the inheritance of this tannin complex. Information such as distribution

of plants for tannin content in the generations following hybridization, number of genes involved, heritability of this character and relationship of tannin content to other characters should prove very useful in a breeding program designed to produce lines with lower tannin content. Other characters that might prove to be of interest in a breeding program are plant height, maturity, plant color, flower color, seed color and seed size. The inheritance of these characters was studied and similar information was obtained as mentioned above for tannin content.

Progress of a breeding program with Lespedeza cuneata might be accelerated by growing two generations each year; one in the field and one in the greenhouse. In many cases both cleistogamous and chasmogamous flowers are desired in the greenhouse population. Experience indicates that this species is very sensitive to photoperiodic treatments, and appears to require a rather narrow range of light to produce both types of flowers. There have been several indications that different strains behave differently when exposed to the same photoperiod. It is also possible that plants placed under a given photoperiod at different stages of growth will respond differently. In this study the effects of various photoperiods on plant growth, total seed production, proportion of seed produced by cleistogamous and chasmogamous flowers, date of blooming, and tannin content of the leaves were determined.

In a breeding program concerned with tannin content of sericea lespedeza a relatively short method of tannin analysis is needed. It is desirable to use a method with which a relatively large number of small samples can be analyzed. Since the tannin in lespedeza is almost entirely of the catechol class, the formaldehyde-hydrochloric acid precipitate method described by Clarke, Frey and Ryland (5) appears

to be a reliable method. The fact must be kept in mind, however, that this method yields consistently higher results than the official hide powder method of tannin analysis.

Extraction of the tannin is very difficult when using small samples. Stitt[1] indicated satisfactory extractions of tannin from Lespedeza cuneata by refluxing one-gram samples in Smalley extraction tubes for sixteen hours with 100 ml. of hot water. Under our conditions this method produced varying results. Variations of such factors as circulation of water through the sample, rate of percolation, temperature of extraction water, and prolonged heating of the extract solution were believed to affect the amount of tannin extracted from the sample. The effects of these factors, along with various methods of tannin extraction, were investigated.

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[1] This method was described by Dr. R. E. Stitt, Assoc. Agronomist of the Montana Agricultural Experiment Station, Bozeman, Montana, by correspondence of May 9, 1950.

REVIEW OF LITERATURE

Inheritance Studies

The value of sericea lespedeza for feeding livestock has received considerable attention and results of various trials have conflicted. In 1935 Pieters (22) stated: "It is clear that hay from Lespedeza sericea is palatable but how the feeding value will compare with alfalfa we do not yet know." He reported that cattle and sheep grazed sericea readily, though in a few instances cattle refused to graze the plants early but grazed them later in the season.

Since that time trials have shown that sericea hay and pasture is not as desirable as some other species for feeding livestock. During preliminary trials in North Carolina, Grinnels (8) found that cattle fed sericea lespedeza hay lost considerable weight, and cows fed alfalfa hay produced 18.3 percent more milk and butter than those fed sericea hay. He concluded, however, that its low lime requirement and its ability to withstand drought are two cultural characteristics that favor sericea in competition with other legumes in that state.

Wylie and Hinton (30) reported that cows consumed less sericea hay than mixed alfalfa hay and produced slightly less milk and butter fat. The cows did not seem to relish or eat as much sericea hay as was expected. Best gains with sericea were obtained when it was fed alone. Edwards (6) concluded that sericea hay is somewhat unpalatable to animals not accustomed to it. He pointed out that the tannin content is rather high and increases rapidly as the plant matures. He estimated that 744 pounds of total digestible nutrients were required to produce 100 pounds gain in weight for lambs fed sericea lespedeza as compared

to 654 pounds required for those receiving cowpea hay. The lambs consumed the hay readily and showed no bad effects as regards health, appetites, or bowel action.

Henson, et al. (12) compared sericea lespedeza pasture with permanent grass mixture. In 1941 and 1942 average beef gains per acre were 43 pounds for sericea and 104 for permanent grasses. Sericea produced 2,099 pounds of dry forage per acre compared to 901 pounds produced by the permanent pasture. After four years they concluded that sericea by itself is not a satisfactory grazing crop.

Marshall (18) experienced somewhat more favorable results. He found that large heifers averaged 1.11 and .66 pounds gain daily per animal in 1945 and 1946 respectively. The difference was attributed to unfavorable moisture conditions in 1946.

Unpublished data obtained by Wilkins, et al., of the Bureau of Plant Industry and Agricultural Research Center, Beltsville, Maryland, showed that during a short trial sheep ate over 200 percent more green forage from a low-tannin strain than from a high-tannin strain of Lespedeza cuneata.

The poor palatability of sericea has generally been related to the high tannin content of this species. In 1933 Helm and Etheridge (11) reported that sericea lespedeza contains from 5 to 9 percent tannin, which they believed may partly explain why animals do not readily graze the plant. In 1936 Ogden (21) stated, "There is an astringent quality thought to be associated with the high tannin content in sericea." He found from 2.2 to 10.0 percent tannin in plants as determined by a colorimetric test. In 1939 Clarke, Frey, and Hyland (5) stated, "It is evident that sometimes something is present in Lespedeza sericea in

sufficient quantity to lower its palatability and interfere with digestion by livestock. One constituent that might be responsible for these objectional features is tannin, since it is distasteful and astringent." These authors reported that sericea leaves contained as high as 22 percent tannin during July, as determined by the formaldehyde-hydrochloric acid precipitate method of analysis.

The tannin content of sericea lespedeza has been found to vary within a single plant when exposed to different environmental conditions. Ogden (21) reported that time of year, time of day, and amount and character of light affect tannin content. Stitt and Clarke (26) found that tannin content of sericea leaves increased up to June 30, then gradually decreased until September 22. Stitt, Hyland, and McKee (28) reported that tannin content varied significantly with soil type. They also suggested differences due to different light intensities. Manley and Olson (17) found that a complete fertilizer decreased tannin content from 6.7 to 4.5 percent. Unpublished results from H. W. Bennett, Mississippi Agricultural Experiment Station, also show a decrease in tannin content with fertilization, particularly so with heavy applications of phosphorus and potassium.

Even though tannin content of sericea lespedeza has been found to vary with several environmental factors, sufficient evidence has been obtained to indicate that there are inherent differences present. Stitt (25) reported inherent differences among six Lespedeza cuneata clones. He concluded that enough variation was found in tannin content so that selection of clones from different seed sources could be very effective in isolating clones lower in tannin. Tannin content was found to be positively correlated with plant height, and, in some

cases, negatively correlated with leafiness of the plant. Stitt's results also indicated inherent differences in plant height, number of shoots, leafiness, dry matter, and yield. Stitt and Hyland (27) found that tannin content was negatively correlated with protein content of the plant.

Photoperiodic Response

Experimental data concerning the response of sericea lespedeza to photoperiod is meager. Pieters, et al. (23) reported that during a one-month period three plants of the same original size produced 17 inches of growth under a long day, 9 inches under a normal day, and 6 inches under an 8-hour day. No other record could be found of photoperiodic response of this species.

McKee and Hyland (19) pointed out that length of day or amount of light available during the flowering season may be an important factor in determining the type of flower produced. This was indicated by the fact that in the greenhouse during winter months and shorter days only apetalous (cleistogamous) flowers were formed, while under longer days with artificial light some petaliferous (chasmogamous) flowers were formed.

Stitt, Hyland, and McKee (28), while conducting a soil type study in relation to the tannin content of sericea lespedeza, concluded that variations in light might have considerable effect on tannin content of this species.

Two different photoperiod studies have been reported with Lespedeza stipulacea and L. striata. The first was conducted by Smith (24), who found a great photoperiodic response within both species. After 57 days all plants under 7 hours of light had set seed.

Plants under 17 hours of light had no flowers after 217 days. No attempt was made to determine the critical photoperiod for these species. The second study concerning these two species was made by Nakata (20). He found that 42 days after planting all plants from both species exposed to 10 and 12 hours of light set mature seed from cleistogamous flowers, while plants exposed to 14 and 16 hours of light were growing vegetatively without any signs of flowering after 90 days. Further study indicated that the critical photoperiod for flowering is approximately 13.75 hours and photoperiods greater than 14 hours will not result in flowering.

While studying cleistogamy and development of the embryo sac in Lespedeza stipulacea, Hanson (9) concluded that day length is probably one factor in determining whether the flower is cleistogamous or chasmogamous.

Determination of Tannin Concent

Atkins and Thompson (2) described a large number of tests for tannin, and stated that all catechol tannins are precipitated with formaldehyde in the presence of hydrochloric acid. Clarke, Frey, and Hyland (5) showed that tannin in Lespedeza cuneata belongs to the catechol class by the fact that it gives an olive-green color with iron-alum solution and a precipitate with bromine water. It was completely precipitated by hydrochloric acid and formaldehyde. The method used by these authors involved boiling 50 ml. of extract solution with 5 ml. of concentrated hydrochloric acid and 10 ml. of 40-percent formaldehyde for 30 minutes under a reflux condenser.

In correspondence, J. S. Rogers, Head of Hides, Tannin Materials and Leather Division of the Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, discussed briefly a number of short methods of tannin analysis. He pointed out several objectional features of various colorimetric methods, but said little concerning the formaldehyde-hydrochloric acid method. He stated: "Your first problem will be extraction of tannin. We have done some work on a short extraction method but have no suggestions at present. Boiling with water removes about two-thirds of the tannin as a rule."

R. E. Stitt of the Montana Agricultural Experiment Station discussed by correspondence the extraction method he used after several years of study with Lespedeza cuneata. His method consisted of placing 5 grams, or as much material as available, in a glass Smalley extraction tube and refluxing for 16 hours with 100 ml. of water.

Several studies of tannin extraction from commercial sources of tannin have been reported. Bravo (3) was able to show that tannins are extracted by a simple solution process, controlled by volume of liquid, time, temperature, and the bulk of material to be extracted. Hilbert (13), working with chestnut wood, oak bark, Quebracho, and hemlock bark, concluded that volume of extraction water, temperature of extraction water, and time or duration of extraction must be understood, coordinated, and scientifically controlled for efficient extraction of tannin. He pointed out that optimum extraction conditions are different for different tannin materials. Prolonged heating resulted in a loss of either tannin or coloring material. He found that 85 to 90 percent of the tannin was extracted in the first two hours of extraction.

Luvisi and Clarke (14) pointed out that each material has an optimum temperature at which more tannin is extracted than at any other temperature. Luvisi and Rogers (15) found that the extraction of tannin from Rumex hymenosepalus was increased by the use of acetone-water mixtures and smaller particle size of the sample, but was not affected by the amount of extractive collected. The best results were obtained with a 50 percent acetone-water mixture.

In 1928 Frey and Reed (7) presented a practical glass extractor for extracting tannin and stated that raw materials and similar products often prove troublesome to extract because of swelling and clogging of the material. A proposed method of tannin extraction was presented by the American Leather Chemist's Association (1). Enough sample is required to produce $4.00 \pm .25$ grams of tannin per liter of extract solution. The collection of two liters of extract in 7 hours is recommended. A diagram of the extraction tube, along with details of the method, is included.

MATERIALS AND METHODS

Inheritance Studies

Parental Material. During the summer of 1951 five plants were selected from a large spaced planting of seed from various sources. The five plants were chosen to represent extremes in several characters. The plants selected to be parental plants along with brief descriptions of the characteristics are as follows:

Plant number	Tannin content	Plant height in inches	Flower color	Seed color	Plant color	Seed maturity	Grams per 1000 seed	Percent chasmogamous seed
1-10-14	low	24	cream	green	green	late	1.18	7.7
5-38-1	high	14	cream	purple	green	early	1.67	6.1
1-10-20	medium low	27	cream	green	green	medium	1.38	2.6
5-30-7	high	15	purple	green	purple	late	1.57	23.6
1A-62	high	15	cream	purple	green	early	1.68	0.8

The five plants originated as single-plant selections from various sources. Seed lots from which each plant was selected are as follows:

Parent	Accession Number	Origin
1-10-14	F.C. 22986	Commercial seed stock
1-10-20	F.C. 22986	Commercial seed stock
5-30-7	F.C. 17291-P	A leafy, purple-flowered selection
5-38-1	F.C. 23908	Selection from P.I. 82437
1A-62	F.C. 23908	Selection from P.I. 82437

Crossing Technique. The crosses were made by hand pollination. No emasculation was used. The method used was described by Hanson (10), who worked with Lespedeza stipulacea. In L. cuneata young buds 4 to 6 mm. in length were selected on the female parent. Usually this is one or two days before anthesis. The corolla of the bud was split with a dissecting needle and the pollen from the male parent dusted on the stigma. The flowers selected for pollen from the male parents were collected from 9:30 A.M. to 12:00 M., depending upon the amount of dew and sunshine. Fresh pollen was collected each day.

A list of the crosses made along with the number of pollinations, numbers of seed produced, and number of F_1 plants obtained is as follows:

Crosses	Number of pollinations	Numbers of seed	Number of plants
A - 1-10-14 x 5-38-1	282	93	35
B - 1-10-20 x 5-30-7	278	58	16
C - 1A-62 x 5-30-7	73	14	5

Data were obtained for each character listed above from the F_2 populations of two of the above crosses. The crosses involving each character are as follows:

Character	A	B	C
Tannin content	x	x	
Plant height	x	x	
Flower color		x	x
Seed color	x		x
Plant color		x	x
Seed maturity	x		x
% Clasmogamous seed		x	x
Weight of 1000 seed	x		x

Methods of Growing Populations. The F_1 seeds were collected, hulled, and the seed coat of each seed punctured with a dissecting needle. Seed from crosses A and B were placed in soil in 6-inch pots in the greenhouse October 26, 1951. Because of late maturity of the female parent the seed of cross C were planted November 12. At the time the F_1 seed were planted about 200 self-pollinated seed from each parent were also planted. The first group of F_1 and S_1 plants were transferred individually to 3- or 4-inch pots November 21. The second group were transplanted December 4. One hundred S_1 plants from each parent were grown in the greenhouse along with the F_1 plants. These progenies were divided into five replications.

Each pot was fertilized with an equal amount of 5-10-5 fertilizer January 18 and the plants were clipped off to about six inches of height about a week later. In an effort to initiate blooming, the plants were placed under an 8-hour day February 6. About one month later cleistogamous seed were formed. Seed production was scanty, resulting in rather small F_2 populations.

All F_2 seed were harvested by June 1, at which time the F_1 plants were cut back and transplanted to the field for further study. They were placed in the field in five replications in a randomized block design in the same order as they were in the greenhouse.

The F_2 seed and about 400 S_2 seed or as many as available from each S_1 line were seeded in pots about June 1. When they reached a height of one to two inches they were transplanted to individual thumb pots. They remained in the greenhouse until June 25, at which time they were transplanted to the field. They were divided into five replications with equal numbers in each replication. Plants were spaced two feet apart in 40-inch rows.

The number of plants in each S_2 and F_2 population were as follows: (1-10-14) 250, (1-10-20) 200, (5-38-1) 150, (5-30-7) 250, (1A-62) 250, (1-10-14) x 5-38-1) 167, (1-10-20 x 5-30-7) 110, and (1A-62 x 5-30-7) 65. These were small numbers for F_2 populations; however, it is felt that they definitely give an indication of the type of inheritance involved. Since in the case of every character studied both extremes were obtained in the respective F_2 population, it is believed that distributions, gene number estimations, etc. are sufficiently indicative of each cross to warrant consideration. Figure 1 shows some of the S_2 and F_2 plants growing in the field.

Figure 1. A section of the spaced F_2 and S_2 plants of Lespedeza cuneata. The picture was taken September 16, just before plants began flowering.

Methods of Evaluating Characters. 1. Tannin content. Tannin content was evaluated for individual plants by use of ferric ammonium citrate. Small strips of coarse white filter paper were treated with a 2.5 percent solution of the compound. One or two leaves were placed between the folded strip of treated paper and squeezed with a pair of smooth-jawed pliers. A piece of nontreated paper of about the same size was folded inside the strip of treated paper before squeezing to act as a strainer for chlorophyll and pieces of leaf material. The juice produces a gray to black spot on the treated paper; the darkness depending upon the tannin content of the leaves squeezed. The spots were rated from 1 to 10 by the uses of the chart shown in figure 2. The figures from 1 to 10 do not denote the percent of tannin but are merely relative ratings.

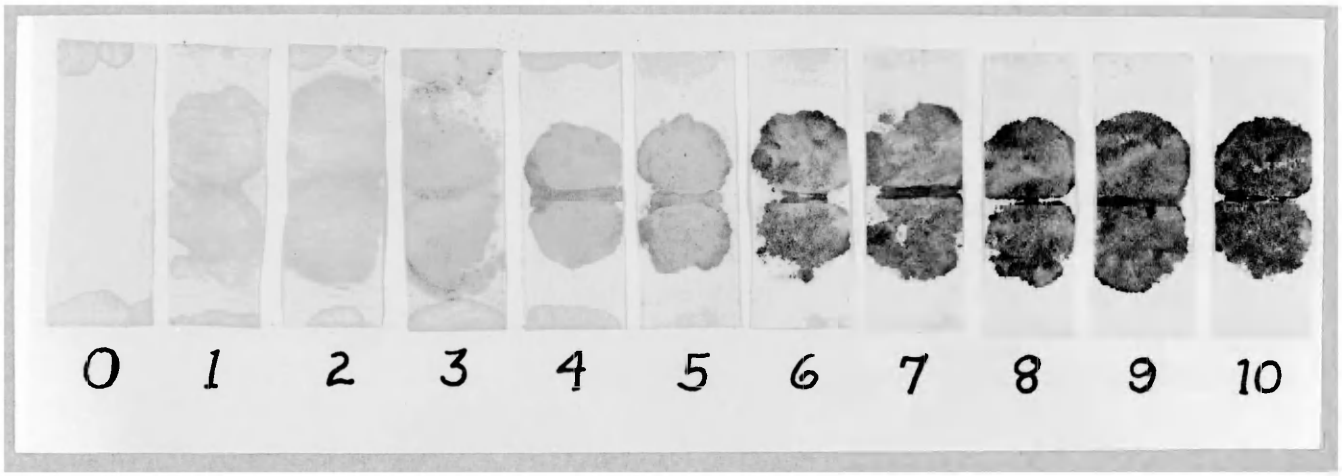


Figure 2. Relative densities of coloring on filter paper treated with ferric ammonium citrate used for evaluating the tannin content of Lespedeza cuneata.

This method showed a significant correlation with the formaldehyde-hydrochloric acid and the official hide powder method of tannin analyses. When used on the same plants grown in the greenhouse and in the field, highly significant correlation coefficients were obtained between the two environments. More information is given in the resultsof this paper, under Determination of Tannin Content.

2. Plant Height. Plant height was recorded in the F_1 and S_1 populations twice in the greenhouse and twice in the field. The greenhouse measurements were in millimeters and the field measurements were in inches. The F_2 and S_2 plants were measured only once and were recorded in inches.

3. Flower color. Flower color was rated from 1 to 5, with 1 being cream flowers and 5 being purple flowers. Intermediate scores refer to intermediate flower color. Figure 3 shows small portions of flowering branches from plants rated from 1 to 5.

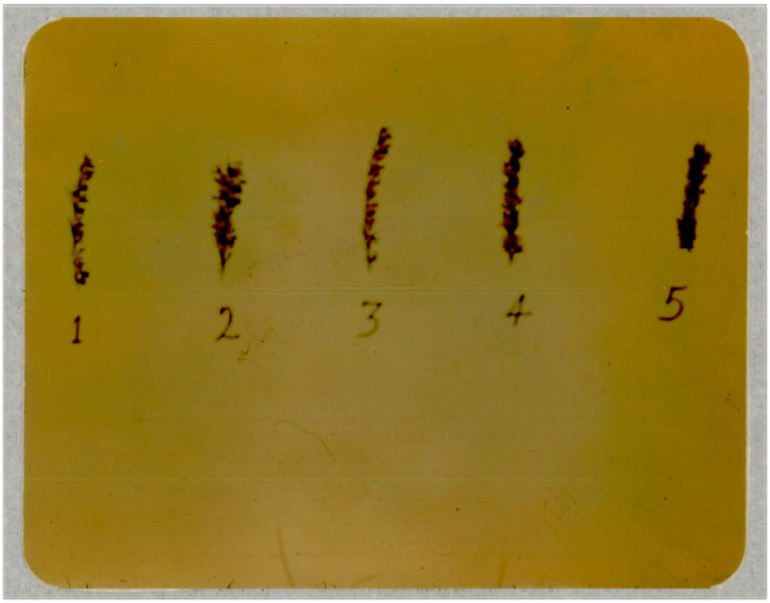


Figure 3. Flowering branches from five Lespedeza cuneata plants showing range of flower color and ratings used.

4. Seed color. Seed color was rated from 1 to 5, with 1 being all green seed and 5 being all purple seed. Intermediate scores refer to intermediate proportions of green and purple seed.

5. Plant color. Plant color was also rated from 1 to 5, with 1 being green plants and 5 being purple plants. Intermediate scores refer to intermediate plant colors. Differences in plant color became evident under cooler temperatures in October.

6. Seed maturity. On October 16 the early maturing parents had almost entirely matured seed, whereas the late maturing parents had all green seed and some plants were still blooming. On this day all the F_1 plants from both crosses involving this character were intermediate, having some immature seed and some mature seed in about equal quantities. The F_2 plants showed variations from one extreme to the other; therefore they were rated from 1 to 5 on October 16 for seed maturity, with 1 being all immature seed and 5 being all mature seed.

7. Seed size. Seed size was determined by weighing 300 cleistogamous seeds from each F_1 and F_2 plant. Weights were reported in grams per 1000 seeds.

8. Percent of seed produced from chasmogamous flowers. Approximately 500 seed from each plant were examined for seed produced from chasmogamous flowers. The chasmogamous seed were counted and the percentage determined.

Methods of Analysing Data. 1. Estimation of the number of genes involved. Gene number was estimated by the use of one of Wright's formulas which was used by Burton (4) in quantitative inheritance studies with pearl millet. The formula is as follows:

$$N = \frac{.25\ (.75 - h + h^2)\ D^2}{\sigma^2_{F_1} - \sigma^2_{F_1}}$$

where:

$$h = \frac{\bar{F}_1 - \bar{P}_1}{\bar{P}_2 - \bar{P}_1}$$

$$D = \bar{P}_2 - \bar{P}_1$$

$\bar{P}_1$ = mean of smallest parent

$\bar{P}_2$ = mean of largest parent

$\bar{F}_1$ = mean of F_1 population

$\bar{F}_2$ = mean of F_2 population

This formula will furnish an estimation of the minimum number of genes controlling the expression of a single character if certain assumptions apply. These assumptions as outlined by Burton are as follows:

a. No linkage exists between pertinent genes.

b. One parent supplies only plus factors and the other only minus factors among those in which they differ.

c. All genes are equally important.

d. The degree of dominance of all plus factors is the same for all.

e. No interaction exists between pertinent nonallelic genes.

2. Estimation of heritability. A number of methods have been used for calculating heritability. They all are designed to estimate the amount of variation present due to genotype of the plants. For the calculations in this study the formula presented by Mahmud and Kramer (16) was used. The formula is as follows:

$$H = \frac{\sigma^2_{F_2} - \sqrt{\sigma^2_{P_1} \cdot \sigma^2_{P_2}}}{\sigma^2_{F_2}} \times 100$$

where:

$\sigma^2_{P_1}$ = variance of one parent

$\sigma^2_{P_2}$ = variance of other parent

$\sigma^2_{F_2}$ = variance of the F_2 population

3. Correlations. Correlation coefficients were calculated between all characters studied in each cross.

4. Frequency distributions. Bar graphs were constructed showing the percent of plants found in each class or rating of the characters studied in the F_2 populations.

Photoperiodic Response

The trial was set up to determine the behavior of Lespedeza cuneata plants under the following photoperiods: 8 hours, 10 hours, 12 hours, 13 hours, 14 hours, 15 hours, and normal day plus 3 hours at about midnight. During the duration of this study the natural day length ranged from 13.3 to 15.1 hours in the latitude of Beltsville, Maryland. Plants under the first six photoperiods remained under natural light for eight hours each day. The additional light was furnished by incandescent bulbs which produced from 100 to 200 foot-candles of light on the plants. Temperature during the study ranged from 80° to 90° F.

Eight strains were exposed to the different photoperiods. Plants of seven strains were obtained from S_1 seed of single plants selected at Beltsville in 1951. Plants of Ga. 13 were from cleistogamous seed from Mr. J. E. Elrod of the Georgia Experiment Station, Experiment, Georgia.

A brief description of each strain is as follows:

1-10-14 -- Late maturity, vigorous growth, excellent seed producer, low tannin content.

1-10-20 -- Medium to late maturity, very vigorous growth, excellent seed producer, medium low tannin content.

1-25-12 -- Medium maturity, blooms for a long period of time, medium vigor, medium tannin content.

5-30-7 -- Late maturity, blooms for a long period of time, good vigor, high tannin content.

5-38-1 -- Early maturity, poor seed producer, blooms for a short period of time, fair vigor, high tannin content.

1A-62 -- Very similar to 5-38-1.

82433 -- Medium maturity, fair seed producer, good vigor, high tannin content.

Ga. 13 -- Late maturity, good seed producer, fair to good vigor, medium to low tannin content.

The characteristics of each plant were determined under field conditions at Beltsville, Maryland. It is possible that some strains would behave differently at other locations.

Six plants of each strain were placed in each photoperiod (except the normal day plus 3 hours of light at night) at 2 inches, 12 inches, and 20 inches of growth, which gave a total of 1,008 plants in the trial. The selfed seed were placed in 3-inch pots in the greenhouse on April 15, 1952, and resultant seedlings were transplanted singly to individual 4-inch pots April 30. The plants were allowed to remain under normal day until May 5, at which time the 2-inch group was placed under the different photoperiods. The 12-inch and 20-inch groups were placed under the different photoperiods June 3 and June 27 respectively. The plants were placed on hand carts and rolled into and out of six dark chambers. Each chamber was equipped with a time switch set to govern the length of a given photoperiod.

Data collected included records of plant height, number of days from planting to the time chasmogamous flowers and cleistogamous seed first appeared, total seed production per plant, and tannin content of leaves from plants under selected photoperiods.

Tannin content was determined by the formaldehyde-hydrochloric acid precipitate method. This method was briefly discussed by Clarke, Frey, and Hyland (3), and gives consistently higher values than the

official hide powder method. It is considered, however, to make possible very reliable comparisons among a number of samples. According to the authors, results may be obtained which are usually about 1.25 times those obtained using the official hide powder method. In the present study tannin was extracted by the use of an extractor described in the results of this thesis. Duplicate 1-gram samples were extracted and analysed.

Determination of Tannin Content

Correlation of Analytical Methods. Correlation studies among the ferric ammonium citrate, formaldehyde-hydrochloric acid, and hide powder methods of analysis were made. Leaf samples were taken similarly for the various methods at the same time and from the same plants.

Correlation coefficients were calculated for tannin content of plants grown in the greenhouse compared with the same plants after being transferred to the field. Two forms of ferric ammonium citrate were tried.

Methods of Extracting Tannin. Most of the extraction studies were conducted with Lespedeza cuneata samples harvested during the growing seasons of 1950 and 1952. For comparison leaf samples from plants of Lotus corniculatus and Coronilla varia harvested in 1950 and 1952 respectively were analysed. The samples were ground through a 30-mesh screen and mixed in a mechanical mixer.

The methods of tannin extraction investigated are as follows:

1. Refluxing the sample in a glass Smalley extraction tube for 16 hours with 100 ml. of water. Several types of extraction thimbles were compared, including alundum thimble, coarse glass thimble, very coarse

glass thimble, and glass thimble with a perforated plate. In an effort to control the temperature of the extraction water, both water condensers and air condensers were used. Comparisons were made between .5, 1, and 2 gram samples.

2. Refluxing the samples suspended in a glass thimble with perforated plate bottom by wire inside the boiling flask. One hundred ml. of water were used. This method was tried in an effort to maintain the temperature of the sample at 100° C. during extraction. The extraction times varied from .5, 1, 2, 5, 8, 16, and 25 hours. As in the preceding method, .5, 1, and 2 gram samples were compared.

3. Boiling the sample in water for 4 and 16 hours. Comparisons were made of the three sample sizes mentioned above, and 4-hour versus the 16-hour extraction. In order to avoid splashing of the sample, it was enclosed in a closely woven cloth bag.

4. Delivering the extract solution from the extraction tube by the use of the official extractor or by a similarly designed tube. Water was boiled beneath the extractiontube, which contained the sample and which was fitted with either an air or water condenser. Temperature of the sample while extracting, amount of water passed through the sample, and filtration of water through the sample were varied, and the effects noted.

The extract solutions obtained by all methods mentioned above were precipitated with 5 ml. hydrochloric acid and 10 ml. of 40-percent formaldehyde. Aliquots of 200 ml. each were precipitated, since very little difference was found between 50, 100, and 200 milliliter portions. The precipitates were boiled for 30 minutes, allowed to cool, filtered through a fritted-glass crucible, washed with 2-percent HCl solution, and dried at 110° C. for four hours.

EXPERIMENTAL RESULTS

Inheritance Studies

Tannin Content. Two crosses were made in which plants with a relatively low tannin content were crossed with plants with a high tannin content. In cross A leaves from the female and male plants contained 5.3 and 9.0 percent tannin respectively according to the official hide powder method of tannin analysis. In cross B leaves from the female and male plants contained 6.6 and 12.0 percent tannin respectively. Due to the impossibility of analyzing all the F_2 plants by the official method, or even by the hydrochloric acid-formaldehyde method, the plants were given relative ratings of their tannin content based on the use of ferric ammonium citrate paper. This method is described in the materials and methods section of this paper.

Figures 4 and 5 show the distribution of F_2 plants for tannin content as scored by the ferric ammonium citrate method. More variation was found in the F_2 populations for tannin content than in the selfed or F_1 populations. By comparing the distributionof the various populations, one will notice that the majority of the F_1 and F_2 populations of both crosses lie between the selfed populations of the parental plants. Of the 167 F_2 plants in cross A, 7, 50, 92 and 15 were scored 5, 6, 7 and 8 respectively; while in cross B, which had 110 plants in the F_2, 6, 29, 60 and 15 were scored 5, 6, 7 and 8 respectively.

The evaluations for tannin content were made just before the plants began blooming. The same type of tannin rating was made on 700 plants when they were in the seedling stage (6-8 inches tall)

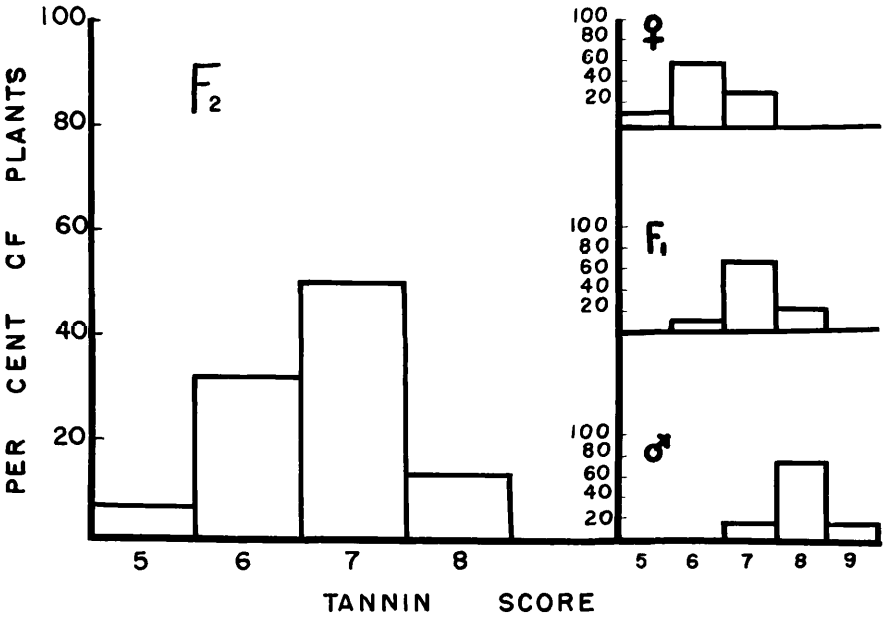


Figure 4. Frequency distributions of plants for tannin content in the F_2, F_1, and S_2 populations of cross A (1-10-14 x 5-38-1) of Lespedeza cuneata. (S_1 plants in ♀ population.)

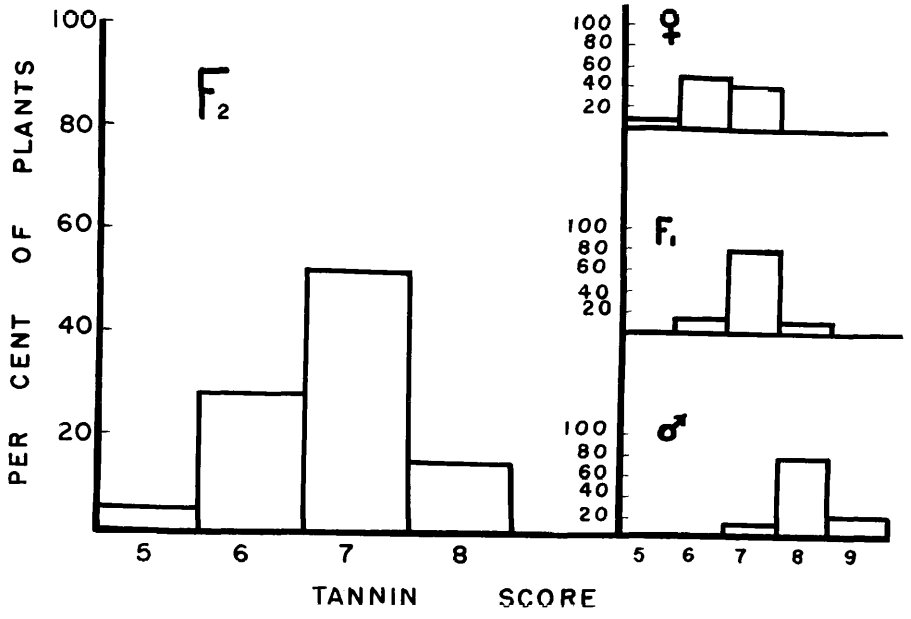


Figure 5. Frequency distribution of plants for tannin content in the F_2, F_1, and S_2 populations of cross B (1-10-20 x 5-30-7) of Lespedeza cuneata.

prebloom stage (18-22 inches tall), and blooming stage. Simple correlation coefficients between these three stages were all significant at the .05 level, indicating that the relative tannin content of different plants is very nearly the same at different stages of growth. As reported before, tannin content of a given plant was found to increase during the season to mid-season and then decrease again. Observations made during the course of this study indicate that the tannin content of some strains increased more during the season than that of other strains. In most cases tannin content was lower in plants that were stunted and growing very slowly. This was especially true in seedling plants. In general the young leaves of a given plant were a little higher in tannin than older leaves. Chlorophyll in the older leaves interfered with the tannin test in some cases; therefore, the younger leaves were used for this test. The terminal buds appeared to contain more tannin than any other part of the plant.

Even though several environmental factors are known to influence tannin content there is no doubt that it is also controlled to some extent by hereditary factors. The estimated heritability as determined by using the F_2 population was 43.0 percent for cross A and 34.0 percent for cross B. The number of genes involved in tannin inheritance was estimated to be 24 for cross A and 19 for cross B. A comparison of the heritability and gene number for tannin content with that for other characters is given in tables 1 and 2 respectively. Tannin content of plants in the F_2 population was positively correlated with seed color in cross A. This correlation did not exist in cross B. No apparent relationship existed between tannin content and plant height, maturity,

flower color, plant color, seed size, or percent of chasmogamous seed. Table 3 gives the correlation coefficients between the various characters studied in L. cuneata.

TABLE 1. Heritability for seven characters in Lespedeza cuneata.

Character	Cross*		
	A	B	C
Plant height	53.8	59.8	
Tannin content	43.0	34.0	
Maturity	91.2		89.8
Flower color		93.0	91.7
Plant color		91.3	89.9
Weight of 1000 seeds	91.2		75.3
Percent chasmogamous seed		35.1	36.6

*Crosses: A = 1-10-14 x 5-38-1; B = 1-10-20 x 5-30-7; C = 1A-62 x 5-30-7.

TABLE 2. Estimated number of genes involved in the inheritance of eight characters in Lespedeza cuneata.

Character	Cross*		
	A	B	C
Plant height	34	13	
Tannin content	24	19	
Maturity	22		10
Flower color		11	8
Plant color		16	12
Seed color	1		1
Weight of 1000 seeds	4		
Percent chasmogamous seed		102	58

*Crosses: A = 1-10-14 x 5-38-1; B = 1-10-2 x 5-30-7; C = 1A-62 x 5-30-7.

TABLE 3. Correlation coefficients between several characters in F_2 populations of Lespedeza cuneata.

Character	Cross 1/		
	A	B	C
Tannin content and maturity	.1473		.2502
Tannin content and flower color		.0439	.1124
Tannin content and plant color		.0824	.1068
Tannin content and seed color	.2864**		.0983
Tannin content and weight of 1000 seeds	.0006		.1376
Tannin content and percent chasmogamous seed	.1126	.0534	
Plant height and tannin content	.0565	-.0281	
Plant height and maturity	.0057		-.3019*
Plant height and flower color		-.0900	-.2429
Plant height and plant color		.0021	-.0141
Plant height and seed color	-.0176		-.1182
Plant height and weight of 1000 seeds	.0348		-.2922*
Plant height and percent chasmogamous seed		.0769	-.1134
Maturity and flower color			.0314
Maturity and plant color			-.2293
Maturity and seed color	.5303**		.4853**
Maturity and weight of 1000 seeds	.2528**		.3423*
Maturity and percent chasmogamous seed	.0719		.1897
Flower color and plant color		.7685**	.6379**
Flower color and seed color			.2983*
Flower color and weight of 1000 seeds		-.0409	-.1836
Flower color and percent chasmogamous seed		.0089	.0497
Plant color and seed color			.0592
Plant color and weight of 1000 seeds			-.2118
Plant color and percent chasmogamous seed		.0913	.2962*
Seed color and weight of 1000 seeds			.2264
Seed color and percent chasmogamous seed	-.0416		-.2192
Weight of 1000 seeds and percent chasmogamous seed	-.0168		.1515

1/ Crosses: A = 1-10-14 x 5-38-1; B = 1-10-20 x 5-30-7; C = 1A-62 x 5-30-7.

Plant Height. The distributions of F_2 plants for plant height in crosses A and B are shown in figures 6 and 7. The patterns of distribution for the two crosses are very similar. Height measurements were made in the field while the plants were blooming. The plants were measured from the ground to their highest point without straightening them. Of the 167 F_2 plants in cross A 4, 12, 34, 68, 43, and 6 measured -10, 11-15, 16-20, 21-25, 26-30, and 30+ inches respectively. In cross B, which had 110 F_2 plants, 5, 13, 22, 39, 27, and 4 measured -10, 11-15, 16-20, 21-25, and 30+ inches respectively.

In the field F_1 plants from cross A were intermediate in growth type, but were more like the female; in fact, the mean height of the F_1 population was greater than the mean height of the S_1 population of the female parent. In the greenhouse the same F_1 plants, before they were transplanted to the field, had growth habits much like the male parent. Figure 8 shows two representative plants from the F_1 population of cross A. Two plants from each of the parental lines are shown with the hybrids. It is evident that the F_1 plants were very similar to the male plants. All plants in the F_1 population were very uniform in type of growth.

After the plants had been cut back and transplanted to the field, regrowth of the F_1 plants was very similar to that of the female parent. Figure 9 shows a typical F_1 plant from cross A with a plant from each parental line. It is apparent that the hybrid has a coarse main stem and a branching pattern much like the female parent. The plant from the paternal line does not have a single main axis but instead sends up numerous branches from the base of the plant.

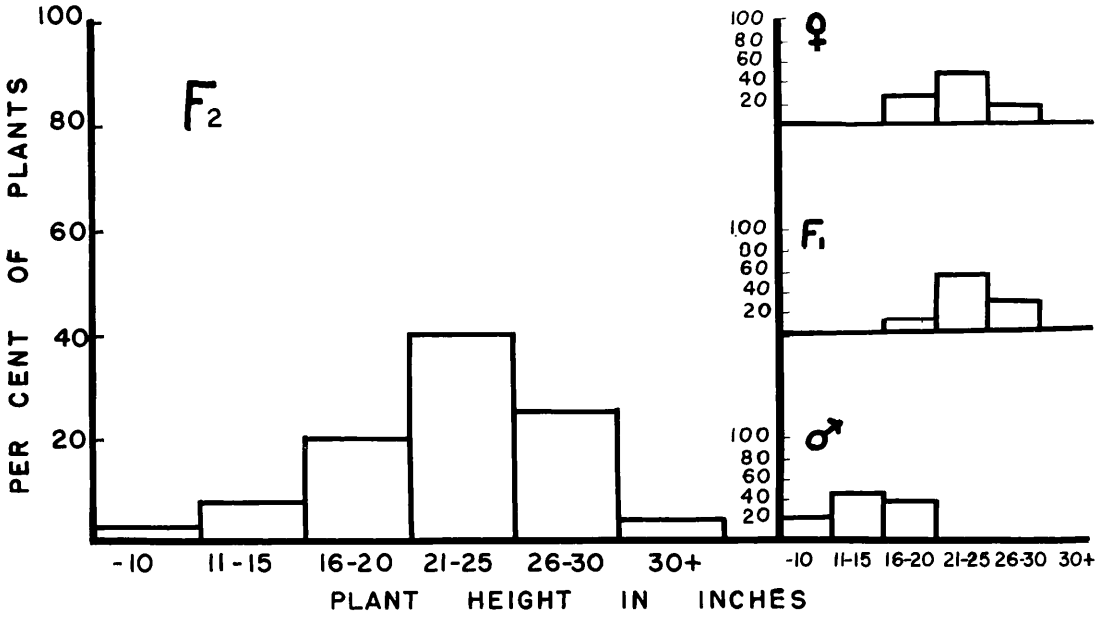


Figure 6. Frequency distribution of mature plants for plant height in the F_2, F_1, and S_2 populations of cross A (1-10-14 x 5-38-1) of Lespedeza cuneata. (S_1 plants in ♀ population.)

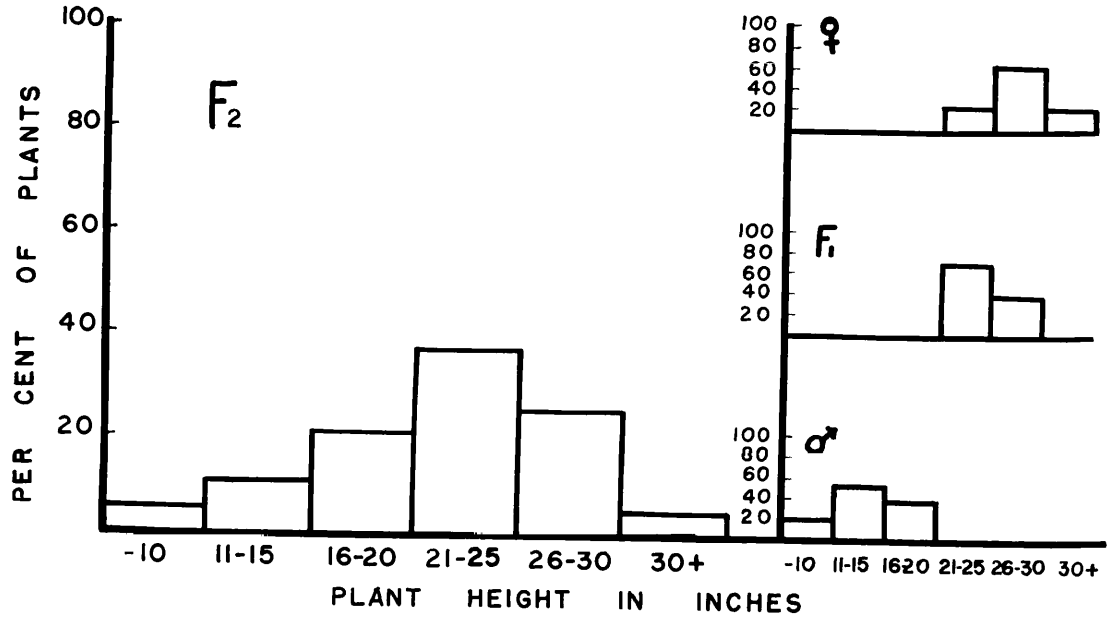


Figure 7. Frequency distributions of mature plants for plant height in the F_2, F_1, and S_2 populations of cross B (1-10-20 x 5-30-7) of Lespedeza cuneata.

Figure 8. Grown F_1 progenies of Lespedeza cuneata from cross A between two plants of each parental S_1 line.

Figure 9. A grown F_1 progeny of Lespedeza cuneata from cross A between a plant of each parental S_1 line.

In cross B plants of the maternal and paternal lines were very similar in the greenhouse. The male parent, however, had larger and darker green leaflets. Figure 10 shows that the F_1 plants of cross B are more like the female. After these plants were transplanted to the field they continued to resemble the female parent. In the field, however, there was a great difference between the female and male parents.

In the F_2 population of both crosses, when grown to maturity in the field, segregates were recovered which appeared identical to each parent involved. Most of the progenies, however, were intermediate in their growth habit and plant height. In the greenhouse seedling plants of cross A in the F_2 population also showed segregation. Some progenies were very similar to each of the parents, while the majority were intermediate.

The estimated heritability for plant height was 53.8 percent for cross A and 59.8 percent for cross B. The estimated number of genes involved was 34 for cross A and 13 for cross B. These values are given in tables 1 and 2. Plant height was not correlated with any of the other characters in these two crosses. In a third cross, however, (cross C) plant height was negatively correlated with maturity and seed size. In cross C the difference in plant height between the two parents was small; therefore, the variation in the F_2 population was not studied extensively. The difference appeared to be due primarily to environmental factors. Correlation coefficients between plant height and the other characters studied are given in table 3.

Maturity. The distributions of F_2 plants of crosses A and C for maturity are shown in figure 11. Of 167 F_2 plants in cross A 43, 47, 50, 18, and 19 were scored 1, 2, 3, 4, and 5 respectively; while in

Figure 10. Grown F_1 progenies from cross B between two plants of each parental S_1 line.

cross C, which had only 65 F_2 plants, 28, 13, 13, 7 and 4 were scored 1, 2, 3, 4, and 5 respectively. It is evident that in cross C nearly half of the plants were late to mature, very similar to the male parent. In cross A the percentage of plants in classes 1, 2 and 3 are nearly equal; however, the majority of the plants tended to be late in maturity. In both crosses plants of the maternal, paternal, and F_1 populations were very uniform for maturity. On October 16 the plants scored 5 contained all mature seed while the plants rated 1 had all green seed and were still flowering.

Rather high heritability values of 91.2 and 89.8 percent were obtained for crosses A and C respectively. The number of genes involved in inheritance of maturity was estimated to be 22 in cross A and 10 in cross C. Maturity was positively related to seed color and seed size in both cases, and negatively correlated with plant height in cross C. Maturity was not correlated with tannin content, flower color, plant color, or percentage of chasmogamous seed.

Flower Color. The distributions of F_2 plants for flower color are shown in figure 12. Of 103 F_2 plants in cross B which were evaluated for flower color 22, 24, 32, 18, and 7 were scored 1, 2, 3, 4, and 5 respectively. In cross C, of 48 plants that flowered 21, 10, 8, 6, and 3 were scored 1, 2, 3, 4, and 5 respectively. Cross C has a distribution for flower color very similar to its distribution for maturity. The F_1 plants of cross C had flowers that were darker purple than those of cross B.

The heritability value for flower color was 93.0 percent for cross B and 91.7 percent for cross C. This character had the highest percent heritability of the eight characters studied. The number of

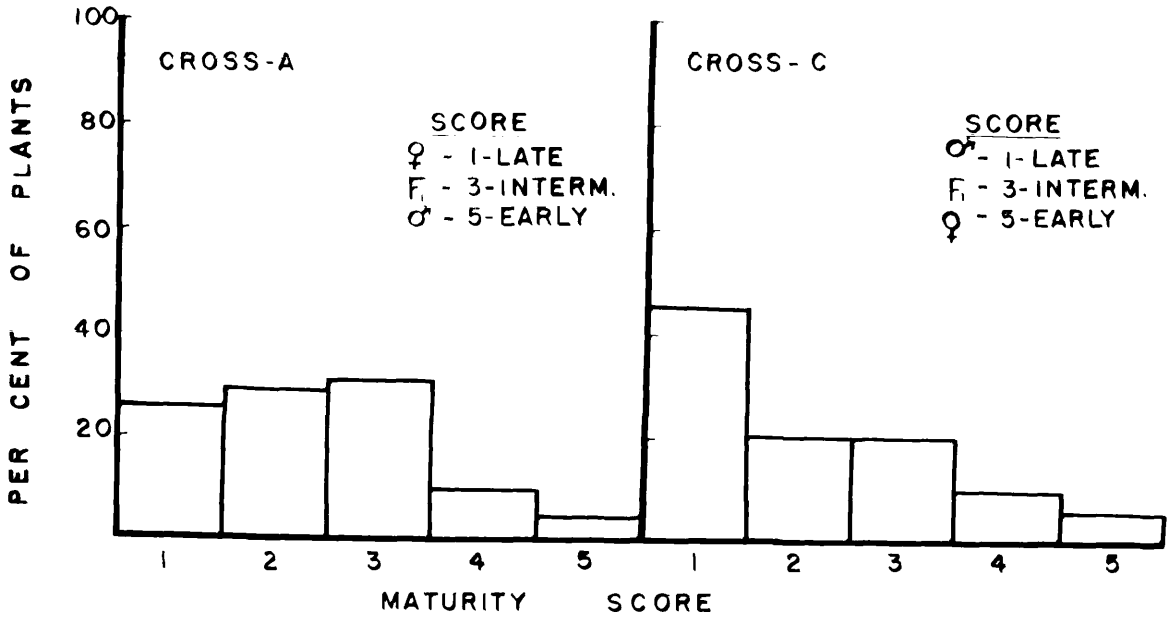


Figure 11. Frequency distributions of F_2 plants for maturity in crosses A (1-10-14 x 5-38-1) and C (1A-62 x 5-30-7) of Lespedeza cuneata.

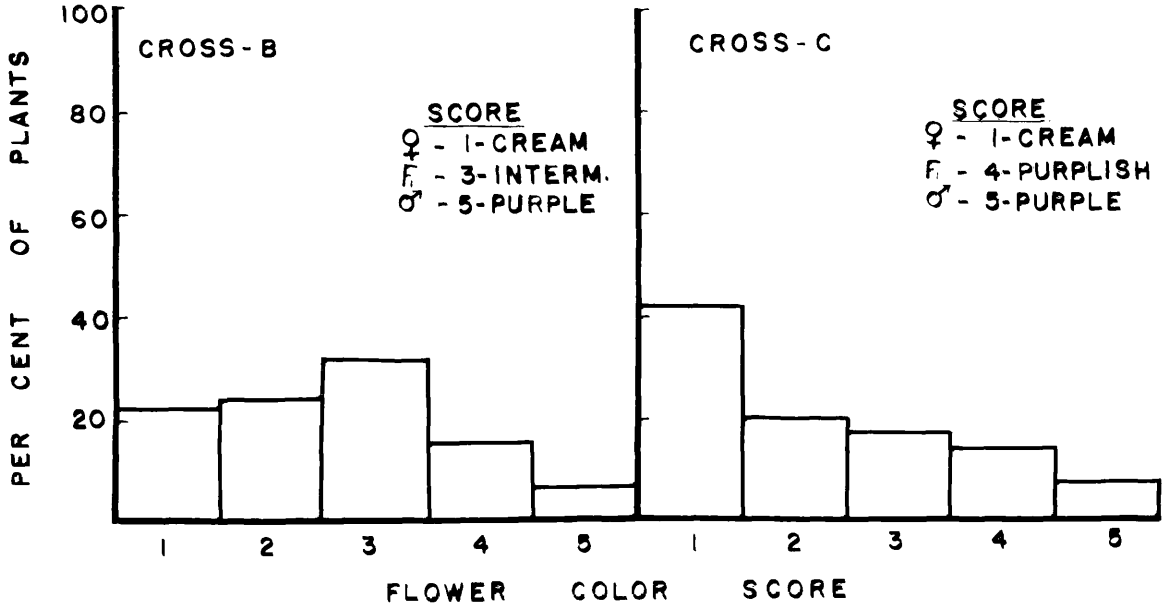


Figure 12. Frequency distributions of F_2 plants for flower color in crosses B (1-10-20 x 5-30-7) and C (1A-62 x 5-30-7) of Lespedeza cuneata.

genes involved in inheritance of flower color was calculated to be 11 for cross B and 8 for cross C. Flower color was highly correlated with plant color in both crosses. In most cases the plants with purple flowers also developed a purple color in their leaves and stems during periods of low temperature. A positive correlation existed between flower color and seed color in cross C. This relationship could not exist in cross B since both parents had green seed. There was no relationship between flower color and tannin content, plant height, maturity, seed size, or percent chasmogamous seed.

Plant Color. This character refers to a purple color produced in the leaves and stems of some plants under certain conditions. Figure 8 shows the distributions of F_2 plants in crosses B and C for this character. The distributions are similar for both crosses, with a few more plants in cross C tending toward the female type. Of 110 plants in cross B 14, 24, 46, 19, and 7 were scored 1, 2, 3, 4, and 5 respectively.

Figure 14 shows a row of S_1 plants of 5-30-7 (center of picture) which turned purple late in the season. The same plants also turned purple for a short period after they were transplanted to the field. In the picture the plants appear to be mature; however, they were not. It is really a purple color that was produced. Every S_1 and S_2 plant of this line, as well as all F_1 plants where it was used as a parent, turned purple.

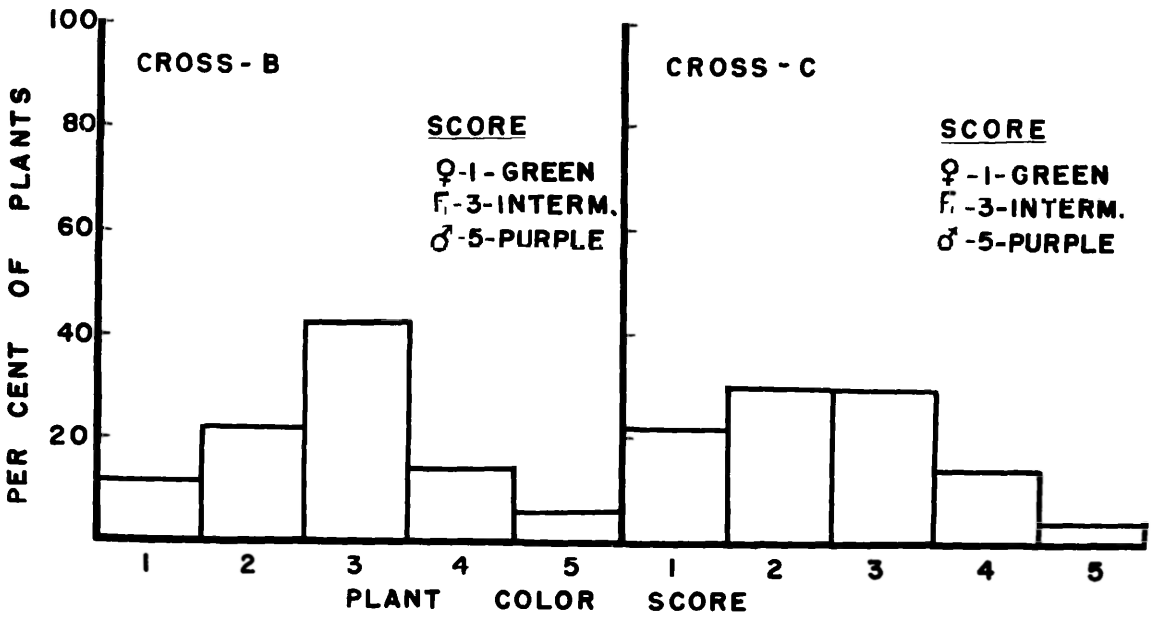


Figure 13. Frequency distributions of F_2 plants for plant color in crosses B (1-10-20 x 5-30-7) and C (1A-62 x 5-30-7) of Lespedeza cuneata.

Figure 14. S_1 plants of 5-30-7 among other S_1 strains of Lespedeza cuneata. The row with a brownish color appears to be more mature than other strains, but actually the plants are still blooming, and they are a reddish-purple color.

In the F_2 populations plants were present with various gradations of color, ranging from solid green to solid purple. Figure 15 shows two F_2 plants from cross B with different degrees of the purple color. In this picture the shadows have a purplish cast; however, some difference can be seen. By comparing the variation in the F_2 population with the variation in the parental lines, plant color was found to be controlled about 91.3 percent in cross B and 89.9 percent in cross C by heritable factors. The number of genes controlling this character was estimated to be 16 for cross B and 12 for cross C.

Figure 15. Two plants from cross B that showed different amounts of purple color.

Plant color was positively correlated with flower color in both crosses. It was positively correlated with percentage of chasmogamous seed in cross C. No relationship existed between plant color and tannin content, plant height, maturity, seed color, or seed size.

Seed Color. In crosses A and C plants with solid purple seed were crossed with plants producing solid green seeds. In the S_1 population

of the purple-seeded parents all the seed were purple. In the S_2 populations of the same parents, however, some green seed were present. The S_1 plants were older and produced seed earlier in the season than the S_2 plants. The green seed from the S_2 plants of the purple-seeded parents are probably due to immaturity of the plants. When seed produced by a single S_2 plant were separated before hulling into immature seed, partly matured seed, and well matured seed it was found that seed from immature seed were green, partly matured seed had a purplish cast, and the well matured seed were dark purple. When seed of some F_2 plants were separated in the same manner the same sort of segregation existed. In other F_2 plants, however, all the seed were green. The number of F_2 plants in cross A with some purple seed was 102, while the number with all green seed was 47. In cross C these numbers were 38 and 10 respectively. If plants with any purple seed are considered to carry the genotype for solid purple seed, the failure for all the seed to be purple may be attributed to lack of maturity of such seeds. If seed on the plants were immature, it is possible that some of the plants which had all green seed might have possessed genes for purple seed.

Heritability for seed color was not calculated since the effect of maturity was so pronounced. The number of plants with some purple seed was roughly three times the number of plants with all green seed. In this case the genotype for seed color would be controlled by a single pair of genes.

In addition to maturity, seed color was positively correlated with tannin content in cross A and with flower color in cross C. No relationship existed between seed color and plant height, plant color, seed size, or percentage of chasmogamous seed.

Seed Size. Seed size is expressed by the weight of 1000 cleistogamous seed in grams. Figure 16 shows the distribution of seed from F_2 plants for seed weight in crosses A and C. There was a great difference in seed size between parents in cross A, but very little difference in cross C. Of 148 F_2 plants in cross A which formed seed 3, 22, 73, 44, and 6 produced seed which weighed 1.0-1.2, 1.2-1.4, 1.4-1.6, 1.6-1.8, and 1.8-2.0 grams per 1000 seeds respectively. In cross C, of 49 F_2 plants 0, 10, 22, 15, and 2 produced seed which weighed 1.0-1.2, 1.2-1.4, 1.4-1.6, 1.6-1.8, and 1.8-2.0 respectively.

The heritability values of 91.2 percent for cross A and 75.3 percent for cross C were obtained. In cross A the estimated gene number involved was 4. The number of genes involved in cross C was not determined since the difference between parents was very small. In both crosses seed size was positively correlated with maturity. In cross C it was negatively correlated with plant height. There was no correlation between seed size and the other characters.

Percentage of Seed Produced from Chasmogamous Flowers. The distributions of F_2 plants for percentage of chasmogamous seed are shown in figure 17. Of 95 F_2 plants in cross B, which produced seed, 47, 18, 13, 11, and 6 produced seed which contained -2, 2-4, 4-6, 6-8, and 8+ percent chasmogamous seed respectively. Of 51 plants in cross C 18, 12, 10, 6, and 5 produced seed which contained -2, 2-4, 4-6, 6-8, and 8+ percent chasmogamous seed respectively. In both crosses B and C the plants had relatively few chasmogamous seed. This is probably because the plants were transplanted to the field late in the season and were late in setting seed. At the time the plants were setting seed, the day length was short and the temperature was relatively low. These are two

conditions which favor seed production from cleistogamous flowers rather than from chasmogamous flowers. As shown in figure 17 there was a great difference between the mean percentage of chasmogamous seed produced by the maternal and paternal lines of both crosses. It is felt that the distributions would not have been skewed toward the female parent as much if the plants had produced seed earlier in the season.

The percent heritability for this character was calculated to be 35.1 for cross B and 36.6 for cross C. An estimated gene number of 102 and 58 were involved in the two crosses respectively. Percentage of chasmogamous seed was positively correlated with plant color in cross C, but was not correlated with tannin content, plant height, maturity, flower color, or seed size.

Photoperiodic Responses

Plant Growth. The effect of photoperiods of different daily durations on plant growth was determined by plant height measurements 73 days after planting. Only the 2-inch and 12-inch groups were measured since the 20-inch group had just been placed under different photoperiods and showed very little difference among photoperiodic treatments. At the time the measurements were made, the 2-inch group had been under the different photoperiods for 52 days, while the 12-inch group had been under different day lengths for 24 days. Table 4 shows the mean plant height in inches for each strain under each photoperiod.

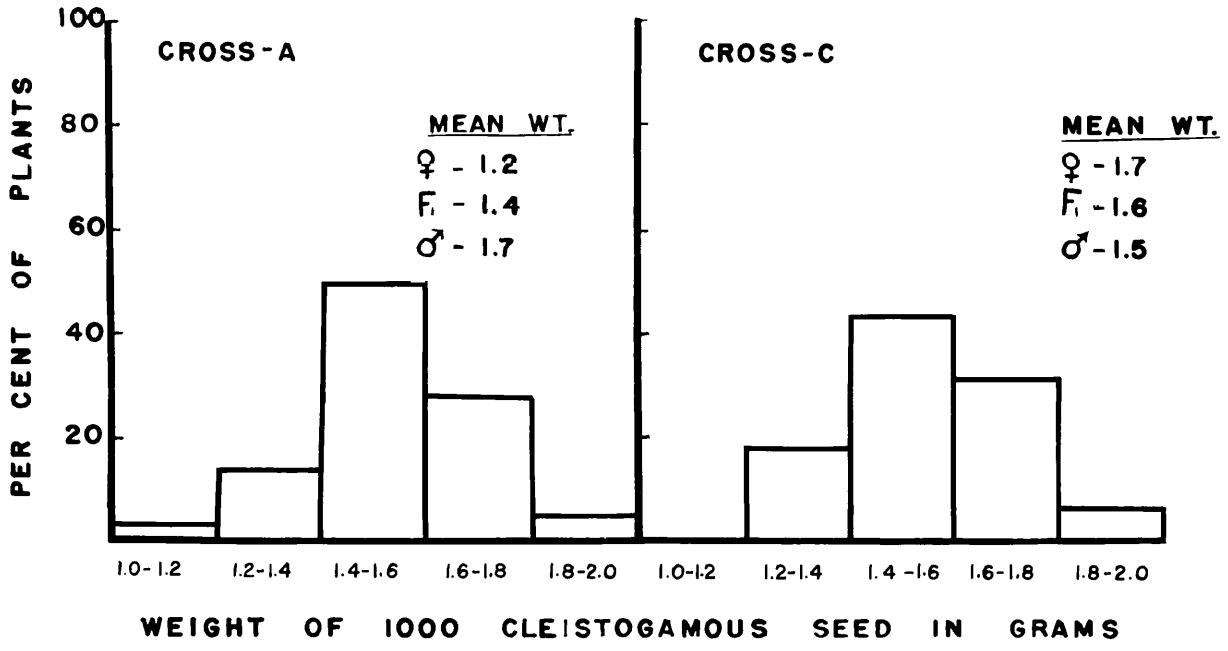


Figure 16. Frequency distributions of F_2 plants for seed size in crosses A (1-10-14 x 5-38-1) and C (1A-62 x 5-30-7) of Lespedeza cuneata.

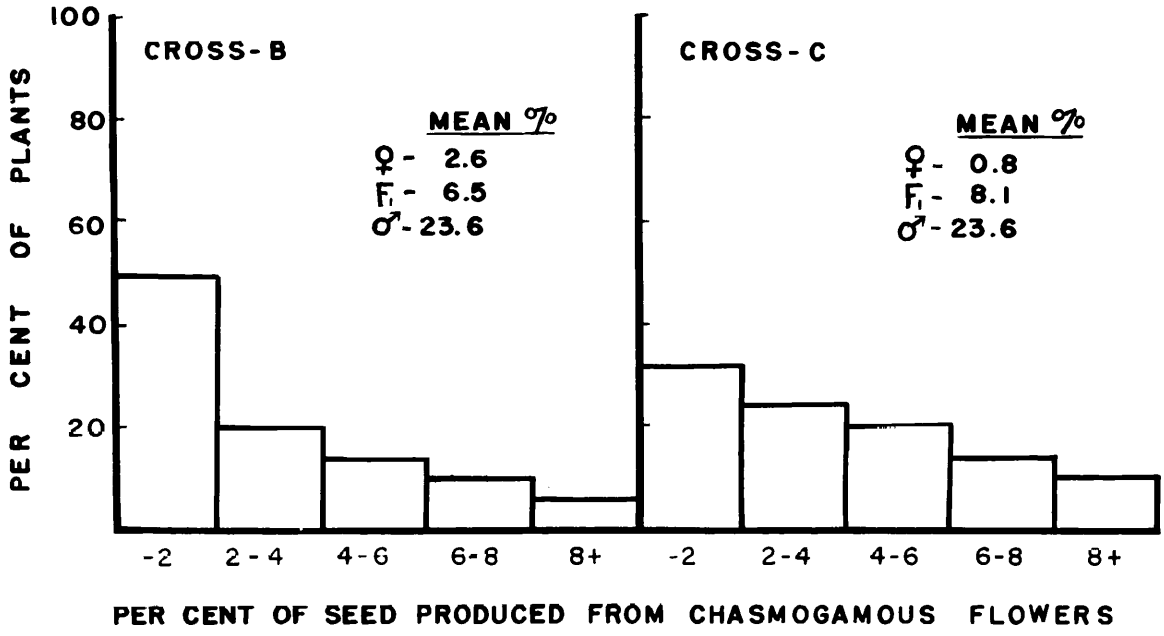


Figure 17. Frequency distributions of F_2 plants for percent of seed produced from chasmogamous seed in crosses B (1-10-20 x 5-30-7) of Lespedeza cuneata.

TABLE 4. Mean plant heights for eight strains of Lespedeza cuneata after growing under various photoperiods.*

Strains	Plant height under different lengths of light periods						
	8 hr.	10 hr.	12 hr.	13 hr.	14 hr.	15 hr.	Means
	Inches	Inches	Inches	Inches	Inches	Inches	Inches
1-10-14	7.5	8.0	10.3	14.0	14.0	13.5	11.2
1-10-20	9.3	9.3	10.8	15.8	18.3	17.3	13.5
1-25-12	9.8	7.8	10.3	14.0	15.8	16.3	12.3
5-30-7	8.8	8.5	11.0	15.5	17.0	15.0	12.6
5-38-1	8.3	8.5	9.3	12.3	16.3	15.5	11.7
1A-62	7.5	7.3	7.3	10.5	14.5	16.0	10.5
82453	8.3	6.0	9.0	12.5	15.8	14.5	11.0
Ga. 13	7.8	7.0	8.5	16.3	15.5	15.5	11.7
Means	8.4	7.8	9.6	13.9	15.9	15.5	11.7
L.S.D. for:			5%	1%			
Photoperiod means			1.0	1.4			
Strain means			1.2	1.6			

* Average of plants placed under photoperiods when 2 inches and 12 inches of height.

An increase of light from 12 to 13 hours had the greatest effect on growth of the plants. By comparing the means, it is noticed that the 8-hour and 10-hour treatments produced similar effects while the 14-hour and 15-hour treatments produced similar effects. Plants under the other two photoperiods behaved differently. When individual strains are considered one will find that most of the strains responded similarly to the means. Figure 18 shows the response of strain 1-10-20 to the different photoperiods. The plants were placed in the respective photoperiods at approximately 2 inches of growth.

A very striking difference was seen among plants grown under 12 and 13 hours of light if they were placed under the photoperiods at 2 inches of growth. This difference was not so great when the plants were placed

under the photoperiods at 12 inches or 20 inches of growth. In a few cases strains behaved differently under a given photoperiod; however, the same trend was noticed in all strains as shown for 1-10-20.

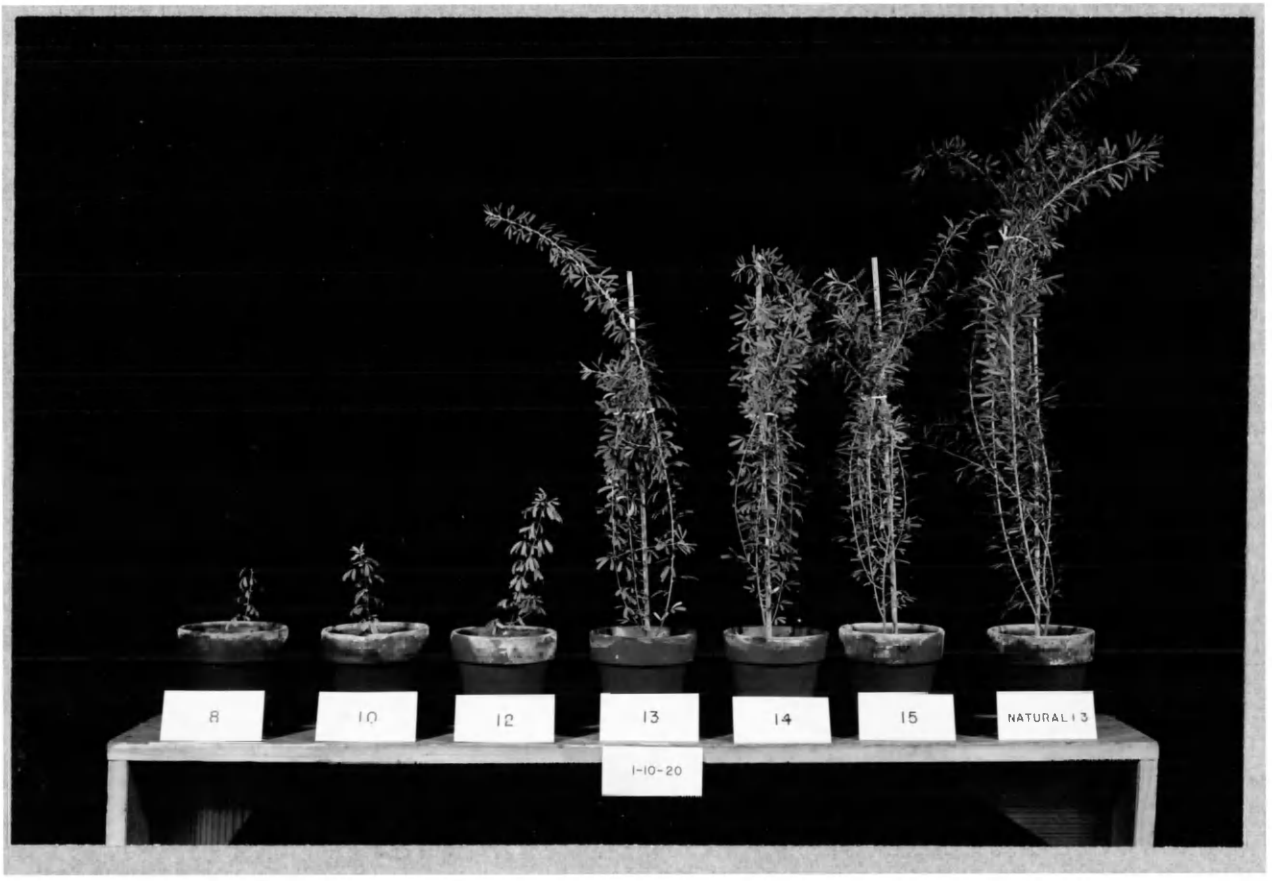


Figure 18. Plants of strain 1-10-20 of Lespedeza cuneata after remaining under seven different photoperiods for 90 days. Plants were placed under photoperiods at 2 inches of height.

The growth of different strains under two photoperiods are shown in figures 19 and 20. The plants were placed under both photoperiods at 2 inches of growth. The greatest interaction of strains as a result of a one-hour change in light appeared to be between 13 and 14 hours. These two pictures of the 13- and 14-hour photoperiods point out the fact that certain strains behaved differently when placed under different daily durations of light. Plants of strain 5-30-1 were the smallest under 13 hours while a plant of the same strain was one of the largest

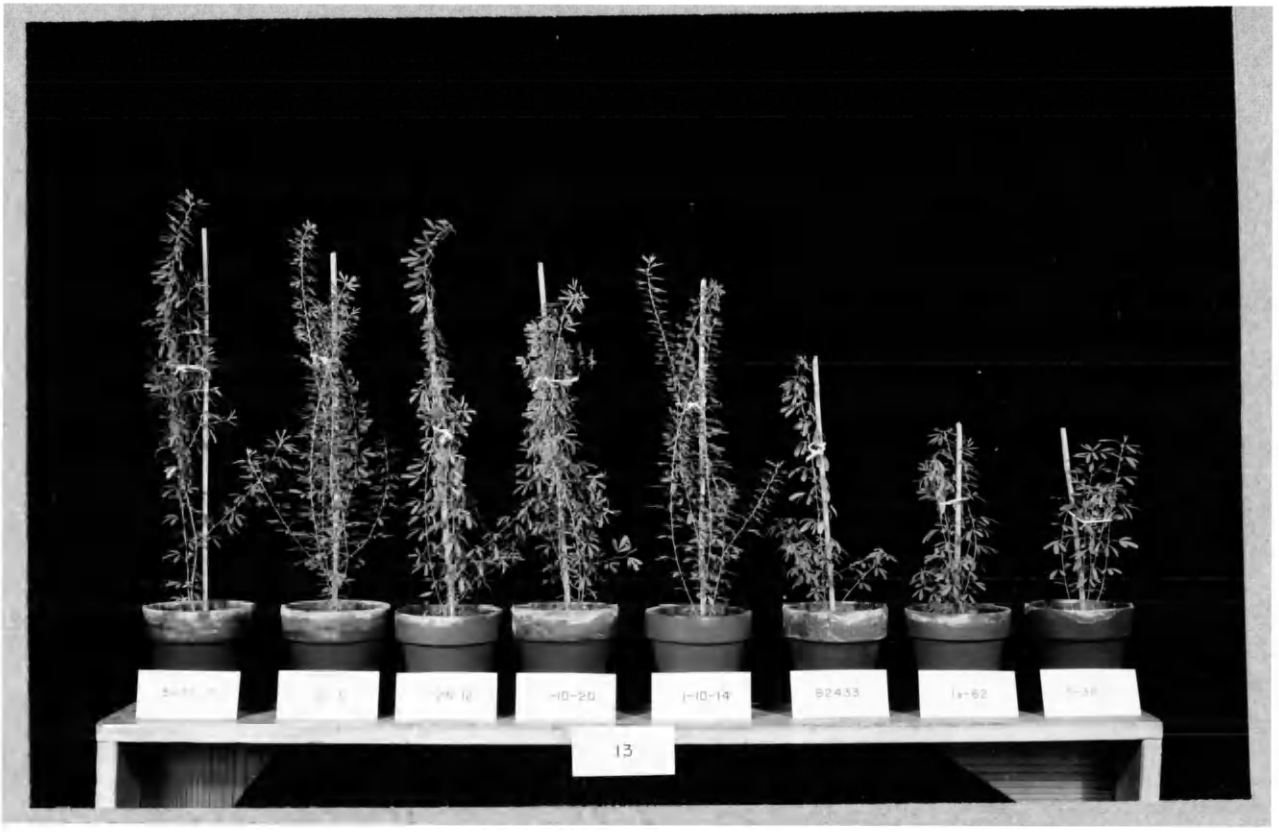


Figure 19. Plants of eight strains of Lespedeza cuneata placed under 13-hour photoperiod, after 90 days of growth. Plants placed under photoperiod at 2 inches of growth.

Figure 20. Plants of eight strains of Lespedeza cuneata after remaining under a 14-hour photoperiod for 90 days. Plants were placed under the photoperiod at 2 inches of growth.

under 14 hours. Plants of strains 5-30-7 and 1-25-12 were about the tallest under 13 hours, but were much shorter in comparison to plants of the remaining strains under 14 hours. It is very likely that other factors such as temperature, moisture and soil fertility may also affect some strains of Lespedeza cuneata much more than they do others.

Plants in the 2-inch, 12-inch, and 20-inch group behaved very differently as expected. The differences became smaller as the daily duration of light increased. Figure 21 shows some of the differences that occurred among age groups of plants of strain 5-30-7.

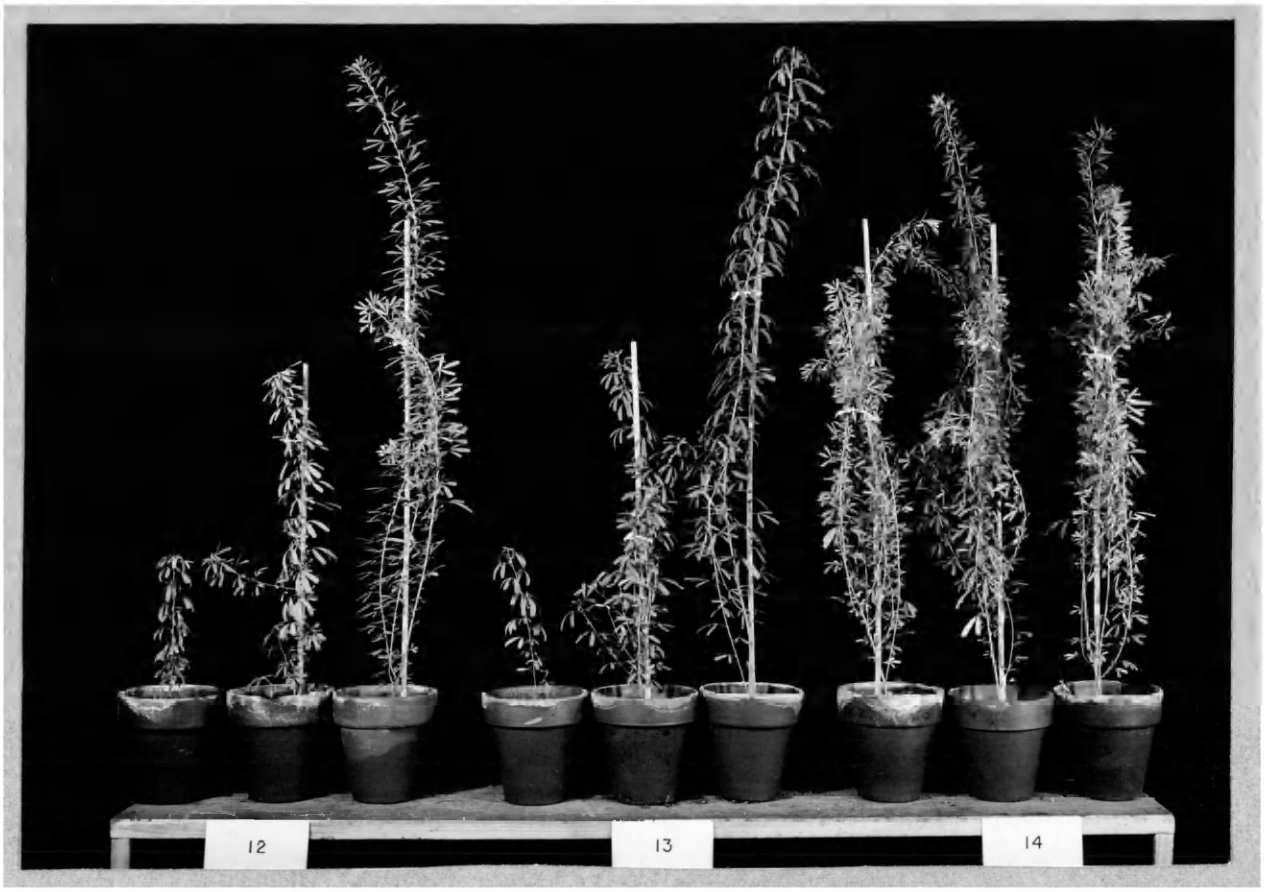


Figure 21. Plants of strain 5-30-7 of Lespedeza cuneata placed under 12-, 13-, and 14-hour photoperiods at 2, 12, and 20 inches of growth (from left to right under each photoperiod).

Table 5 summarizes the effect of stage of growth when the plants were placed in the various photoperiods. Each value represents the average of all strains. The number of days that plants of the 2-inch, 12-inch, and 20-inch age groups remained under the different photoperiods was 66, 40, and 14 respectively at the time measurements were made.

TABLE 5. Mean plant height of Lespedeza cuneata plants placed under six photoperiods at 2 inches, 12 inches, and 20 inches of height.*

Photoperiod (Hours of light per day)	Plant height when placed in photoperiod		
	2 inches	12 inches	20 inches
8	2.7	13.5	20.6
10	2.8	12.3	21.4
12	4.9	14.2	22.1
13	11.3	16.4	22.3
14	14.8	16.8	22.4
15	14.1	16.8	22.8
Mean	8.4	15.0	21.9

* Average of eight strains.

It is apparent that the critical daily duration of light for flowering is between 13 and 14 hours. Figure 22 illustrates the striking difference between plants grown under these two day lengths. The truck of plants on the left was exposed to 13 hours of light, while the truck on the right was exposed to 14 hours. The plants on the bench in the background received natural light plus 3 hours of artificial light during the middle of the night.

Figure 22 also indicates how the plants were handled in the greenhouse. The trucks were left in the greenhouse each day and each evening were pushed into various dark chambers equipped with lamps and electric time switches to supply the additional light needed to furnish a given day length.

Date of Blooming. Date of blooming was affected very greatly by different day lengths. Some strains, in certain cases, did not bloom at all, and only under one photoperiod in conjunction with one age group did they all bloom. Table 6 presents the number of days after planting that chasmogamous flowers first appeared on the plants.

Figure 22. A comparison of plants on the left truck grown under 13-hour photoperiod with plants on the right truck grown under 14-hour photoperiods. Plants were placed under both photoperiods at 12 inches of height. Plants on the bench remained under natural day, with 3 hours of light at midnight.

TABLE 6. Number of days after planting required for first chasmogamous flowers to appear on plants of eight strains of *Lespedeza cuneata* under four photoperiods.*

Strain	8 hours			10 hours			12 hours			13 hours		
	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch
1-10-14	x	x	93	x	x	93	x	x	93	118	x	118
1-10-20	x	x	93	x	x	93	x	79	93	73	79	104
1-25-12	x	x	x	x	x	x	x	x	105	73	83	104
5-30-7	x	x	x	x	x	93	x	79	93	73	79	101
5-38-1	x	x	x	x	x	x	x	x	x	118	x	x
1A-62	x	x	x	x	x	x	x	x	x	118	x	x
82433	x	x	93	x	x	x	x	x	x	118	x	118
Ga. 13	x	x	93	x	x	x	x	96	107	118	93	118

* Plants were placed under the respective photoperiod at 2 inches, 12 inches, and 20 inches of height.
x Indicates no flower formation.

This table shows that the majority of chasmogamous flowers were produced under 13 hours of light. Strain 1-10-20 was the most consistent in producing chasmogamous flowers, followed by strains 5-30-7 and Ga. 13. Strains 5-38-1 and 1A-62 produced flowers only under 13 hours of light, when started at 2 inches of growth. The photoperiods of 14 hours, 15 hours, and natural day plus 3 hours had not produced chasmogamous flowers at the close of this trial, 148 days after seeding.

Cleistogamous fruits were produced about the same time or slightly after chasmogamous flowers were produced. In several cases, however, cleistogamous fruits were produced even though no chasmogamous flowers were formed. Table 7 shows the conditions of photoperiod and initial plant height and number of days required to produce cleistogamous fruit.

TABLE 7. Number of days after planting required for cleistogamous fruit to be formed on plants of eight strains of Lespedeza cuneata under four photoperiods.*

Strain	8 hours			10 hours			12 hours			13 hours		
	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch
1-10-14	x	83	96	x	83	96	x	79	104	86	118	118
1-10-20	x	79	96	x	79	96	x	77	96	73	93	118
1-25-12	x	79	101	x	79	109	x	79	104	73	93	118
5-30-7	x	79	93	x	79	96	x	77	96	73	93	118
5-38-1	x	79	101	x	79	101	x	83	101	73	86	118
1A-62	x	83	101	x	x	101	x	83	104	77	90	118
82433	x	83	96	x	90	109	x	86	101	93	90	118
Ga. 13	x	93	96	x	86	101	x	79	101	93	101	118

* Plants were placed under the respective photoperiod at 2 inches, 12 inches, and 20 inches in height.
x Indicates no seed formation.

From this table it is apparent that when the plants were placed under light periods of 8, 10, 12, or 13 hours at 12 or 20 inches of growth, cleistogamous fruit were formed. There were no great differences among strains as was the case in formation of chasmogamous flowers. At the end of 148 days plants under 14 hours of light and those under natural day plus 3 hours of light at night had produced no cleistogamous seed.

Total Seed Production. All strains showed great difference in seed production in response to photoperiodic treatments. Table 8 shows that most seeds were produced under 13 hours of light, with no great differences among 8, 10, and 12 hours. Some strains produced considerably more seed than others. Ga. 13 showed the largest mean yield, while 1-25-12 and 1A-62 had the smallest. After 148 days no seeds were produced on plants under 14 hours and natural day plus 3 hours of light.

TABLE 8. Total seed production in grams per plant from eight strains of *Lespedeza cuneata* under four photoperiods.*

Strain	8 hours			10 hours			12 hours			13 hours			Means
	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	2 inch	12 inch	20 inch	
1-10-14	x	.016	.213	x	.013	.176	x	.083	.187	.003	.553	.642	.209
1-10-20	x	.028	.178	x	.033	.152	x	.058	.347	.110	.537	.782	.247
1-25-12	x	.003	.031	x	.003	.028	x	.032	.032	.042	.245	.542	.106
5-30-7	x	.020	.133	x	.033	.252	x	.061	.358	.676	.650	.573	.306
5-38-1	x	.004	.021	x	.004	.024	x	.003	.088	.724	.028	.231	.125
1A-62	x	.001	.031	x	x	.023	x	.003	.012	.178	.140	.468	.095
82433	x	x	.150	x	.002	.108	x	.003	.129	.490	.215	.735	.204
Ga. 13	x	.003	.520	x	.007	.172	x	.177	.287	.328	1.41	1.03	.437
Means	x	.009	.162	x	.012	.117	x	.053	.180	.319	.472	.509	

L.S.D. for:	5%	1%
Strains	.170	.230
Photoperiod x age group	.180	.240

* Plants were placed under the respective photoperiod at 2 inches, 12 inches, and 20 inches in height.

x Denotes no seed production.

Proportion of Cleistogamous and Chasmogamous Seed. Very few if any chasmogamous flowers on any of the plants produced seeds. Even the plants with many showy flowers produced very few seed from them. The chasmogamous flowers dropped off without producing seed. The reason for failure of these flowers to develop into seed is not known; however, seed production by chasmogamous flowers is frequently low under greenhouse conditions.

Tannin Content of Leaves. Tannin determinations were made in duplicate for each composite sample of leaves from six plants. Leaf samples were taken from the plants August 8. The formaldehyde-hydrochloric acid method of analysis was used. Table 9 shows the mean percentage of tannin for each strain under light periods of 8, 13, and natural day plus 3 hours.

TABLE 9. Percentages of tannin in eight strains of Lespedeza cuneata grown under three photoperiods, as determined by the hydrochloric acid-formaldehyde method of analysis.

Strain	8 hr.	13 hr.	Normal day plus 3 hr.	Mean
1-10-14	2.96	6.33	8.31	5.93
1-10-20	7.33	10.60	12.15	10.03
1-25-12	6.68	8.67	11.32	8.89
5-30-7	9.93	7.33	12.92	10.06
5-30-1	7.56	6.15	13.23	8.98
1A-62	5.20	7.91	11.23	8.11
82433	6.93	10.31	9.25	8.83
Ga. 13	6.78	9.29	13.54	9.87
Mean	6.67	8.35	11.50	8.84
L.S.D. for:		5%	1%	
	Strains	2.72	3.78	
	Photoperiods	1.52	2.12	

Determination of Tannin Content

Correlation of Analytical Methods. The official method of extracting and determining the tannin content of plants used for commercial sources of tannin requires from 24 to 48 hours. A quantity of material is required which will yield 4.00 ± .25 grams of tannin per liter of extract. About 60 to 80 grams of Lespedeza cuneata leaves are needed per determination of this type. In the inheritance study reported in this paper, neither time nor plant material was available for use of the official procedure.

A comparatively short method of tannin analysis has been used which involves the use of hydrochloric acid and 40 percent formaldehyde solution. A precipitate is produced by this method which is very stable and

probably contains compounds from the plant other than tannin. This method is described in the literature review of this paper. This method, as was modified, involved a 7-hour water extraction and as little as one gram of plant material was used. The method gave constantly higher values than the official method. On the average the values obtained by the formaldehyde method were 1.32 times larger than values obtained from the same samples by the official method. A number of samples of leaves were taken during the 1952 growing season and analyzed by the two methods. The correlation coefficient obtained between the two methods was r = .7929. With 13 degrees of freedom this value is highly significant. Table 10 presents the values obtained from 12 of the samples that were analyzed by the two methods, as well as rated by a third method.

TABLE 10. Value obtained for tannin content of twelve leaf samples as determined by the official hide powder method and formaldehyde-hydrochloric acid method of analysis as well as a relative rating by using ferric ammonium citrate.

Strain	Date sampled	Method of evaluating tannin content		
		Official method	Formaldehyde-hydrochloric acid method	Ferric ammonium citrate rating
4A	6/9/52	9.0	14.6	8
20A	"	10.0	11.1	8
22A	"	8.7	10.9	7
24119	6/11/52	10.9	10.2	8
121119	"	3.9	2.1	3
1-10-14	8/13/52	5.3	5.7	5
1-10-20	"	6.3	9.5	6
5-30-7	"	12.0	15.1	9
5-38-1	"	8.4	12.4	8
1A-62	"	8.9	14.7	8
9A-3	"	7.9	9.9	7
Ga. 35	"	8.8	12.9	8

Even though the formaldehyde method is much shorter and requires only a few grams of plant material, about 9 samples per day were all that could be analyzed. Since the number of plants to be evaluated for tannin in the inheritance study was very large, another method was tried. It was not intended to give the actual percentage of tannin in the plants, but to give only relative ratings among the plants. This method involved treating small strips of paper with a 2.5 percent solution of ferric ammonium citrate, allowing it to dry and squeezing a leaf between the folded paper. The darkness of the spot produced on the paper depends upon the tannin content of the leaf. Ratings of some samples are shown in table 10. A correlation coefficient between these ratings and the values obtained by the official method was $r = .9238$, which is highly significant with 10 degrees of freedom. The correlation coefficient between these ratings and values obtained by the formaldehyde method was also highly significant.

During the inheritance study the problem of whether tannin content of plants could be accurately evaluated in the greenhouse was encountered. Two different correlation coefficients were obtained between greenhouse and field grown plants. The plants were grown in the greenhouse, evaluated for tannin content, and later transplanted in the field; so that the values were on the same plants. Table 11 gives the correlation coefficients obtained as well as several other correlation coefficients.

TABLE 11. Correlation coefficients between several methods of evaluating tannin content of Lespedeza cuneata.

Methods or conditions	r
Hide powder and formaldehyde method	.7929**
Hide powder and ferric ammonium citrate rating	.9238**
Formaldehyde method and ferric ammonium citrate rating (1951)	.6600**
Formaldehyde method and ferric ammonium citrate rating (1952)	.8763**
Green and brown form of ferric ammonium citrate	.7412**
Greenhouse and field (ferric ammonium citrate method)	.7969**
Greenhouse and field (formaldehyde method)	.8986**

** Highly significant.

Extraction of Tannin. Six determinations, using the method described on page 8 under Materials and Methods, gave formaldehyde precipitate values ranging from 6.58 to 8.93 for sample 1-10-20. This range was considered too wide for duplicate determinations to be considered accurate. One obvious difference among the six 1-gram samples during extraction was the rate of filtration of water through the samples. In order to determine if this had any effect, different type extraction thimbles and different sized samples were compared. The results obtained are given in table 12.

TABLE 12. Effects of different extraction thimbles and different sized samples on the extraction of tannin from sample 22A-9 of *Lespedeza cuneata*.

Type of thimble	% formaldehyde precipitate from a 2-gram sample of 22A-9
Alundum	6.56
Glass, with coarse fritted glass plate	4.30
Glass, with perforated plate	9.29
L.S.D. at .05 level	3.05

Size of sample	% formaldehyde precipitate Glass thimble, perforated plate
0.5 gram	11.79
1.0 gram	10.59
2.0 grams	9.29
L.S.D. at .05 level	.95

From the values presented in table 12, it appears that 0.5-gram samples in glass thimbles with a perforated plate solves the problem of extraction. When six 0.5-gram samples of another source (4A) were extracted in the glass thimbles with perforated plates, however, a range of value from 8.22 to 13.32 was obtained. Some other source of variation seemed to be having an effect. Since the construction of the Smalley extraction tube allows entrance of steam only at the top, above the sample, the sample temperature during extraction is well below 100° C. In an effort to maintain the temperature of the sample during extraction near 100° C., the sample was suspended by a wire into the boiling flask.

This method is described on page 15 under Materials and Methods. The samples were treated with boiling water before they were placed into

the thimbles in an effort to prevent swelling and formation of air pockets in the sample during extraction. This eliminated most of the swelling. Different sizes of samples and different lengths of extraction were compared in this method. The results are presented in table 13.

TABLE 13. Comparisons of different sized samples and different lengths of extraction on the amount of tannin extracted from sample 4A of Lespedeza cuneata.

Size of sample	% formaldehyde precipitate 16-hour extraction
0.5 gram	13.01
1.0 gram	12.39
2.0 grams	13.18
Length of extraction	**% formaldehyde precipitate 1-gram sample**
.5 hr.	7.45
1 hr.	7.00
2 hr.	10.48
5 hr.	10.65
8 hr.	10.86
16 hr.	11.85
25 hr.	12.37
L.S.D. at .05 level	4.23

The values given in the size of sample comparison are greater than those given for the sixteen-hour extraction in the second comparison because in the first group fresh water was placed in the boiling flasks after two hours of extraction. This gives slightly higher values.

Three-hour extractions from a Lotus corniculatus and a Lespedeza cuneata sample on which hide powder analyses had been made gave 4.74 and 7.24 percent respectively. The hide powder values were 5.50 and

10.57 percent respectively. Six 3-hour extractions from sample 22A gave values ranging from 7.26 to 9.24 percent. Six 16-hour extractions gave values from 10.87 to 14.10 percent. In a comparison of water condensers with air condensers, while extracting sample 4A for 16 hours, mean values of 12.73 and 14.53 percent respectively were obtained.

The scheme of boiling the sample in water as described in number 3 on page 15 under Materials and Methods gave very low values. One-gram samples of material that analyzed 5.50 and 10.57 percent by the hide powder method gave mean values of 2.18 and 4.12 respectively when boiled loose in water for 16 hours. When the samples were enclosed in closely woven cloth bags, these values were increased to 2.27 and 6.96 percent respectively. Because of the very low values obtained, this method was not studied further.

Extraction with acetone-water mixtures as described on page 23 under Materials and Methods was tried with several samples.

A method as described on page 23 under Materials and Methods whereby the extract solution was delivered from the extraction tube to prevent refluxing was tried with air condensers. In this method the temperature of the sample during extraction seemed to affect the completeness of the extraction. Four slightly different extraction tubes were constructed to allow the sample temperatures to vary while the heater temperatures were the same. The effects of different temperatures on the extraction efficiency are shown in table 14.

TABLE 14. Effects of sample temperatures during extraction on amounts of tannin extracted from a sample that was determined to be 10.57 percent of tannin by the hide powder method.

Temperature of sample	Mls. of water passed through sample	% formaldehyde precipitate
78° C.	410	7.90
88° C.	450	12.65
93° C.	450	13.20
100° C.	350	14.76
L.S.D. at .05 level		1.45

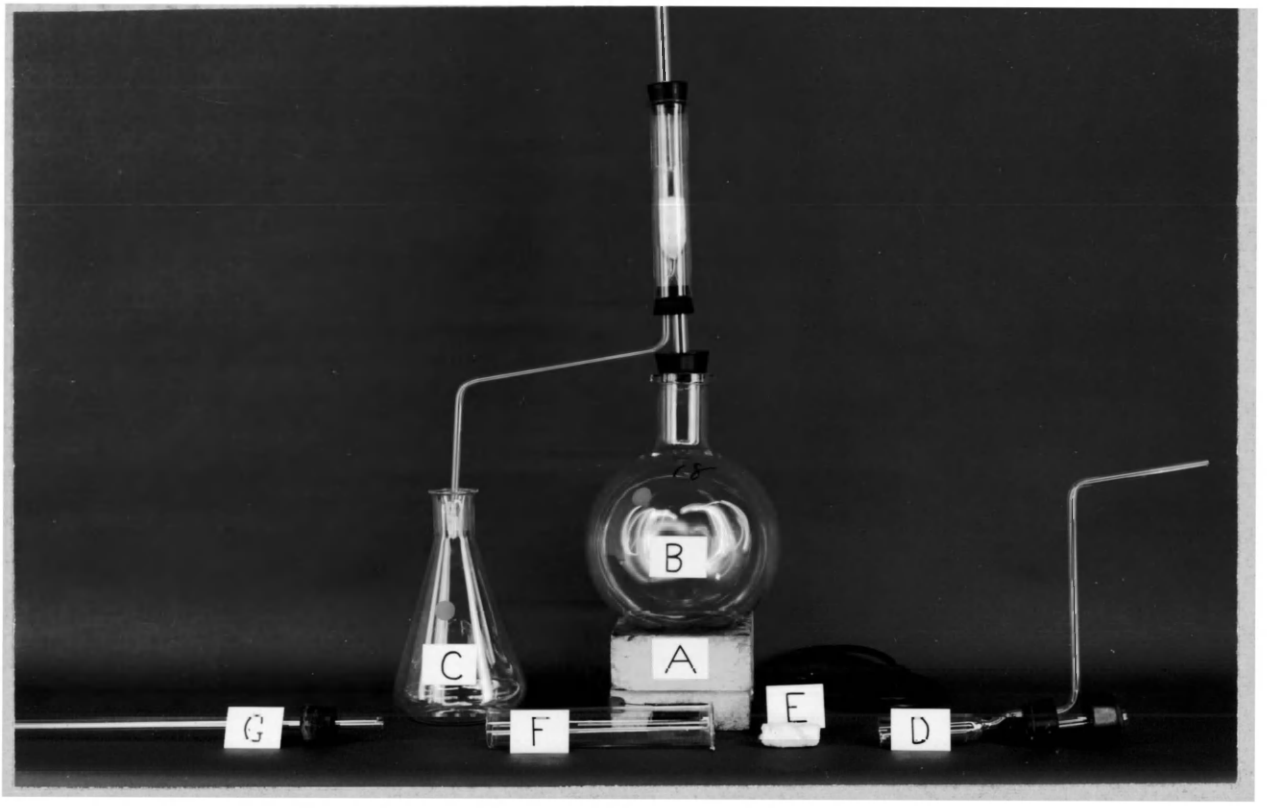
Because of the construction of the tubes some of the water collected was condensed outside of the extraction thimble at the three lowest temperatures and did not pass through the sample. It is felt, however, that at least 350 ml. passed through each sample. Table 15 shows that volume of water had little effect when the sample temperature was maintained at 100° C.

TABLE 15. Effect of volume of extraction water on extraction of tannin from a sample which contained 10.57 percent tannin when analyzed by the official hide powder method.

Volume of water passed through sample	% formaldehyde precipitate
350 ml.	14.76
430 ml.	14.90
500 ml.	14.40
520 ml.	14.28
570 ml.	14.70
670 ml.	14.99

This method gave by far the highest and most uniform results of the methods investigated. An extraction tube was constructed which is similar to the official tannin extractor except that it is smaller and better adapted to small samples. In comparing the small extractor with the large official extractor, duplicate determinations in each type of tube of samples from 13 sources gave mean tannin percentages of 9.16 for the official tube and 9.15 for the small tube. These very similar values were obtained by using 1-gram samples. Larger samples were tried in the official extractor, but results were unsatisfactory. One liter of water was passed through 25 grams of material containing approximately 15 percent tannin, and a value of 12.28 percent was obtained. Two liters of water passed through 50 grams of the same material resulted in a value of 11.52 percent. The small extractor using 1-gram samples gave a mean percentage of 15.05 for the same material. A picture of the small extractor is shown in figure 23.

Mean percentages for sample 4A as determined by a number of the modified methods, along with the range of variations for each are presented in table 16.



A - heater, B - boiling flask, C - receiving flask, D - thimble and delivery tube of small extractor, E - sample rolled in cotton, F - jacket of extractor, and G - air condenser.

Figure 25. The apparatus that gave best extraction of tannin from Lespedeza cuneata.

TABLE 16. Mean and range of tannin percentages obtained from sample 4A by several methods of extraction. Extract was analyzed by the hydrochloric acid-formaldehyde method.

Method	Mean percentage	Range of percentages
Reflux, Smalley tube, .5 gm.	10.42	8.22 to 12.32
Reflux, sample in steam, 1 gm. 3 hr.	10.35	9.04 to 11.48
Reflux, sample in steam, 1 gm. 16 hr.	12.73	11.92 to 14.10
Reflux, same as above, air condensers	14.55	14.00 to 15.06
Boil sample loose in water for 4 hr.	11.07	10.46 to 11.60
Boil sample in cloth bag for 16 hr.	9.07	8.79 to 9.17
Official extractor, 25 or 50 gm.	11.76	11.35 to 12.28
Official extractor, 1 gm. sample	14.04	13.44 to 15.04
Small extractor, 1 gm. sample	15.85	15.22 to 16.20
L.S.D. at .05 level	1.85	

DISCUSSION

Inheritance Studies

The inheritance studies of several characters of *Lespedeza cuneata* gave several indications of what might be expected in a breeding program with this species. Probably the most important character studied was tannin content. This work leaves no doubt that plants can be selected which are lower in their tannin content than the average tannin content of commercial seed stocks. It was also indicated that this character can be transferred by hybridization, and that plants of varying tannin content can be selected from segregating populations following the cross. It was also apparent that the inheritance of tannin content is complex and that genetic factors, in some cases, do not account for as much variation as environmental factors. The genetic factors involved in the inheritance of tannin content do not appear to be strongly linked with genes controlling other characters studied. The only evidence of linkage with tannin content was in the case of seed color. In the F_2 population, plants containing the least tannin had green seed while plants higher in tannin had purple seed. There were some exceptions to this, however, and no doubt plants with low tannin content could be selected which also have purple seed. This type of combination might be desirable for placing a marker of seed color on a low tannin variety. A few plants were recovered in the F_2 populations that appeared to have a tannin content just as low or even lower than the lower parent. This indicates that the hybridization of two plants relatively low in tannin might yield new genetic combinations in the segregating generations that would have a still lower tannin content.

Plant height appeared to be heritable in the two crosses studied. The effects of non-heritable factors, however, were quite large. The heritability percentages obtained for the two crosses (53.8 and 59.8 percent) agree very well with heritability values obtained for the same character in soybeans by Weber and Moorthy (29). Some evidence of hybrid vigor was present in one cross; however, this was not true in the other cross. In the F_2 population following both crosses plants were present which did not resemble either parent and some appeared to be a more desirable type than either parent. This study indicates that such characters as large leaves and increased leafiness can be combined with fine stems to give a more desirable plant type. From this study it appears that by a backcross program plant height and growth type could be combined with any of the other characters studied. The only signs of linkage involving plant height was with maturity and seed size. Both of these cases were in cross C where the difference in plant height between the parents was not very great.

Variation of maturity in plants of the F_2 populations was found to be primarily due to heritable factors. Neither of the segregating populations showed a normal distribution. There were definitely more late maturing plants. The late transplanting of plants to the field probably explains why the F_2 distributions were skewed toward late maturity. This skewness was much more pronounced in cross C. Even though the inheritance of maturity appears to be rather complex, it appears quite evident from this study that maturity date can be transferred from one strain to another in Lespedeza cuneata. The only characters closely related to maturity were seed size and seed color. These relationships probably existed because on the late plants many seed were immature when the seed were harvested.

Distributions for flower color in the F_2 populations were much like those for maturity. Flower color does not appear to be affected by the age of the plant at flowering time; therefore, the skewness of these distributions are not due to the late transplanting of the plants to the field. From the F_2 distributions one might think that the greater frequency of cream flowers was due to dominance. In the F_1 generation, however, the flowers were purple, indicating that purple is dominant. The inheritance of flower color did not appear to be as complex as most of the other characters studied and had the highest percentage of heritability, with the possible exception of seed color. Flower color was strongly associated with plant color, and the independent transfer of flower color without plant color would probably require large populations in generations following a cross.

Plant color variations showed up only in early season just after the plants were transplanted to the field and in late season while the plants were blooming. It is quite possible that in regions where sericea lespedeza is better adapted this characteristic might not become evident. In areas with a longer growing season the plants might be mature before the temperature became low enough to cause formation of the pigment. This color was not correlated with tannin content or growth type and probably would have no importance in the use of sericea lespedeza.

Seed color appeared to be controlled by one or two gene pairs. Due to the late maturity of many F_2 plants, it is possible that some plants had a genotype for purple seed but the phenotype was green because all the seed on the plant were immature. In the F_2 populations the number of plants with all green seed were about one-third the

number with some purple seed, indicating that perhaps one pair of genes is involved in the inheritance. The purple color referred to is a solid deep purple. In Lespedeza cuneata seeds are also present which have purple mottled seed coats. In the F_2 populations of these two crosses none of this mottling was found, indicating that perhaps this is a different set of genetic factors. Due to the lack of time and the unimportance of the mottling character its inheritance was not studied further. The solid purple color might be desirable as a marker in an improved variety of sericea. There was some evidence of linkage between seed color and tannin content. In general the plants with purple seed had a relatively high tannin content; however, there were some exceptions, and probably this character could be combined in a low tannin strain.

Seed size is considered a rather important character from the point of view that increased seed size will result in increased seedling vigor. This has been indicated with sericea seedlings in the greenhouse. In general the seedling vigor of this species is very poor and the establishment is very slow. Very little if any use is made of the first year's growth due to the slow rate of establishment. This study of the inheritance of seed size indicates that this character is controlled largely by genetic factors and that the inheritance is rather simple, involving about four pairs of genes. There appears to be no linkage between seed size and tannin content or growth habit. By hybridization one should be able to transfer this character to low-tannin lines of sericea lespedeza. In this study seed of the large-seeded parent weighed about 1.7 grams per 1000 seeds. Some individual plants have

been selected which produce seed that weigh over 2 grams per 1000 seeds. The weight of an average lot of seed is about 1.4 to 1.5 grams per 1000 seeds.

A character in Lespedeza cuneata that is probably not important agronomically, but which might have some importance in a breeding program is the proportion of seed produced from chasmogamous and cleistogamous flowers. In a breeding program cleistogamous flowers provide an easy way of obtaining selfed seed, while if hybridization is practiced a rather high proportion of chasmogamous flowers are desired. Several workers have indicated that the proportion of these two types of flowers occurring on a plant is controlled by a number of environmental factors. During this study temperature and day length was found to greatly affect this character. When several strains were grown under the same conditions a great difference in the proportion of the two types of flowers existed. Since two crosses involved parents with extremes of this character, an effort was made to determine whether the character is inherited. This study indicates that the proportion of seed produced by chasmogamous and cleistogamous flowers is inherited, but the inheritance is very complex. The heritable portion of the variation comprised only about one-third of the total variation.

Photoperiodic Response

Lespedeza cuneata is extremely sensitive to photoperiodic treatments. The results of this study show much variation in plant growth, date of blooming, seed production, and tannin content due to different lengths of day. Plant growth was almost inhibited by placing plants under an 8-hour day. Very little increase in growth was made with

10 and 12 hours of light. In some strains plants under a 13-hour day made considerable growth, while plants of other strains grew very slowly. When some of these slow-growing strains were given an additional hour of light they grew very rapidly. The difference in response of the different strains indicates that strains could probably be developed that are better adapted to a given location. The fact that some strains made good growth under several photoperiods indicates that some strains might have a wider range of adaptation than others.

This study indicates that Lespedeza cuneata produces chasmogamous (showy) flowers under about a 13-hour day. Some strains behave differently, but in general most strains will probably bloom under this photoperiod. Likewise, the 13-hour day seems to favor production of cleistogamous flowers of all strains. Total seed production was also greater under this day length. There were large differences in the ability of strains to flower and produce seed. These studies were under greenhouse conditions, and whether the same relative amounts of seed would be produced under field conditions is not known.

Tannin content appeared to be affected greatly by different day lengths. Plants grown under three different photoperiods were analysed for tannin. The mean percentage of tannin in leaves of the eight strains under 8 hours, 13 hours, and normal day plus three hours of light at midnight was 6.7, 8.4, and 11.5 respectively. All strains did not behave the same under the three photoperiods. By an increase in the day length, some strains were affected much more than others. This interaction between strains and photoperiods indicates that low-tannin lines might have to be developed in the area in which they are to be used, and also that palatable lines may be developed more successfully in some areas than

in others. Many other environmental factors will enter into determining the tannin content of a given field of sericea lespedeza.

Determination of Tannin Content

No very accurate short methods have been devised for the determination of tannin content in plant material. To date probably the best method to use for single plant selections from a large, variable population is the relative test with filter paper treater with ferric ammonium citrate. The use of this method could very well be supplemented with the hydrochloric-acid, formaldehyde method for checking various selections and differences among strains. The ferric ammonium citrate method does not give the actual percentage of tannin in a plant but merely the amount in comparison to other plants. The hydrochloric acid-formaldehyde method does give a fairly good estimation of the tannin content if the fact is kept in mind that it yields slightly higher values than the hide powder method. If a few samples of the material being studied are analyzed by the hide powder method a conversion factor can easily be obtained There is need for a more accurate and convenient method of tannin analysis with rather small samples of plant material. More information is also needed on the compounds involved in the tannin complex and factors affecting the formation and break-down of this complex.

One problem encountered in the hydrochloric acid-formaldehyde method was the extraction of tannin from small samples of herbaceous material. Too much variation occurred among several determinations of the same sample. Rate of percolation, circulation of water through the sample, and temperature of the sample and extraction water appeared to account

for most of the variation. A small extractor was made that resembles the official tannin extractor, except it is better adapted to small samples. With this extractor variation among a number of determinations of the same sample was reduced greatly. Sometimes, however, there was more variation than at others. The extraction of tannin from small samples was very erratic but merits improvement.

The determination of tannin content of plants growing in the greenhouse under long days and rather high temperatures appears to give a good indication of the relative tannin content of the plants when grown under field conditions. Tannin content appears to remain relatively the same in different strains at different stages of growth. In a breeding program involving tannin content of Lespedeza cuneata the screening out of high tannin plants in the greenhouse, while the plants are 12 to 14 inches of height, before they are transplanted to the field seems to be one method of increasing the number of plants evaluated.

SUMMARY

Three crosses were made between individual plants of Lespedeza cuneata. Parents were selected so that some indication could be obtained of the inheritance of eight characters in two different crosses. In cross A tannin content, plant height, maturity, seed color, and seed size were involved. In cross B tannin content, plant height, flower color, plant color and proportion of seed produced from chasmogamous flowers were studied. Cross C involved maturity, flower color, plant color, seed size, and proportion of seed produced from chasmogamous flowers.

The F_2 distributions for each character are given along with the estimated heritability and gene number. Correlation coefficients were calculated between all characters. Tannin content appears to be inherited with the number of gene pairs involved being about 20 to 25. Approximately 40 percent of the variation in the F_2 populations was accounted for by heritable factors. In one cross tannin content was associated with seed color. This was the only indication of linkage involving tannin content. Plant height of mature plants was controlled by about 34 gene pairs in cross A, while cross B involved about 13 pairs. About 55 to 60 percent of the variation in the F_2 was due to genetic factors. In cross C, plant height was negatively correlated with maturity and seed size. There was no indication of linkage between plant height and any of the other characters. Approximately 90 percent of the maturity variation in the F_2 populations was accounted for by heritable factors. The estimated gene number in cross A was 22 while

in cross C it was only ten. There was some evidence of linkage between maturity and tannin content, seed color, and seed size. Inheritance of color appeared to involve about ten pairs of genes, and about 92 to 93 percent of the variation was due to genetic factors. Flower color was rather strongly linked with plant color, and showed some evidence of linkage with seed color. Plant color was also correlated with percentage of seed produced from chasmogamous flowers in cross C. The heritability for plant color was estimated to be about 90 percent, while the number of gene pairs involved in the inheritance of this character appeared to be 12 to 16. The solid purple seed color appeared to be simply inherited. No plants were found in the F_2 with a flecked seed coat which is often present in Lespedeza cuneata. This indicates that the solid purple and flecking colors might be controlled by different genetic factors. Seed size appeared to be rather simply inherited, with about four gene pairs involved. The heritability values for seed size were 91 and 75 percent. The proportion of seed from chasmogamous flowers was very complex in its inheritance, with the number of gene pairs being 102 and 58 for the two crosses. Only about 36 percent of the variation in the F_2 populations was due to genetic factors. The difference in the estimated gene number involved in some of the characters between the two crosses indicates that in this species a given character might be controlled by different sets of genetic factors, depending upon the plant material being crossed.

Lespedeza cuneata was extremely sensitive to various photoperiods. Very little plant growth was obtained under photoperiods under 13 hours. The 13-hour day gave fair growth and brought about the most flowering

and seed production. The 13-hour photoperiod was the only treatment which resulted in the production of chasmogamous flowers by all eight strains. Very few, if any, seed were produced by chasmogamous flowers. The increase in day length caused an increase in tannin content of the leaves. Under 8 hours, 13 hours and natural day plus 3 hours at midnight the mean percentages of tannin in the leaves were 6.7, 8.4, and 11.5 percent respectively. The strains of Lespedeza cuneata behaved differently under different photoperiods.

In a breeding program where a large number of individual plants are to be evaluated for tannin content, the ferric ammonium citrate method of rating the plants appears to be a desirable method to use. Another method such as the hydrochloric acid-formaldehyde technique could very well be used for evaluating strains and varieties. In this method the tannin extraction appears to be the greatest problem. In order to reduce variation in extraction such factors as percolation of extraction water, temperature of water and sample, and filtration of water through the sample must be kept constant. In an effort to achieve this goal a small extractor was developed which greatly reduced this variation.

From this study it appears that plants can be accurately evaluated for tannin content in the greenhouse. The tannin content is lower under greenhouse conditions, but appears to remain relatively the same among plants when grown under field conditions.

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