AN ABSTRACT OF THE THESIS OF

Thomas Benenati for the Master of Science in Biological Sciences presented on December 8, 2000.

Title: Effect of plant density on chasmogamy and cleistogamy in *Lespedeza cuneata* (Fabaceae).

Abstract approved: 

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Lespedeza cuneata (commonly "sericea") is an invasive, exotic, perennial legume. Sericea can produce two types of flowers on the same plant: self-pollinating cleistogamous (CL) and open-pollinating chasmogamous (CH). The purpose of this study was to determine if the ratio of CH to CL flowers is affected by plant density. Flowering in low-, medium-, and high-density stands of sericea in a Greenwood County, Kansas, pasture was monitored over the 10-week flowering period, August to October, 1999. Two-way analysis of variance of these data indicated no difference among density treatments in CL flowering ($P = 0.168$). Significant variation was found among density treatments in CH flowering ($P = 0.041$). Cleistogamous and CH flowering varied significantly among weeks ($P < 0.001$). Pastures in which sericea density is reduced by weed control treatments that fail to eliminate all sericea plants will likely produce a higher proportion of CH flowers in the remaining plants than

found prior to the control treatment. Heterosis in these remaining plants could produce a more vigorous infestation than that prior to weed control treatment.

Effects of plant density on chasmogamy and cleistogamy in *Lespedeza cuneata* (Fabaceae)

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A Thesis Presented to

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Introduction

The purpose of this study was to determine if the ratio of chasmogamous to cleistogamous flowers in *Lespedeza cuneata* (also known as sericea lespedeza and Chinese bush clover) (McGregor, 1986) is affected by plant density. *Lespedeza cuneata* is a perennial legume introduced from Asia into the southeastern United States (Clewell, 1966) in the 1930s (McGregor, 1986). It is used in the southeastern U.S. as forage and in soil erosion management (Clewell, 1966). *Lespedeza cuneata* was introduced into Kansas in the 1930s and planted land surrounding federal and state reservoirs in the 1940s and 1950s (Ohlenbusch, 2000). *Lespedeza cuneata* is less palatable to cattle than other range plants, and can replace more favorable species (Ohlenbusch, 2000). In the 1980s the State of Kansas provided counties the option to declare *L. cuneata* a noxious weed; on July 1, 2000, it became a statewide noxious weed (Kansas Statutes Annotated 2-1314). This places it in the unique position of being both a commercial crop in the southeastern U.S. and a noxious weed in Kansas.

Lespedeza cuneata can produce two types of flowers on the same plant: cleistogamous (CL) and chasmogamous (CH) (Hanson and Cope, 1955) (Fig. 1). In *Lespedeza*, CL flowers are very small (1.0-1.5 mm long at maturity) and self-pollinating within a closed bud. Chasmogamous flowers are larger (approximately 6-10 mm long) and potentially out-crossing (Hanson and Cope, 1955). It is unknown to what extent CH flowers self-pollinate.

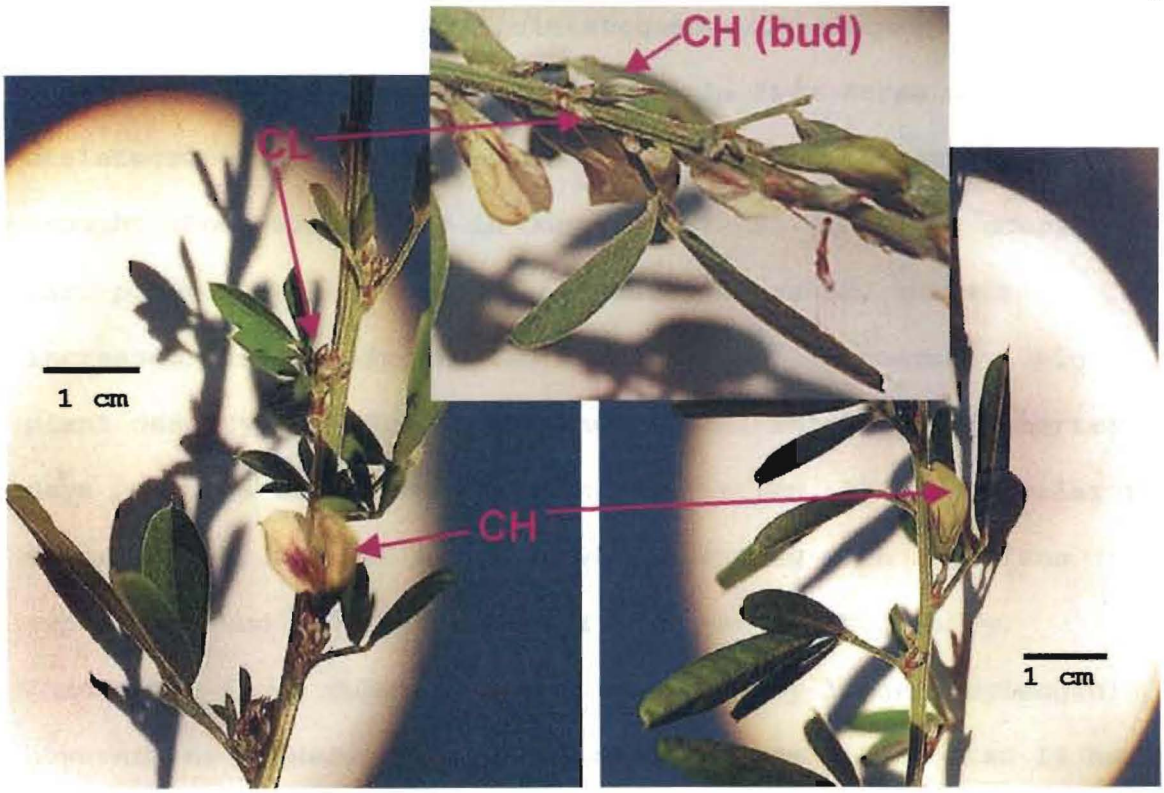

Figure 1. Cleistogamous (CL) and Chasmogamous (CH) flowers of *Lespedeza cuneata*. The axillary inflorescence can contain various combinations of CL and CH flowers.

Flowering response of cleistogamous/chasmogamous plants to their environment varies among taxa. In five *Stipa* species, cleistogamous flowering results from cooler temperatures and drought (Ponomarev, 1962 *in* Donnelly, 1979). In *Viola odorata* var. *praecox* Gregory, increased shade favors CH, whereas increased sunlight favors CL (Madge, 1929). Conversely, higher plant density (crowding), poor nutrition, shading, and shorter days promotes CL in *Bromus carinatus* (Harlan, 1945). Similar to *B. carinatus*, Schmitt et al. (1987), working with *Impatiens capensis* found an increase in CH at lower plant density. Chasmogamy in *L. cuneata* might be favored by longer daylength; however, no flowering occurs in photoperiods longer than 14 hours (Bates, 1955). Cleistogamy in *L. cuneata* is favored by repeated mowing (Donnelly and Patterson, 1969).

Study of *L. cuneata*'s flowering strategy is important for two reasons. First, an increase in plant vigor, heterosis, may result from CH outcrossing. Donnelly (1979) found that in *L. cuneata* grown in northern and central Alabama, CH flower outcrossing produced more vigorous plants (exhibiting shorter internodal space and a more erect stem habit). This has not been confirmed in Kansas. Whereas Donnelly's studies aimed at improving the vigor of cultivated *L. cuneata*, the research is also applicable to weed control. Altering the ratio of CH to CL flowers might alter the vigor of weedy *L. cuneata* populations. Weed control methods, which thin population densities but do not affect all plants, might instead promote CH flowering and

facilitate heterosis among the remaining plants, thus improving the vigor of the offspring of the surviving population.

Further, Donnelly (1979) showed that chasmogamy in *L. cuneata* is a "highly heritable" condition. Thus, the continued progeny of plants arising from survivors of an incomplete herbicide application, mowing, or burn treatment would also likely have high percent chasmogamous flowering, further encouraging heterosis. *Lespedeza cuneata* recovery in incompletely-treated *L. cuneata* stands might be accelerated and result in a more vigorous population than in completely treated stands. Additionally, chasmogamy in *L. cuneata* populations of different densities might be more common during a certain time of the flowering period.

A second justification for this research is to identify if less dense, scattered populations of *L. cuneata*, such as those found on the edge of the population's range, are more likely to produce CH flowers than more established, dense stands. If this were the case, it would underscore the need to address a *L. cuneata* invasion into a pasture quickly, before heterosis among the early invaders creates a vigorous population of plants. The possibility of a high degree of CH flowering exhibited by early *L. cuneata* invaders into a pasture or rangeland is alarming. These early-invading "shock-troops" might exhibit a high-CH flowering strategy more dangerous and potent, in terms of offspring vigor, than established, more densely crowded, CL-flowering plants.

Preliminary Study

Materials and Methods: I undertook a preliminary study to make observations concerning cleistogamous (CL) and chasmogamous (CH) flower phenology, morphology, and development, and to prepare observational methods for the primary study. Of special concern was the need to develop a field-practical method to easily distinguish vegetative buds, CH buds, and CL flowers (recalling that CL flowers undergo anthesis within a closed bud). On January 20, 1999, I transplanted five *Lespedeza cuneata* plants from a population near Melvern Lake, Osage County, Kansas into 25-cm pots containing only soil from the collection site. I clipped the dried stems (ramets) from the previous season to approximately 5 cm and placed the plants in the Emporia State University greenhouse in Emporia, Kansas. Approximately every 3 d I watered the plants, labeled A-E, until water flowed from the bottom of the containers. To supplement the available natural daylight, on February 9, 1999, I activated one 400-watt Phillips C400S34 USA70M-2 lamp in a P.L. Light Systems-Canada LR4887712 fixture above the plants, and set it to a 12 h photoperiod. Total light intensity at mid-ramet level measured on February 22, 1999, at 0900 hrs under overcast cloud conditions was approximately 150 microeinsteins$\cdot m^{-2} \cdot s^{-1}$.

I sequentially numbered ramets as they developed from the crown of each plant (e.g., the first ramet of plant A was A1, the second A2, etc.). Throughout the early growing period, I

attempted to identify CH and CL buds with a hand lens. One week after anthesis of the first CH flower, I collected a ramet containing multiple unknown buds and open CH flowers. I dissected the open CH flowers and unknown buds using a Nikon Type 102 dissection microscope. I recorded the dissection using the 30x and 200x magnification modules of a Scalar VideoLoupe VL-7EX hand-held microscope and a Quasar VV-2009 monitor/VCR.

Preliminary Study Results: Plant E failed to develop any vegetative growth, and was discarded. Plants A-D developed between four and 22 ramets by March 19, 1999. Some axillary stem growth also occurred. Hand lens observation of the longest ramets revealed two type of buds: small buds (approximately 2 mm in length) with approximately 0.5 mm calyx teeth, and larger buds (to 4 mm in length) with 1-2 mm calyx teeth. The ramet harvested for dissection was 68 cm long and exhibited axillary closed buds over the lower 58 cm of the ramet. Moving acropetally, this lower region was followed by a 5-cm intermediate region of opening CH flowers, large, long-toothed buds, and small, short-toothed buds. The terminal 5 cm stretch of the ramet had only closed buds.

The immature size of the buds in the proximal 58 cm and distal 5 cm made it difficult to distinguish the two bud types from each other, and flower buds from axillary vegetative buds. Distinguishing bud type was possible only in the intermediate region of the ramet. The first signs of flowering occurred in

this intermediate region of the ramet, and flowering proceeded acropetally and basipetally along the ramet from this region.

Dissection microscopy of the small, short-toothed, closed buds from the intermediate region of this ramet revealed young ovaries with sharply curved, persistent styles. Further, the anthers were withered, indicating dehiscence had already occurred within the closed bud. Sharply-curved styles are evidence of cleistogamy; the style is curved down towards the anthers of the same flower to allow self-pollination (Hanson and Cope, 1955). Dissection of the larger, long-toothed buds and open flowers revealed a straight or only slightly curved style protruding beyond the androecium.

These preliminary results established a field-practical protocol that defined the two flower types for the primary study. I designated the larger buds with longer calyx teeth and emerging corolla, as well as the subsequently-opened petaliferous flowers as chasmogamous. I designated the smaller non-opening buds with shorter calyx teeth as cleistogamous. This is consistent with McGregor's (1986) description that calyx teeth of CL flowers of sericea are typically 2-3 mm long, while teeth of CH flowers are 5 mm long. I relied on the calyx teeth length and bud sizes heavily when distinguishing CL buds from closed CH buds. Microscopy also revealed axillary inflorescences composed of developing CL seed, as evidenced by the hooked persistent style, and CH flowers.

Primary Study

Materials and Methods: In May 1999 I selected a 50 m^2 study site at the United States Army Corps of Engineers Fall River Water Resources Development Project in Greenwood County, Kansas (Fig. 2). The site was located at T27S R12E S22 NW1/4 SW1/4 SW1/4, or LAT: 37°41'12.04" LNG: 96°05'17.01" (Ashtech Reliance Submeter FS/2 GPS System). The site is an upland pasture on Woodson soil series, ungrazed since 1991. The elevation is approximately 303 meters. The study site is outside the flood control pool of the reservoir. Table 1 describes the plant species present at the site.

I chose three *L. cuneata* densities as treatment groups. The low-density treatment (LD) contained 60-75 stems/m^2, the medium density (MD) contained 200-250 stems/m^2, and the high density (HD) contained 650-800 stems/m^2 (values include all elongated axillary stems, as well as primary axes). I randomly selected three replicate 1-m^2 plots of each treatment by blindly throwing a flag into a stand of the appropriate density. I noted the flag's resting position as the NE corner of the plot, and staked the corners of the plots with 0.5 m wooden stakes. I then adjusted two graduated meter sticks, connected at the zero mark, to a 90° angle and placed along the edge of each plot, creating a 10,000-cm^2 grid. I used a Casio FX-260 calculator with a random number function was used to define coordinates for ten points within each plot. I then located the stem at the selected point and labeled it with a 4-cm^2 paper tag. I labeled a mixture of

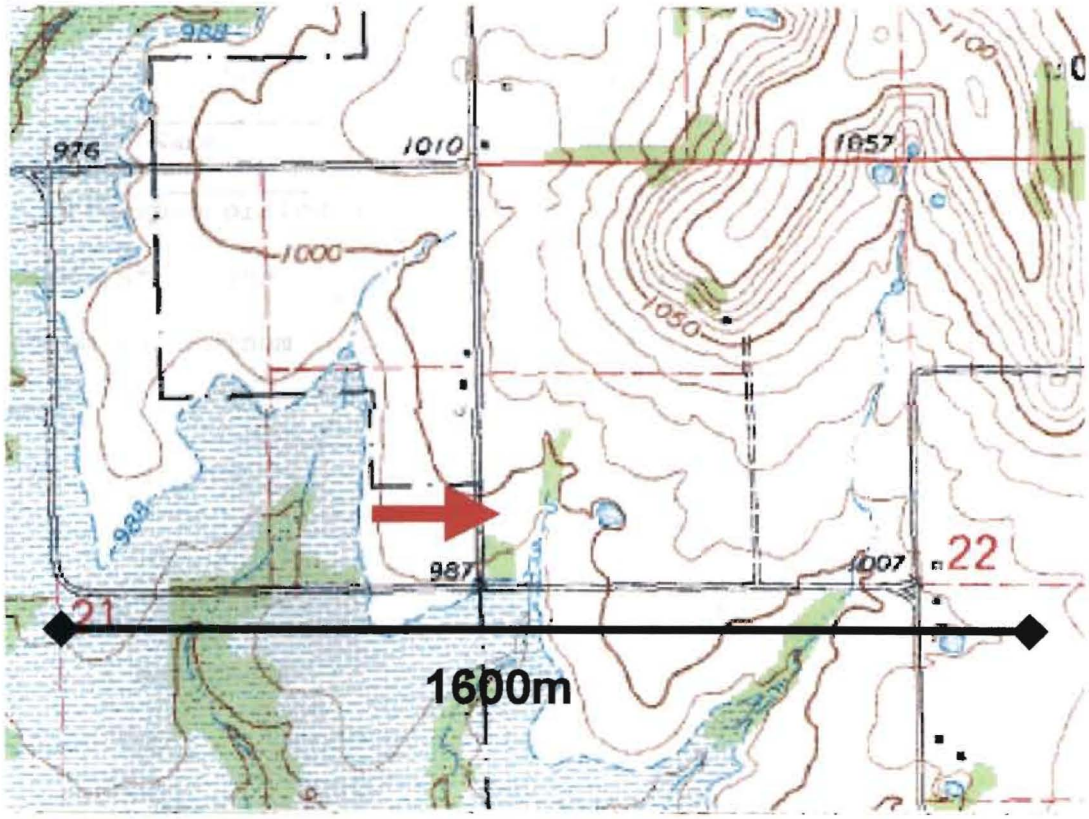

Figure 2. 1:25,000 topographic map of study area. The Greenwood County study site (T27S R12E S22 NW1/4 SW1/4 SW1/4) is indicated with the red arrow.

Table 1. Plant species observed at study site.

Scientific Name	Common Name
Symphoricarpos orbiculatus	Coralberry
Erigeron strigosus	Daisy fleabane
Apocynum cannibinum	Hemp dogbane
Bromus inermis	Smooth brome
Maclura pomifera	Osage orange
Andropogon gerardii	Big bluestem
Andropogon scoparius	Little bluestem
Euphorbia marginata	Snow-on-the-mountain
Solidago spp	Goldenrod
Helianthus maximilianii	Maximillian sunflower
Helianthus annuus	Annual sunflower
Vernonia baldwinii	Inland ironweed
Bouteloua curtipendula	Side-oats grama
Gutierrezia sarothrae	Broomweed
Ambrosia artemisiifolia	Common ragweed
Sorghastrum nutans	Indian grass
Desmanthus illinoensis	Illinois bundleflower

primary and secondary ramets, as identified by the random coordinates. I numbered the tags sequentially with permanent ink within each plot. Thus, each density treatment contained 3 plots containing labeled stems numbered 1-10, 11-20, and 21-30, respectively.

From August 7, 1999, to October 9, 1999, I made a weekly record of numbers of CL and CH flowers on each selected stem. I identified and marked the flowers (see Preliminary Study Results for identification protocol) using a #1 sable brush and India Ink. The ink persisted on the flower throughout the study period, and I recorded only new flowers each week. Thus, data for each succeeding week were discrete. I re-checked a small proportion (<10%) of the sample flowers designated as the week after identification to verify no bud opening, and thus correct identification. I recorded soil temperature weekly between 1600-1900 hrs for each study plot using a Weksler Type 2R08J Bi-metal Soil Thermometer. I also recorded soil pH and percent relative saturation weekly for each study plot using a Kelway HB-2 Soil Acidity and Moisture Probe, according to the manufacturer's instructions. I made soil pH and moisture measurements by inserting the probe around the perimeter of the study plot, so as to minimally disturb the vegetation in the plot. The Fall River Area Office (approximately 8 kilometers from the study site) provided records of minimum and maximum air temperature and daily precipitation.

I used SigmaStat 2.0 (Jandel Scientific) to analyze the

data. The CL and CH flowering data were analyzed separately with Two-Way Analysis of Variance (ANOVA) and Tukey's test at $P < 0.05$. The factors for the ANOVA were stem density and week-of-flowering. I tested three null hypothesis for CL and CH flowering, respectively:

1. H_{o1}: mean (μ) flowering LD treatment = μ flowering MD treatment = μ flowering HD treatment
2. H_{o2}: μ flowering week one = μ flowering week two = μ flowering week three... = μ flowering week ten.
3. H_{o3}: There is no interaction between *L. cuneata* density and week of flowering.

Primary Study Results: I made the first flower observations August 7, 1999, at 13 hrs 49 minutes of daylength. I recorded 1,362 CL flowers (40.3% of total) and 2,021 CH flowers (59.7% of total) during the 10 week flowering period. Flowering peaked for both types of flowers in week six of the study (Fig. 3). Production of CL flowers showed a decline after week six. Chasmogamous flower productions continued at high levels through week seven before dropping sharply. Little precipitation occurred during the first five weeks of the study, relative to rainfall increases during weeks six and nine (Fig. 3). Mean low air temperature decreased throughout the study from a peak in week two (Fig. 3).

Soil pH and relative saturation fluctuated during the flowering period, but variations among study plots were synchronized. Soil pH averaged 6.1, 5.9, and 5.8 in the LD, MD, and HD treatment groups, respectively. Soil temperature averaged

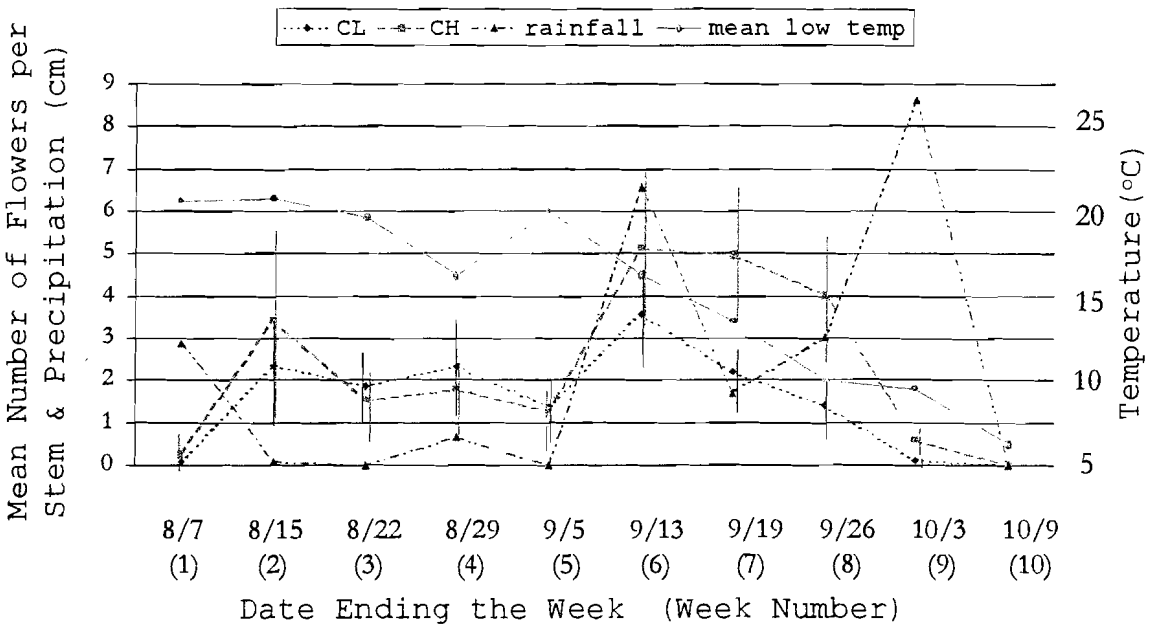

Figure 3. Cleistogamous (CL) and chasmogamous (CH) flowering in *Lespedeza cuneata* from 7 August to 9 October, 1999. Vertical bars on flowering data indicate 95% confidence interval.

21.9°C, 21.4°C, and 21.3°C in the LD, MD, and HD treatment groups, respectively. Percent relative soil saturation (percent of field capacity) averaged 56.3%, 59.2%, and 58.3% in the LD, MD, and HD treatment groups, respectively.

Although CL flowering among the study weeks varied significantly ($P < 0.001$), there was no difference in CL flowering among the three density treatments ($P = 0.168$) (Fig. 4). There was no interaction between density and week of CL flowering ($P = 0.920$).

Chasmogamous flowering was significantly different among study weeks ($P < 0.001$). Significant variation in CH flowering was also found among the three density treatments ($P = 0.041$) (Fig. 5). There was an interaction between density and week of CH flowering ($P = 0.004$).

Tukey's Multiple Comparison Test indicated that the low density treatment produced significantly-higher levels of CH flowering than the high density treatment ($P < 0.05$). The difference was most dramatic at week two. However, CH flower production in the medium density treatment was not significantly different from either the low or high-density treatments ($P > 0.05$).

Discussion

Flower initiation at approximately 13 h 49 min of daylength is consistent with *L. cuneata*'s <14 hours short-day designation (daylength on August 1, 1999 was 14 hours). Most of the flowers in this study (59.4%) were CH, similar to 2,475 *L. cuneata* plants

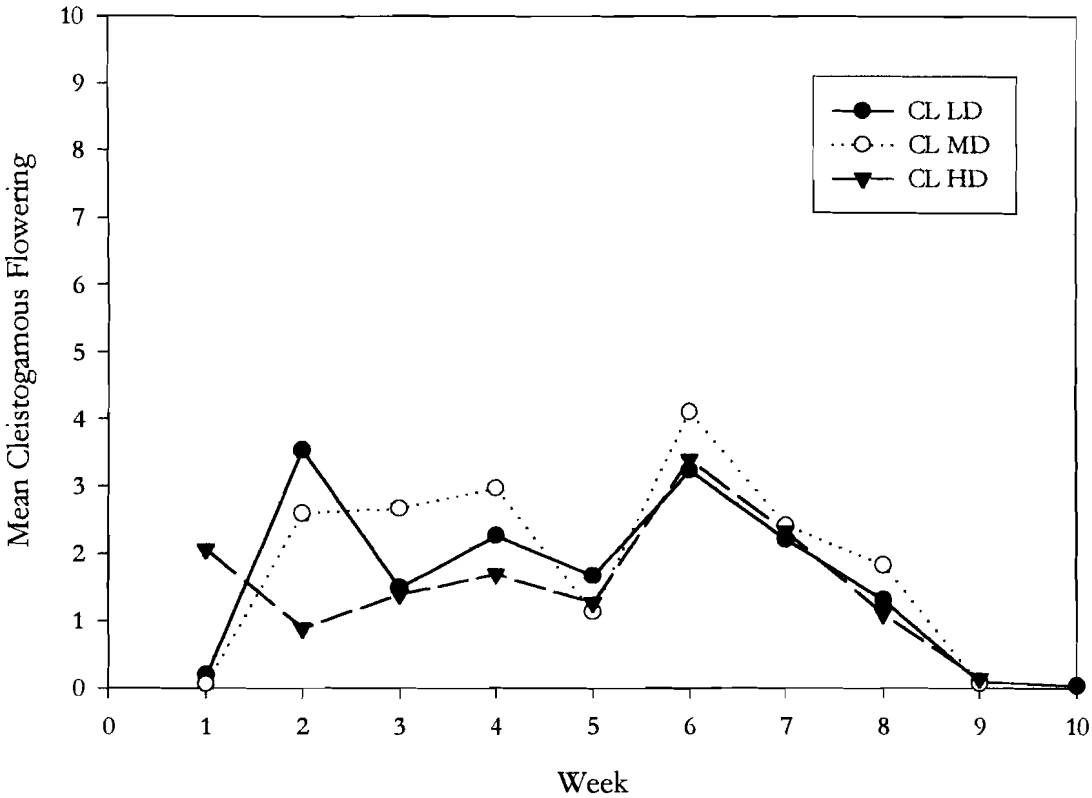

Figure 4. Cleistogamous flowering of low, medium, and high-density stands of *L. cuneata* over 10 weeks. Variation in mean flower production among the three density treatments was not significant ($P = 0.168$).

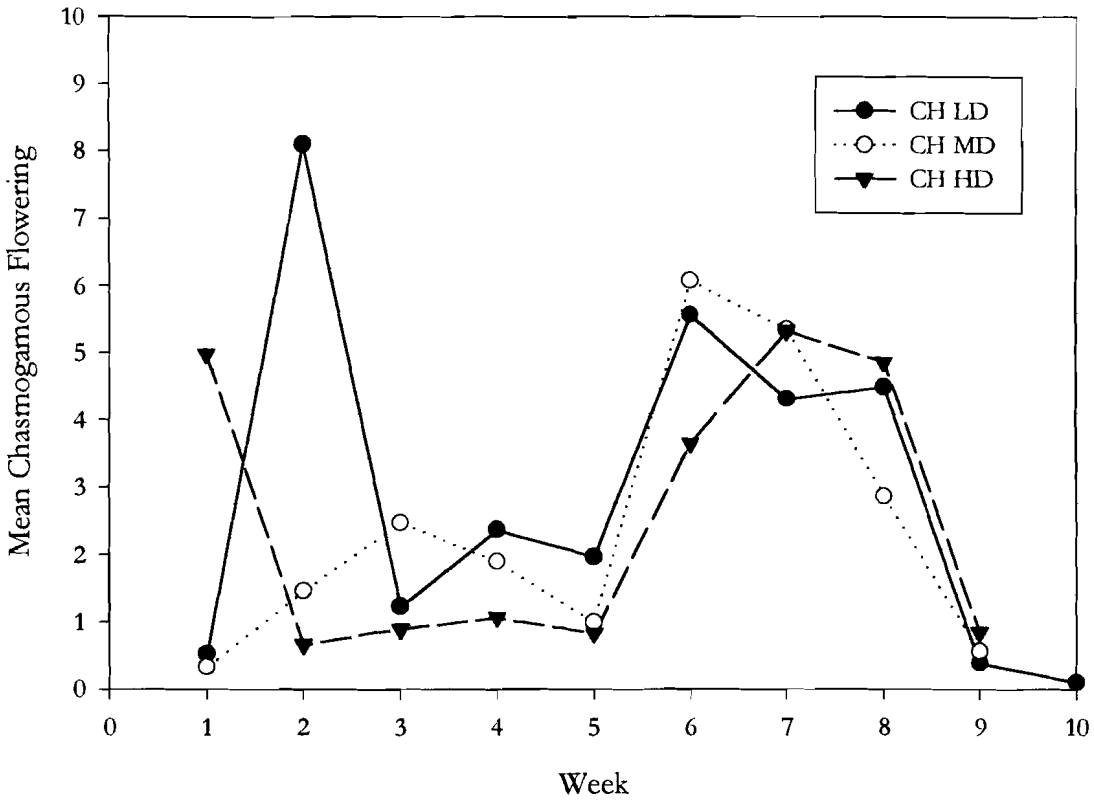

Figure 5. Chasmogamous flowering of low, medium, and high-density stands of *L. cuneata* over 10 weeks. Mean flower production in the low density treatment varied significantly from the high density treatment ($P = 0.041$).

examined by Donnelly, which averaged 52% CH flowers (1979). This is somewhat counter-intuitive, because petaliferous flowers would seem to represent a greater dedication of available growth resources per-flower than CL flowers. Examination of whether this high level of CH is typical of weedy sericea populations in Southeast Kansas, or is restricted to a local variety, would be valuable. Chasmogamous and cleistogamous flowering rates varied significantly during the 10-week flowering period (both $P < 0.001$). The greatest mean number of CL and CH flowers occurred during week six. More precipitation occurred between weeks five and six than cumulatively in the first month of the study (Fig. 3). The week six flowering peak appears to be associated with this increase in available water. During weeks two, three, and four, flowering rates also increased and decreased with precipitation, which supports the suggestion that water availability is an important factor in controlling flowering. These results suggest an examination of flowering rates and leaf water potential as a measure of drought stress to better describe the relationship between available water and flowering.

Referring again to Fig. 3, after the flowering peak during week six, CL flowering dropped precipitously. During the same time period, CH flowering plateaued before declining rapidly during early October. Even though the highest weekly precipitation of the study period was recorded during this time, CL and CH flowering declined abruptly. This suggests that late

in the flowering period some factor other than water availability was limiting flowering. Temperature is a possible candidate, and this would be consistent with the results observed in five *Stipa* species (Ponomarev, 1962 *in* Donnelly, 1979). During early October, air temperature fell to 10°C for the first time during the study period. The abrupt drop in flowering suggests that approximately 10°C may be a critical threshold for flowering in *L. cuneata*.

The plants in low density plots produced significantly more CH flowers than in high density plots ($P = 0.041$) (Fig. 5). Pasture stands of sericea in which either the ramet density or plant density is reduced due to incomplete mowing, grazing, burning, or chemical treatment will likely produce a higher proportion of CH flowers in the remaining plants than what was found prior to the control treatment. Given Donnelly's (1979) documentation of heterosis through CH flowering, and the assumption that CH flowers in southeast Kansas sericea populations are predominantly outcrossing, these results describe a mechanism whereby sericea might re-populate an incompletely-treated pasture with more vigorous plants than were present in the original infestation.

The above assumption of outcrossing is an important one, because heterosis might be overestimated if CH flowers are self-pollinating in local populations. In the 40 hours of field time I spent in close proximity to CH flowers, at no time was an insect pollinator witnessed in or on a CH flower. Regrettably,

there is little information available in the literature on insect pollinators in association with sericea.

There was no difference in CL flowering among the three density treatments ($P = 0.168$) (Fig. 4). Because CL flowering does not play a role in outcrossing, this lack of a relationship with density does not affect the pasture invasion/re-population hypothesis described above.

Finally, there was no interaction between density and week of CL flowering ($P = 0.920$), however there was a significant interaction between density and week of CH flowering ($P = 0.004$). Early in the study, low densities produced significantly more CH flowers than medium and high densities in the same period ($P < 0.05$) (see Fig. 5, week two). Because *L. cuneata* is a short-day species (and will not flower in greater than 14 hours of light), the increased light exposure of low-density stands of plants might result in a greater sensitivity to the daylength threshold. Shading provided by crowding in more dense clusters might inhibit CH flowering.

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