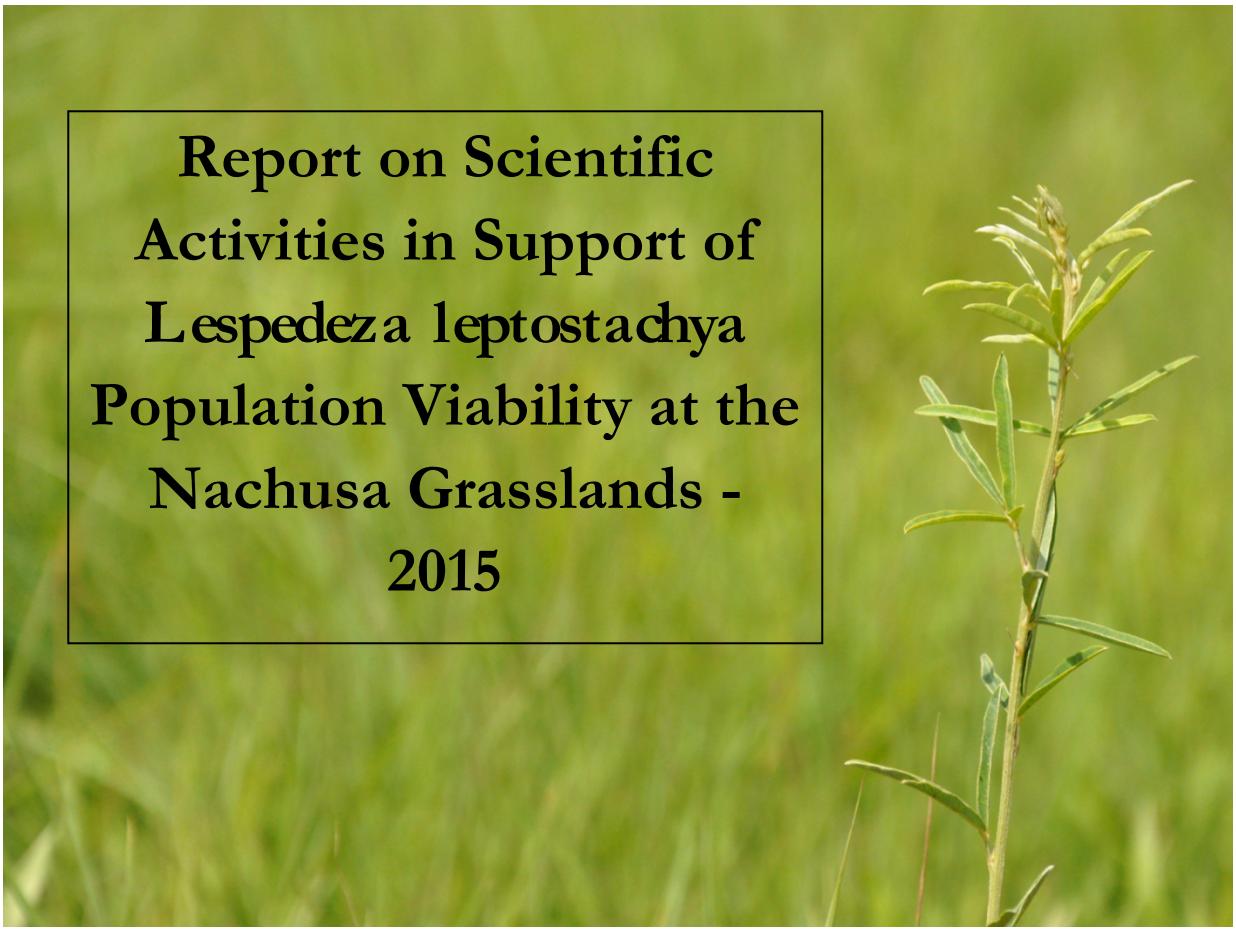

Report on Scientific Activities in Support of Lespedeza leptostachya Population Viability at the Nachusa Grasslands - 2015

Submitted to: The Friends of Nachusa

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Report slightly edited from that submitted to the Illinois Chapter of the Nature Conservancy

Prairie bush clover*, Lespedeza leptostachya*, is a federally threatened species endemic to gravel hilltops and knobs in a narrow portion of the tall grass prairie, which enjoys its largest presence in the southern most portion of the range at the Nachusa Grasslands, owned and managed by The Nature Conservancy. Research conducted in 2015 was designed to determine the best locations to introduce new Prairie Bush Clover (PBC) populations, and to uncover greater insight into seed germination dynamics. For example, understanding the habitat at Nachusa Grasslands that is currently inhabited by PBC, and establishing characteristics of that habitat that are correlated with population performance, is important to determining the best sites to establish new populations. In addition, understanding patterns in seed germination will help determine how and when to use seeds to introduce PBC to new sites, as well as inform the management of these new populations in terms of prescribed burn intervals and the timing of new plantings.

Vegetation and soil analysis of existing populations at Nachusa Grasslands in 2014 revealed that "large" populations are correlated with soils that have lower sand content and with reduced litter cover. In an effort to determine the effects of litter cover on seed germination, seedling establishment and subsequent plant growth and reproduction, seeds were collected from 7 populations in fall 2014, and subsequently cleaned, counted, and weighed. There were no significant differences in seed weight between the sites, although seeds from Management Area 2 were not well filled. (Figure 1.)

Site	**Total Number of Plants (including plants in demographic plots)**	**# of Seeds Collected**
Management Area 1	271	187
Management Area 2	73	125
Management Area 3	84	92
Lee-Ogle 4	23	54
Lee Ogle 6	10	0
Lee Ogle 7	31	85
Lee Ogle 1	4	10
Total	496	553

A small subsample of seeds were placed in cold stratification for 5 weeks and grown out in the greenhouse in the summer of 2015 to test for germination and growth differences among the 7 sites, and to determine if soil rhizobium increase seedling growth rates. Because of limited seed number, no analysis of the effect of soil rhizobium was possible. Germination differed between the subpopulations, although small sample sizes reduced the power to determine significance, but not in the direction expected. I expected that germination and subsequent growth would be highest from seeds collected at Management Site 1 (along old Pussy Toes Lane), because this is the largest extant site and therefore the least "inbred" subpopulation. I also expected that seeds from Lee Ogle 4

would be inbred. However, seedlings from seed collected at Lee Ogle 4 had the highest growth rate, as well as the highest germination rate.

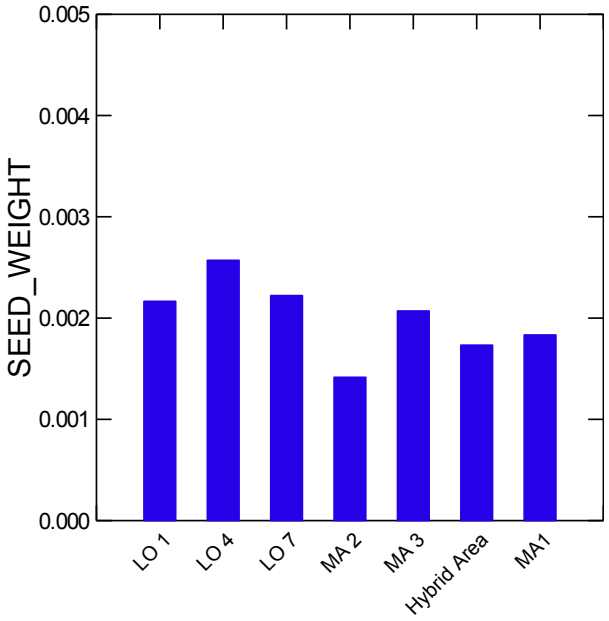

Figure 1. Mean Seed Weight of all seeds collected from 6 sites at Nachusa Grasslands in the fall of 2014.

Teasing apart the relationship between litter cover and population size requires an experimental approach to determine several things. First, litter cover may be important to seedling survivorship by retaining soil moisture content. This would have the effect of increasing population size, regardless of the negative effects that litter has on overall plant size and seed production. Second, litter may physically suppress the growth of small plants, reducing their overall size and fecundity. Third, litter cover may be associated with lower levels of soil nutrient availability. This last effect can work in one of two ways: litter is very carbon rich, which makes soil microbes very happy and active. Soil microbial activity often results in reduced nitrogen availability, which is already low in these gravel hill prairies, resulting in smaller plants, and suppressing seed set. The second way that litter cover may affect plant growth is via nutrient release during a burn. Reduced litter is correlated with more recent burning, as well as the associated nutrient pulse increasing soil nutrient availability, resulting in larger and more productive PBC plants.

Research Activities - In 2015, I continued to explore relationship between plant size and litter for its effect on germination and establishment of young PBC plants. In addition, permanent plots established in 2000 were censused and demographic data, as well as vegetation data, were collected. In addition, censuses were conducted of all of the new introductions across the Nachusa Grasslands with the help of the Staff of the Chicago Botanic Garden's Plants of Concern (Figure 5 & Table 4). 2015 was a terrific year for introducing this species by planting plugs because it was cool

and very wet. Survivorship during the course of the summer appeared to be quite high. In addition, newly established seedlings in the permanent plots achieved total heights that had not been previously observed at this site. Generally, seedlings do not acquire a total plant height greater than 8.0-8.5cm, and generally much lower. In 2015, however, several seedlings were observed with total plant heights of 9.5cm. The increased height was likely due to the fact that these new plants never experienced the usual late summer drought, and were therefore able to continue growing until they became dormant. This increased growth will likely result in higher than usual survivorship and emergence rates in 2016.

Table 2. Design of treatments in the Seed Basket Array in the Nursery at the Chicago Botanic Garden.

Treatment	Tag#	Treatment	Tag#	Treatment	Tag#	Treatment	Tag#	Treatment	Tag#
Low Litter	1228	High Litter	672	Low Litter	49	Low Litter/Burn	1269	High Litter	1258
Low Litter/Burn	543	Low Litter	734	High Litter/Burn	673	Low Litter/Burn	1260	Control	1236
Low Litter/Burn	1369	Control	535	Control	1255	High Litter	1486	Control	671
Low Litter	1403	Low Litter	746	Low Litter/Burn	94	High Litter/Burn	689	High Litter/Burn	1392
High Litter/Burn	1457	High Litter	1471	High Litter/Burn	779	High Litter	1474	Control	1243

Figure 2. Photograph of seed basket array in the nursery at the Chicago Botanic Garden.

After the small sample germination trials were complete, and no statistically significant differences were detected in overall seed weight, germination, and growth, seeds were subsequently pooled for further experimentation and analysis. 25 subsamples of 20 randomly drawn seeds were randomly assigned to one of five (1 of 5) treatment groups: 1. Control; 2. High Litter, No Burn; 3. High Litter, Burn; 4. Low Litter, No Burn; 5. Low Litter, Burn. There are 5 replicates for each treatment, placed into randomly assigned places in a 5x5 array of seed baskets sunk into the sand in a sand-bed in the nursery at the Chicago Botanic Garden. Low Litter seed baskets received

7.48 grams of litter, while High Litter seed baskets received 22.88 grams of litter. Litter amounts for treatments were determined by collecting all standing (dead) biomass from 5 plots in each of the three management sites with permanent Lespedeza leptostachya monitoring plots. A meter-square plot frame was laid down surround each plot and the enclosed litter was then cut out and lifted away from the plot. A total of 15 plots, 5 from Management Area 1 (MA1) – just west of the road (See Figure 2), which was burned in the spring of 2015. And 5 each form Management Area 2 (MA2), just west of MA1 and another 5 from Management Area 3 (MA3), the furthest west permanent study area. Neither MA2 nor MA3 have been recently burned – i.e. within the last 3 years – and have subsequently accumulated high litter loads. The litter samples were placed in a drying cabinet for two weeks, and were then weighed. There was no statistically significant difference for litter weights for MA2 and MA 3. Litter weight was significantly different from MA1. The weight/area relationship was determined and replicated to produce the litter weights for the Low Litter (replicating the litter amounts found in MA1, 1 growing season after a spring burn) and High Litter (replicating cumulative litter with no recent burn history found in MA2/MA3). A portable propane torch will be used to simulate a spring burn of the appropriate treatment plots in the Fall of 2016, and germination rates will be determined beginning in June 2016. Resulting surviving seedlings will be grown in the nursery bed to determine growth rates and survivorship, and litter will be supplemented throughout to determine the effects on these parameters. These plants will ultimately become the seed source for subsequent reintroduction efforts and further experimentation, if necessary.

Dates on site: July 14-15, 2015

July 21-22, 2015

July 25, 2015

September 10-13, 2015

September 20, 2015

October 13, 2015

December 7, 2015

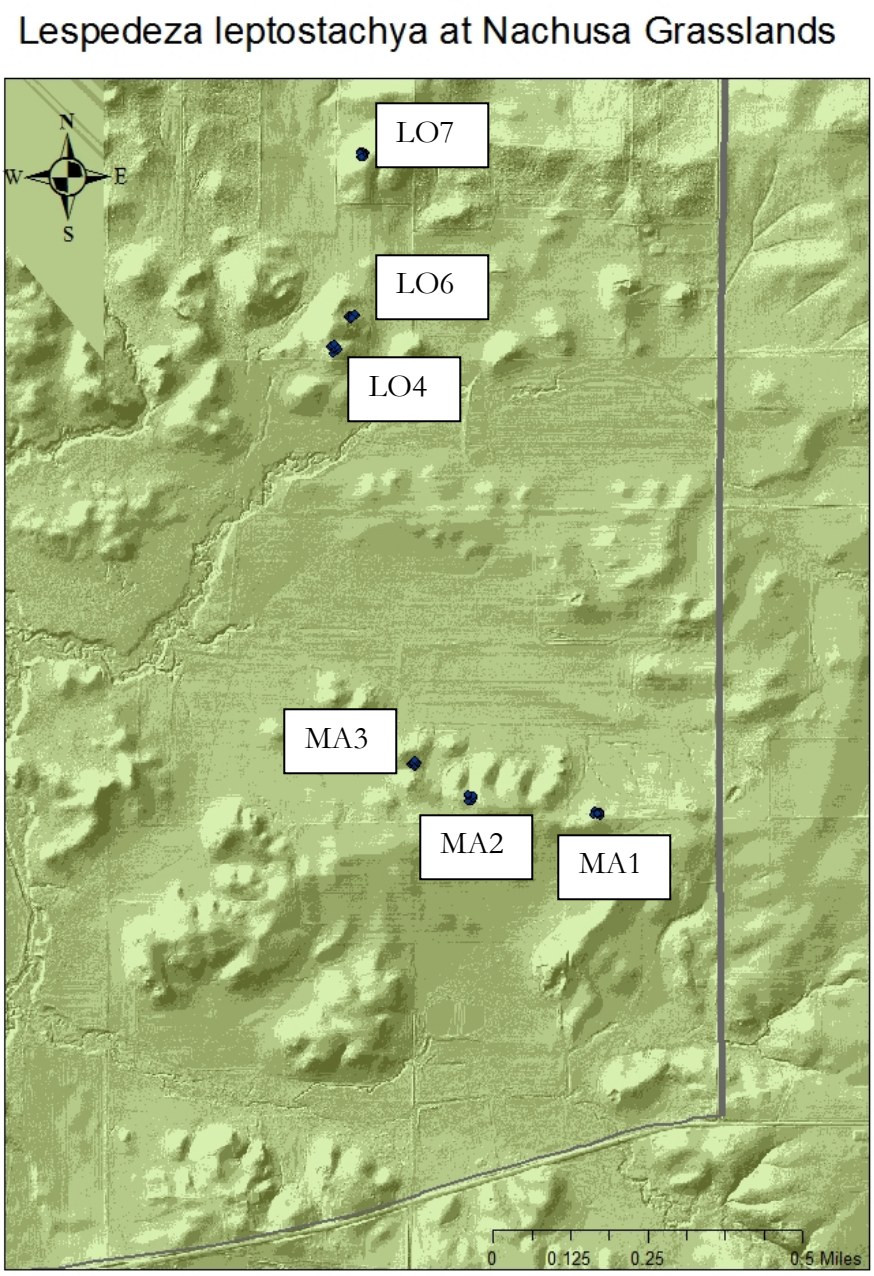

Figure 3. Sites censused for all *Lespedeza leptostachya* plants in 2014, and the source of seeds for seed germination trials begun in 2015. MA1, MA2 and MA3 are the sites of 57 permanent monitoring plots. Demographic data collection has occurred in these sites annually since 2000.

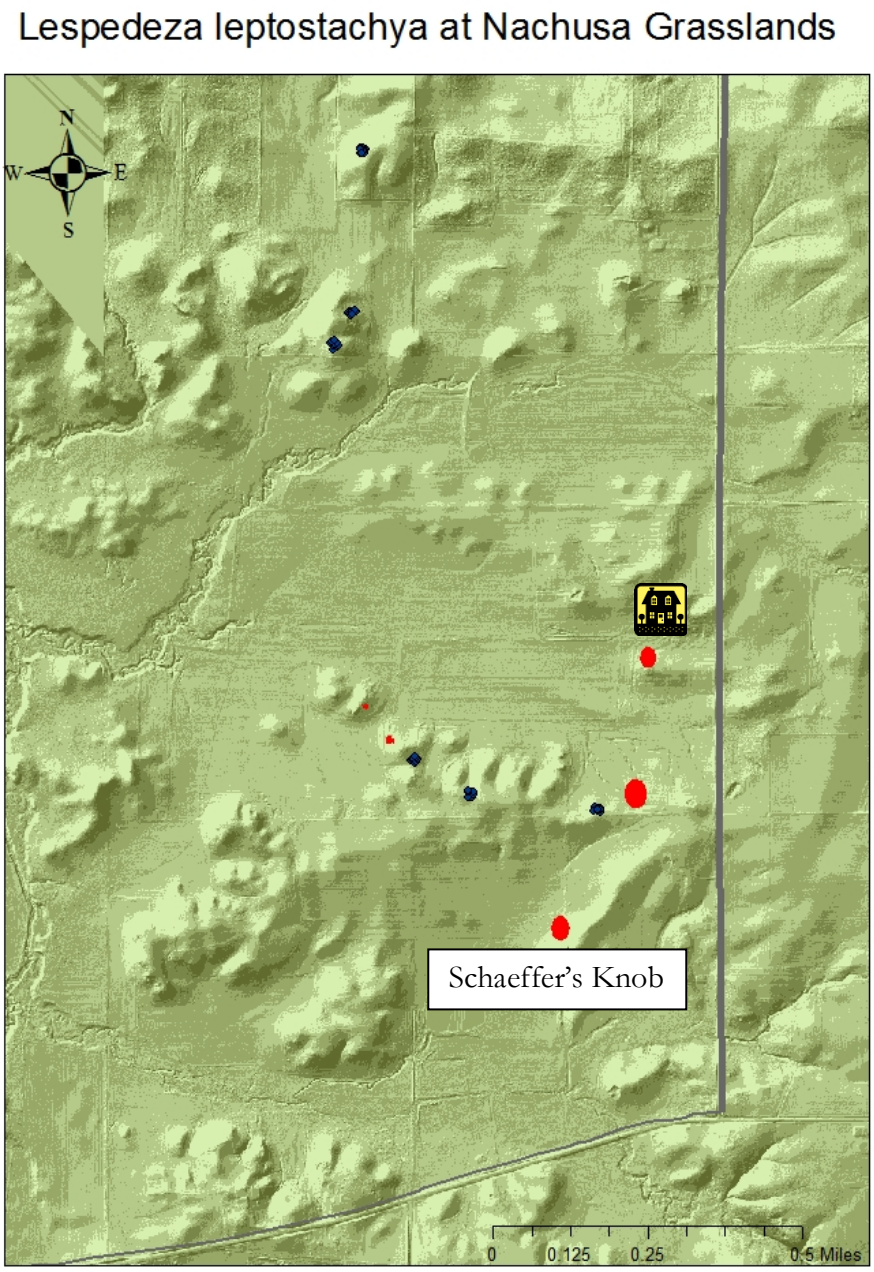

Figure 4. Red areas indicate sites surveyed for subsequent reintroduction of Lespedeza leptostachya at the Nachusa Grasslands. Sites were chosen by visual inspection for further investigation and were chosen on the basis of slope, aspect, visible soil texture, and surrounding vegetation. Easily apparent vegetation matrix was important in determining subsequent habitat delineation, including further soil analysis. Size of the red area signifies best habitat matching to natural populations. Of special note, Schaeffer's Knob, which was chosen as reintroduction site by stewards, and although the site looked promising, there were several invasive species present, and it appeared to need additional management to be a viable site for reintroduction. Several apparently naturally occurring plants were found on the lower west facing slope of Schaeffer's Knob during the fall census. The most promising introduction site is the small knob just south of the Little Yellow House site.

Lespedeza leptostachya at Nachusa Grasslands

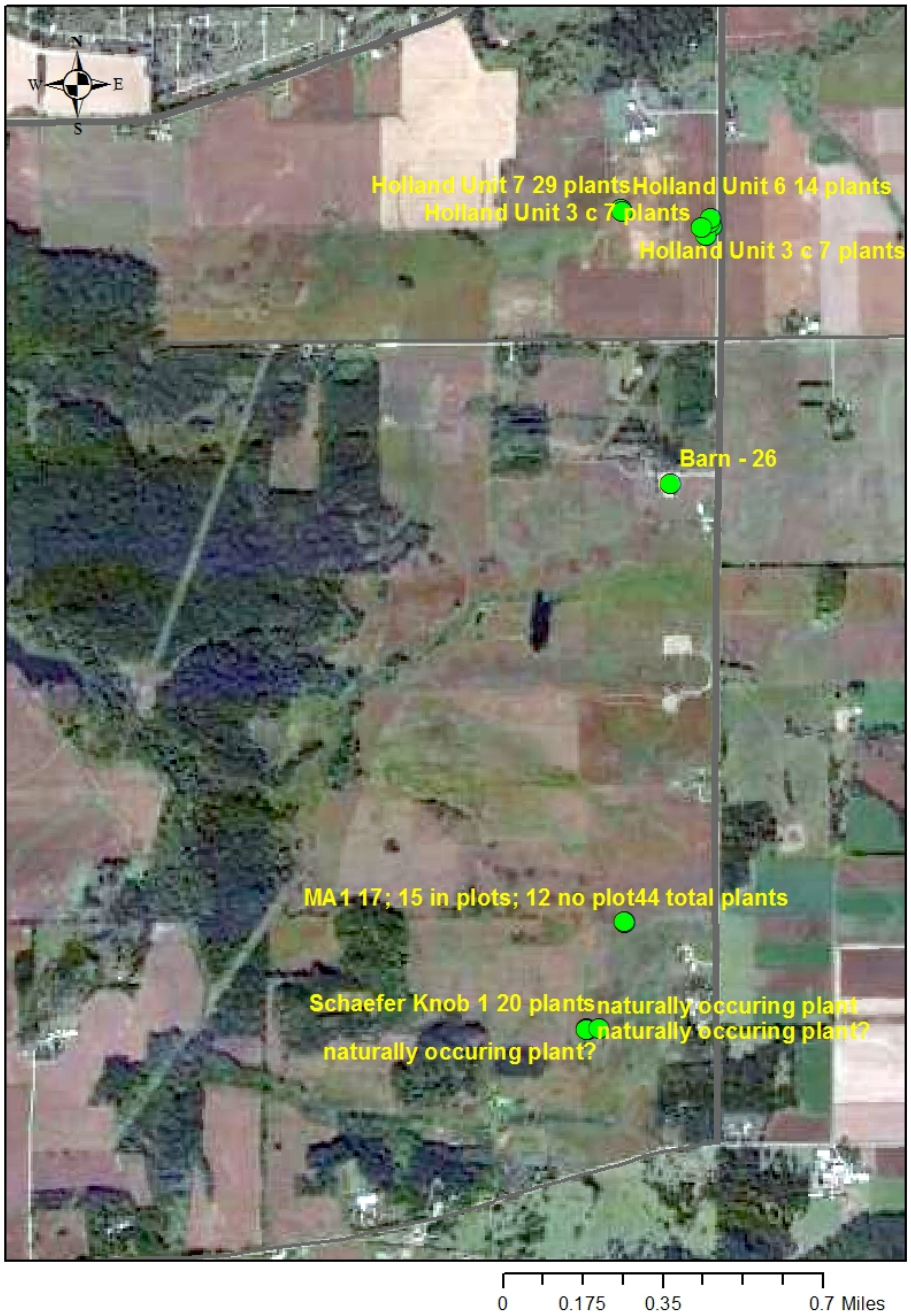

Figure 5. Reintroduction Sites, Census Fall 2015.