Section 6 (Texas Traditional) Report Review

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TPWD signature date on report: 8/31/2010

Project Title: Habitat requirements of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), a rare plant of central Texas

Final or Interim Report? Final

Grant #: TX-E-96-R

Reviewer Station: Austin ESFO

Lead station concurs with the following comments: NA (reviewer from lead station)

Interim Report (check one):

- ☐ Acceptable (no comments)
- ☐ Needs revision prior to final report (see comments below)
- ☐ Incomplete (see comments below)

Final Report (check one):

- ☒ Acceptable (no comments)
- ☐ Needs revision (see comments below)
- ☐ Incomplete (see comments below)

Comments:

The report is very thorough. My only question is whether there should have been captions for the two photographs that follow figure 7 and figure 8.

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-96-R

Endangered and Threatened Species Conservation

Habitat requirements of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), a rare plant of central Texas

Prepared by:

Norma Fowler

Carter Smith
Executive Director

Clayton Wolf
Division Director, Wildlife

31 Aug 2010

FINAL REPORT

STATE: ____Texas_______________ **GRANT NUMBER:** ___TX E-96-R___

GRANT TITLE: Habitat requirements of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), a rare plant of central Texas

REPORTING PERIOD: ____6 Sep 07 to 31 Aug 10_

OBJECTIVE(S):

To provide new ecological information for successful establishment of persistent populations of *Streptanthus bracteatus* to prevent its listing under the Endangered Species Act.

Segment Objectives:

a. Laboratory and wild seed of *S. bracteatus* will be sown in experimental plots to determine the effect of an overstory of woody plants on *S. bracteatus* on two geologically distinct substrates;
b. Plants will be monitored regularly and their size and fecundity measured non-destructively.
c. Results will be incorporated into strategies regarding reintroduction and management of *S. bracteatus.*

Significant Deviation:

None.

Summary Of Progress:

Please see Attachment A (pdf file).

Location: Travis County, Texas.

Cost: ___Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.__

Prepared by: _Craig Farquhar_____________ **Date:** _31 Aug 2010

Approved by: ______________________________ **Date:**_____31 Aug 2010_____

C. Craig Farquhar

FINAL REPORT

Habitat requirements of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), a rare plant of central Texas

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Section 6 Program
Contract No. 187255

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Abstract

A better understanding of the habitat requirements of *Streptanthus bracteatus*, a rare annual of central Texas woodlands, is critical to attempts to establish new populations, and may also help manage existing ones. We conducted a transplant experiment to determine the effects of different amounts of cover on the performance of this species. A range of covers was provided by pruning existing woody understory plants, and also by natural canopy gaps and by using an area where oak-wilt had killed *Quercus buckleyi* trees. In general, lower levels of cover improved plant performance, especially fecundity. The optimum level of cover at the height of a *Streptanthus bracteatus* plant (i.e., < 0.5 m above ground) was no more than 50%, and perhaps less. This is consistent with the hypothesis that this species was a 'fire-follower' rather than a true understory species. The results suggest that attempts to establish new populations should use woodland or shrubland sites where cover is no more than 50% and should include ongoing cover reductions. Managers of existing populations may wish to try reducing cover, especially where natural populations are declining without an apparent cause.

Introduction

Streptanthus bracteatus (bracted twistflower; Brassicaceae) is a rare annual wildflower endemic to the eastern and southern Edwards Plateau of central Texas (Poole et al. 2007). Known threats include the development of private land, recreational activities on public land, and deer herbivory everywhere (Zippin 1997, Pepper 2010). In addition, some populations are declining for reasons that are unclear. It has been suggested that these unexplained declines may in part be the result of habitat change related to increases in woody plant cover and consequent reductions in light levels (Zippin 1997; members of the Bracted Twistflower Working Group pers. comm.). **The goal of the experiment reported here was to quantify the effect of the light environment (cover) on the growth and reproduction of *S. bracteatus*.**

S. bracteatus has a G2S2 NatureServe ("imperiled") rank but is not listed under the US Endangered Species Act. However, as its known populations decline in size or disappear entirely, the risk of extinction is increasing. All but one of the populations in Travis County are on private land without any legal protection, or are being negatively impacted by uncontrolled recreation (especially mountain bikes) on public land, and/or are known to be declining. The establishment of populations in protected sites will therefore probably be a critical part of ensuring the survival of this species. Although seeds of the species have been successfully germinated and grown in greenhouses and gardens, at least ten efforts to establish persistent populations in apparently suitable sites have all failed. **More knowledge about the habitat requirements of *S. bracteatus* is needed if new populations are to be established where they can persist.** A better understanding of its habitat requirements could also be used **to improve the management of the remaining populations** and thereby increase their likelihood of persisting.

Understanding the ecological requirements of an endangered plant species is often essential to its preservation and successful recovery (Menges and Dolan 1998, Pfab and Witkowski 2000, Brys et al. 2004, Colling and Matthies 2006). It is especially important when the species has become restricted or nearly restricted to sub-optimal sites, as often happens when a species has become rare. Currently occupied sites may even be completely unsuitable for long-term persistence: many species can persist in a site long after substantial changes in an ecosystem have reduced recruitment below the replacement rate (Hanski and Ovaskainen 2002; Eriksson 1996). When the mismatch between current and optimal habitat is not obvious, the ecological requirements of the species may be misunderstood, reducing the effectiveness of conservation actions. This is especially likely to be the case where grazing, fire suppression, hunting, and other human activities have changed plant and animal communities in ways that are less obvious than the conversion of woodland to office building. Note that research on rare species in relation to habitat change was listed as a high priority topic in the RFP.

In the case of *S. bracteatus*, we hypothesized that its present habitat is sub-optimal due to low light levels. Most of the existing Travis County populations are in closed-canopy woodland (Fowler, pers. obs.), although this may not have been true in the past (P. McNeal, pers. comm.) A human activity likely to have caused this is fire suppression. The role of fire in central Texas woodlands has just begun to be studied (Reemts and Hansen 2008), although fires are thought to have been common enough in the region in the past to maintain its savannas (Smeins 1980, Smeins et al. 1997). Recent studies in the eastern United States and in the Ozarks suggest that both crown fires and surface fires may have been responsible for the dominance of oaks in many forests, and that fire suppression is one of the reasons why oak regeneration is not occurring in many places (Cutter and Guyette 1994, Brose et al. 1999, Guyette et al 2006, Nowacki and Abrams 2008). Surface fires in oak woodlands can create more open understories (Dey and Hartman 2005) and can increase abundances of some herbaceous species (Elliott et al. 1999, Bourg et al. 2005). If fire played a similar role in the woodlands of central Texas, *S. bracteatus* could have had the ecological niche of 'fire-follower', that is, a species adapted to germinate and grow in the conditions that follow a fire. Some California species of *Streptanthus* are fire-followers (Moreno and Oechel 1991, Hickman 1993, A. Pepper, pers. comm.), and others live in the high-light environment of serpentine outcrops (Kruckeberg 1986, Mayer et al. 1994, Dolan 1995, Rodriguez-Rojo et al. 2001, Harrison et al. 2006).

Objective (as described in the proposal)

The proposed research will provide new ecological information that is essential for the successful establishment of persistent populations of *Streptanthus bracteatus* to prevent its listing under the Endangered Species Act.

Location

The field experiment was conducted at Vireo Preserve (30.31222 N, 97.81927 W, approximately 275 meters asl), which is part of the Balcones Canyonlands Preserve system. It is managed by the City of Austin. See Appendix I for the coordinates of the experimental plots.

Methods

Seed germination and pre-transplanting care

Because of the very limited number and size of populations of *Streptanthus bracteatus* in the Austin area, we did not use field-collected seed for this experiment. Instead, seeds were collected from greenhouse-grown plants of Valburn provenance, stored at 4ºC until planted. The present experiment involved two separate batches of transplants (see below). Some (22 of 110) of the first batch of transplants were planted indoors in September 2008 and moved out-of-doors on 4 November 2008 to harden off. The remainder of the first batch of transplants (88 of 110) were planted indoors in November 2008 and moved out-of-doors on 23 December 2008. All seeds of the second batch of transplants were planted outside on 1 February 2009, where they remained until transplanted into the field site.

Seeds were germinated, and transplants grown, in 250ml styrofoam cups with a hole punched in the bottom of each (Fig. M1). The outside of each cup was covered with aluminum foil to make it opaque. Cups were filled with MetroMix® 702 potting soil. All cups were watered with a weak solution of a complete fertilizer (Dyna-Gro® , 0.3255 ml/l) once or twice per day as needed. While indoors, all cups received 16 hr/day of lighting from fluorescent bulbs (Starcoat® T5 Ecolux® bulbs, manufactured by GE; sold under the name of T5® 6500K by Hydrofarm, Petaluma, CA to retailers). While out-of-doors, each plant received direct sunlight 2-4 hours of each sunny day. All plants were sprayed regularly with a sulfur (Safer Brand Garden Fungicide Concentrate® , 12% S by weight, diluted to 7.8 ml/l) and neem oil (Green Light Rose Defense® , 70% neem oil, diluted to 7.8 ml/l) solution to prevent and control powdery mildew. Neem oil may also provide some protection against insect herbivory. Pots received a weekly soil drench of *Bacillus thuringensis israelensis* (Gnatrol®, 600 ITU/mg, diluted to 19.87 ml/l) to control fungus gnats. The number of leaves of each transplant was counted just before transplanting.

Field site preparation

This field experiment was conducted at Vireo Preserve (30.31222 N, 97.81927 W, approximately 275 meters asl), which is part of the Balcones Canyonlands Preserve system managed by the City of Austin (Map 1). On 28 October 2008 ten pairs of plots were selected in an area of the Preserve dominated by *Quercus buckleyi* (Texas red oak, Spanish oak) and *Juniperus ashei* (Ashe juniper). Six of the ten plots to be treated ('thinned plots') were deliberately located in canopy gaps along a southwest-facing hillside and the other four plots to be thinned were located on a nearby 'saddle' where oak wilt (*Ceratocystis fagacearum)* had killed *Q. buckleyi* (Map 2). On the same day, ten control plots were also selected. One control plot was selected for each thinned plot so as to be no more than a few meters away from it and to have similar slope, aspect, and vegetation, but with a continuous canopy. All twenty plots had thin soil over limestone bedrock and were located very close to the boundary between the Walnut and Glen Rose Formations (Garner and Young 1976).

An additional criterion for locating thinned plots was that we were permitted to prune their understory species. Plots to be thinned were located so as not to contain *Diospyros texana* or *Ungnadia speciosa*, which we were not permitted to prune. Species that were pruned in one or more thinned plots were *Forestiera pubescens*, *Rhus virens*, *Ilex vomitoria*, *Quercus sinuata*, *J. ashei* (small plants and side branches only), *Vitis* spp., and *Rhus radicans*. The exact location of each plot was adjusted until it contained 5 points, at least 0.5 m apart, where the soil was deep enough to plant a transplant.

Transplanting

In November and December 2008 cover was measured in all plots with a densiometer at ~1 m above the ground. Understory plants were then pruned by hand until total cover had been reduced by 50% from its initial value, as measured by the densiometer. Deer fencing was constructed around each plot (Fig. M2). We saw no evidence of deer browsing within any plot during the experiment.

Batch 1: Between 19 and 21 January 2010, 100 transplants were planted, five per plot. Ten replacement transplants were planted on 18 February. Herbivory was intense in most plots: leaves were repeatedly removed, in part or entirely, and some plants were entirely removed. All but 37 of the original plants and all but one replacement plant died, often after being repeatedly damaged. Herbivore damage did not cease until each plant was protected by an individual cage of poultry wire (2.54 cm hexagonal mesh) ~ 30 cm diameter and ~ 60 cm high. Because neither bird netting over the deer exclosures nor poultry wire around the bases of the deer exclosures were effective, but cayenne was partially effective (although temporary and difficult to apply thoroughly, cayenne appeared to reduce the amount of leaf area removed), we infer that climbing mammals, probably tree or ground squirrels, were responsible. *Streptanthus bracteatus* does not usually suffer herbivory of this sort (Zippin 1997), but the severe drought may have reduced other sources of food and water enough to make it attractive.

Batch 2: These were transplanted 23-25 March 2010 to replace the 62 empty points where no plant of batch 1 had survived. Each plant was protected by an individual cage immediately after transplanting. All of these transplants survived.

Transplant care

In addition to protecting the transplants from mammalian herbivores, we continued the sulfur and Neem spraying, primarily to control powdery mildew. As needed, fungus gnats and other root-eating insects were controlled with soil drenches of *Bacillus thuringensis israelensis* or imidacloprid (Bayer Advanced Insect Killer for Soil and Turf Concentrate® , 0.72% imidacloprid, diluted to 3.9 ml/l and applied sparingly at the base of each plant).

Transplants were watered daily for at least a week after transplanting to prevent transplant shock. We continued to provide lesser amounts of supplementary water during the rest of the experiment because the soil was exceptionally dry. Rainfall during the six months from January through June 2009 was 66% (28 cm)of the average 42 cm for January-June (NOAA NCDC). Furthermore, this followed another, even drier, period: rainfall July-December 2008 was only

36% (14 cm) of the Austin average of 39 cm for July-December. We therefore provided supplementary water throughout the experiment, first by hand watering and later by a gravity-driven drip irrigation system (Figs. M3 and M4). Each plant had its own drip line. Holes in the drip lines were adjusted to deliver the same amount of water to each plant. We estimate that the extra water did not quite bring the total up to an average year. For example, in May and June the average Austin rainfall is ~19 cm, which is more than the total of rainfall in 2009 (7 cm) + plus irrigation (estimated to be 10 cm, assuming that a drip line making a 25 cm circle around a plant watered an area 50 cm in diameter).

Data collection

Weekly measurements of plant size were taken after transplants were established. Each week, we measured rosette diameter (greatest leaf tip to leaf tip distance) and counted the number of rosette leaves of each transplant. For analysis, we used only the maximum number of leaves each plant had and its maximum diameter. Typically, a plant's maximum leaf number and diameter occurred just before its first flowering stalk was visible. The date on which the first flowering stalk was visible was recorded; this is the 'date of first reproduction' referred to in the analyses. The height of each flowering stalk and the length of each seed pod (silique) were also recorded weekly. For analysis, we summed the height of all flowering stalks on a plant on each date, but only analyzed the maximum sum attained by that plant, regardless of which date it occurred on. Likewise, we summed the length of all flowering pods on a plant on each date, but only analyzed the maximum sum attained by that plant. We did this because flowering stalks and pods lengthen as seeds ripen. A plant's maximum summed pod length was the best non-destructive surrogate for its total seedset (i.e., its fecundity). Seed pods were left to ripen and split open on the plants and seeds were allowed to disperse naturally, in the hopes that the species would persist in this site.

To measure cover during the experiment, hemispherical photographs were taken at each of the 100 planting points on 3-7 March and again on 22 June (Figs. M5 and M6). Photographs were taken with a Sigma 4.5mm F2.8 EX DC Circular Fisheye lens after leveling the camera. Each photograph was taken directly above a transplant, no more than 0.5 m above the ground. Images were analyzed using Gap Light Analyzer© Version 2.0. Hemispherical photographs were also taken above plants in two natural *S. bracteatus* populations, Cat Mountain and Mount Bonnell, on 27 May 2009.

Statistical analyses

Cover was analyzed with analyses of variance (ANOVAs) that had treatment, plot pair, and treatment x pair as independent variables. A contingency table with a χ^2-test was used to analyze survival rate. The proportion of plants reproducing was analyzed with a Wilcoxon test.

Other plant responses were analyzed with analyses of covariance (ANCOVAs), ANOVAs, and regressions. Separate analyses were made of each response variable (diameter, number of leaves, etc.) in each batch. For each response variable in each batch, the first step in its analysis was an ANCOVA with six independent variables: initial leaf number (analyses of diameter and maximum leaf number only) or maximum number of leaves (analyses of date of first reproduction, summed flowering stalk height, and summed pod length only), average cover per plot in March, average cover per plot in June, treatment, plot pair, and treatment x pair. The F-tests of the SAS type I (hierarchical) sums of squares were examined to determine which covariates were significant.

For each response variable in each batch, the next step was to construct a statistical model that included the leaf number covariate, if significant, and plot. (If the model did not include a covariate, this became a simple one-way ANOVA comparing the 20 plots.) Least-squares means were calculated and contrasts between the least-squares means of the two plots of each pair calculated. Least-squares means are averages adjusted for differences among plots in the values of the covariate. By including initial leaf number in the analysis of batch 1 maximum leaf number, latter differences in plant sizes were adjusted for initial size differences among plants. Similarly, the three reproduction response variables of batch 1 were adjusted for differences in adult plant size by including in their analyses the covariate maximum leaf number. Using maximum leaf number as a covariate in the analyses of the three reproduction response variables of batch 1 removed much of the variation in size due to early herbivory, making the effects of treatment on reproduction more detectable. Due to lack of significance in the initial ANCOVAs, no covariates were included in the model of batch 1 diameter or in the models of any of the batch 2 plant response variables. Note that all contrasts were made between pairs of plots, and that each batch was analyzed separately. For example, average diameter of batch 1 plants in plot 1-control was compared to average diameter of batch 1 plants in plot 1-thinned, average diameter of batch 1 plants in plot 2-control was compared to average diameter of batch 1 plants in plot 2-thinned, and so on. The least-square means and the results of the contrasts are reported in Figs. 3 and 5.

The effects of cover, as measured by hemispherical photographs, on plant responses were analyzed with regressions. Separate analyses were again made of each response variable (diameter, number of leaves, etc.) in each batch. A regression with March cover is only reported if the initial ANCOVA described above found a significant effect of March cover on the particular response variable; likewise for June cover. Maximum leaf number was included as a covariate in the regression analyses of the batch 1 reproduction variables. The results of the regressions are reported in Figs. 4 and 6. In some cases cover was non-significant although it was significant in the initial ANCOVA. There are two reasons for this discrepancy: the regression models had different terms than the initial ANCOVAs, and I have reported in Figs. 4 and 6 the results of significance tests based on SAS type III rather than SAS type I sums of squares, because they are more conservative.

Results

Survival rates

Most, if not all, of the early deaths of the initial 100 transplants were due to herbivory, probably by squirrels. Herbivory rates varied greatly among plots. Two pairs of plots had no deaths and five pairs had no survivors (Fig. 1). In the remaining three pairs of plots, 13 of 15 plants in control plots and 4 of 15 plants in thinned plots survived, a significant difference (χ^2 = 10.9955, P = 0.0009). However, because these 30 plants were too few to compare survival rates among plots or among pairs of plots, we cannot rule out the likely possibility that the apparent difference between treatments was actually due to plot-to-plot differences in herbivory rates. All but one of the 10 replacement plants of the first batch also were killed by herbivory. Deaths due to herbivory continued until each transplant was in its own poultry-wire cage. Each of the plants in the second batch was individually caged at transplanting, and all of them survived.

Light environment

Plots to be thinned were deliberately placed in canopy gaps or in areas where the canopy cover had been reduced by oak wilt. The understory plants in each of these plots were then pruned until total cover had been reduced by 50%, as measured by a densiometer at ~1 m above the ground. Nevertheless, once the canopy began to leaf out, cover in thinned and control plots was very similar in all but pairs 7, 8, and 10 (measured in hemispherical photographs; Fig. 2). The thinned plots of pairs 7, 8, and 10 were all located where oak wilt had killed the overstory *Q. buckleyi* trees. Because of these three plots, cover differed significantly between treatments in both March and June ($F_{1,80}$ = 62.21, P < 0.0001, and $F_{1,80}$ = 68.15, P < 0.0001). Differences among pairs of plots and the treatment x pair interaction term were also significant (P < 0.0001, both terms, both dates).

Most of the variation in cover was between plots (March: R^2 = 79%; June: R^2 = 86%), rather than among planting points within plots (March: R^2 = 21%; June: R^2 = 14%). This allowed us to do the following: For each plot on each date, the cover values from its five planting points were averaged. These averages (one average value per plot per date) were used in all subsequent analyses. Using these averages made cover on each date available as a potential covariate in all subsequent analyses. Average cover per plot on the two dates was positively correlated (r_s = 0.66, N = 20 plots).

Plant responses - batch 1

These plants were larger in the control plots than in the thinned plots, although the differences did not reach significance in all pairs of plots (Figs 3a and 3b). Because biomass was not measured, it is not possible to know whether these differences represent differences in plant biomass or merely re-allocations of resources to leaf area in response to shading. Plants in the control plots also reproduced earlier (Fig. 3c). Consistent with these differences, plant diameter tended to have a positive relationship with June cover (Fig. 4a), and date of first reproduction (initiation of bolting) had a significant negative relationship with June cover (Fig. 4b).

However, despite their apparent advantages of larger size and earlier reproduction, control plants did not have greater fecundity (Fig. 3e). All transplants in this batch initiated reproduction (i.e., had flowering stalks). The relationships between cover and both summed flowering stalk length (Fig. 4c) and summed seed pod length (Fig.4d) were negative, with a highly significant relationship between summed seed pod length and March cover ($F_{1,33}$ = 14.80, P = 0.0005, slope = -5.56, N = 36; number of leaves was included in the model as a covariate but was not significant [$F_{1,33}$ = 2.34, P = 0.14, type III sums of squares table]).

Plant responses - batch 2

These plants tended to be larger in thinned plots (Figs. 5a and 5b), although the differences reached significance only for number of leaves and only in two pairs of plots (7 and 9). Both pair 7 and pair 9 were located in the area where oak wilt had removed the overstory and therefore differed in cover throughout the experiment (Fig. 2). Rosette diameter tended to be greater in plots with lower cover (Fig. 6a). The number of leaves per plant had a strongly significant negative relationship with March cover ($F_{1,60}$ = 16.95, P = 0.0001, slope = -0.59, N = 62; Fig 6b) and with June cover ($F_{1,60}$ = 19.38, P < 0.0001, slope = -0.34, N = 62; Fig. 6c).

Only 18 of the 62 plants in batch 2 had produced flowering stalks by the time the experiment was terminated: 9 of 27 plants in control plots and 9 of 35 plants in thinned plots (χ^2 = 0.4294, P = 0.5123). There was a non-significant trend for plants that initiated reproduction before the end of the experiment to be in plots with lower cover than plants that did not initiate reproduction (only plot pairs with batch 2 plants in both plots were included in an analysis comparing June cover between treatments, N = 54; Wilcoxon χ^2 = 3.1124, P = 0.0777). Although plants in thinned plots tended to have greater summed flowering stalk length (Fig. 5c) and greater summed pod length (Fig. 5d), sample sizes were too small for these differences to be significant. Summed pod length had a negative but non-significant relationship with cover ($F_{1,4}$ = 1.82, P = 0.25, slope = -2.27, N = 7).

Cover at two existing populations

Average cover over *S. bracteatus* plants at Mt. Bonnell was 59.8% (N = 8, standard error = 2.2) and at Cat Mountain it was 63.95% (N = 14, standard error = 1.1) (Figs. 7 and 8).

Discussion

Overall, greater light availability (that is, lower cover) had a positive effect on *Streptanthus bracteatus* transplants. In an annual species like *Streptanthus bracteatus*, the measure of plant performance most relevant to future population size is individual fecundity. In the first batch of plants, which was transplanted into the field early enough for all survivors to initiate reproduction, individual fecundity was greater where cover was less. Most of the transplants in the second batch did not have time to initiate reproduction. However, they were larger where cover was less, and those that did initiate reproduction had a non-significant trend towards greater fecundity where cover was less.

These results are consistent with the results reported by Ramsey (2008). She compared plants grown out-of-doors in pots in three treatments: full sun, shaded 35% of daylight hours, or grown under 50% shade cloth. Like the batch 1 plants in the present experiment, plants in her most shaded treatment (50%-shade) had the greatest rosette diameter. However, while no 50%-shade plants reproduced, 45% of 35%-shade plants and 25% of full-sun plants reproduced. Seedset did not differ between the reproductive plants in the latter two treatments. The results of the present experiment are also consistent with my own qualitative experiences growing this species in pots, and with the unpublished results of Wendy Leonard (pers. comm.).

Although all of these studies agree that *S. bracteatus* can thrive in less than 50% shade, we do not yet know what degree of shading is optimal for this species. It is likely that there is not a single optimum. Instead, the optimal degree of shading probably depends on the amount of water available to the plants. Nothing is known about the physiological ecology of *S. bracteatus*. However, as a general rule shading a plant reduces its transpiration rate (i.e., rate of water loss). Therefore, if no other plant takes up the water it would have used, shading can allow a plant to continue to photosynthesize instead of wilting or shutting its stomates. As a C_3 species, one would expect the rate of carbon fixation in well-watered *S. bracteatus* plants to reach a maximum at light levels less than full sunlight. Inspection of the graphs of Figs. 4 and 6 suggests that the optimum degree of shading might have been close to 50% cover in the present experiment, although there is too much variation among plots and plants to rule out a much lower optimum value, possibly less than 40% cover. However, it is very unlikely that the optimum was more than 50% cover. This is consistent with the results of Ramsey (2008), a pot experiment in which optimum shading was certainly much less than 50%.

The negative effects on *S. bracteatus* of cover over 50% are consistent with the hypothesis that this species is adapted to grow in central Texas woodlands after fires, although they do not prove this hypothesis. We do not know what the likely range of cover would have been after surface or crown fires in these woodlands. However, qualitative observations of woodland sites that have experienced wildfires suggest that woodland fires would have been quite patchy and likely produced a wide range of cover values (K. Doyle, pers. obs.). Woodlands in the area today have relatively dense canopies. (See Section II. Significant Deviations, below; I was not able to find woodland sites with canopies more open than those at Vireo Preserve.) It may be that optimal *S. bracteatus* habitat no longer exists because all remaining woodlands have too dense a canopy.

We cannot rule out the possibility that some of the positive effects of lower cover that we observed were due to associated reductions of underground competition for water and nutrients rather than to reductions in shading. A plant's uptake of water and the nutrients dissolved in that water is limited by its stomatal area, which is determined primarily by its total leaf area. Pruned plants therefore removed much less water from the soil than unpruned plants, and oaks killed by oak wilt of course removed none at all. To the extent that a fire reduces the total leaf area of a plant, it will have an initial effect on its water uptake similar to pruning. Whether competing perennials are completely killed or merely lose some or all of their above-ground leaf area, 'fire-following' annuals will experience less competition for both light and water from them after a fire. However, from a management point of view, the mechanism underlying the positive effects of lower cover is not necessarily critical.

Are existing natural populations experiencing cover too high to be optimal? Our limited data suggest that they may be: average cover for *S. bracteatus* plants at two natural populations in Travis County were ~ 60% and ~ 64% (Figs. 7 and 8). However, this interpretation depends upon our estimate that the amount of soil water available to the experimental transplants was similar to the amount of soil water available to plants in natural populations in years wet enough for seeds of this species to germinate. Our estimation of water availability could be wrong in either direction. For example, the drip irrigation system probably put more water near the plant than further away from it. On the other hand, drought conditions before and during the experiment probably caused surrounding woody plants to grow roots into the watered area, which would have decreased water availability to the transplants.

Management implications

The results of this experiment and the results of the other studies cited above, together with discussions with land managers and with the members of the Bracted Twistflower Working Group, support the following recommendations:

• Sites selected for attempts to establish new *Streptanthus bracteatus* populations should not be in dense *Juniperus ashei* stands or other closed-canopy vegetation.

• Regardless of the initial cover at a site selected for establishing a new population, management should include ongoing thinning or pruning to counteract expected increases in woody cover, especially cover of *Juniperus ashei*. In some sites prescribed burns might be an alternative tool for accomplishing this.

• These recommendation in no ways obviate the need to address all the other known threats facing this species and to conform to all of our present knowledge and inferences about this species' other habitat requirements. Therefore, sites selected for attempts to establish new *Streptanthus bracteatus* populations also
  - should have legal, lasting protection from development;
  - should be protected from recreational use, especially mountain bikes;
  - should be fenced to exclude deer;
  - should be at or near the boundaries between the Edwards, Walnut, and Glen Rose Formations; and
  - should be in woodland or shrubland vegetation rather than in dense grassland or on barren rocky outcrops.

• Managers of existing populations, especially those of populations declining for no apparent reason (e.g., Bright Leaf), should estimate cover ~ 0.5 m above the ground. (A densiometer is an inexpensive, easy-to-use tool to do this.) If cover is > 50%, thinning or pruning of woody plants should be considered for a portion of the site. The effects on *S. bracteatus* in the thinned portion of the site should be assessed to determine whether further thinning is desirable at that site.

• If cover reduction is undertaken, the treated area <u>must</u> be deer-fenced at the same time. Zippin (1997) showed that deer herbivory on flowering stalks has substantial negative impacts on population growth rates of this species. Other understory plants and low-lying branches may be providing *S. bracteatus* plants with some physical protection from deer, as they do for oaks in this region (Russell and Fowler 2005). (Recall that in the present experiment deer fences were in place before any transplants were put out.) If understory plants and low-lying branches are removed without providing protection from deer, *S. bracteatus* plants could become more vulnerable to deer browsing.

• Reducing cover should not be considered a substitute for protecting this species from other threats, especially from deer and recreational use.

Acknowledgments

Alyson Center designed and built the cages and irrigation system, measured the transplants in the field, took the photographs, and calculated cover. Dana Price and Alan Pepper were co-investigators on the original proposal and contributed substantially to it.

We especially thank Lisa O'Donnell, manager of the Vireo Preserve, for facilitating this project in many ways, including working with us to position the experimental plots. We thank all the people who provided information about all the field sites we considered using, particularly Bill Carr (TNC), John Mahan (Bright Leaf), and Bill Reiner (City of Austin). Finally, we thank the members of the unofficial Bracted Twistflower Working Group, whose suggestions, ideas, and encouragement provided the impetus for this project.

This project was supported by a U.S. Fish and Wildlife Service Section 6 Grant, awarded and administered by Texas Parks and Wildlife Department.

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Appendix I. Coordinates of pairs of plots at Vireo Preserve

plot pair	latitude	longitude
1	30.31222	97.81927
2	30.31249	97.82001
3	30.31392	97.82023
4	30.31316	97.82043
5	30.31332	97.82076
6	30.31345	97.82111
7	30.31468	97.82115
8	30.31459	97.82135
9	30.31460	97.82156
10	30.31461	97.82156

Appendix II. Methods originally proposed. This is a copy of the text in the 'Approach' section of the proposal.

To determine the effect of an overstory of woody plants on *S. bracteatus*, sets of three plots will be located along short transects, four transects per site. Each transect will contain one plot under the canopy of woody plants, one plot at the edge of a stand of woody plants, and one plot in the open. (For an example of the use of such transects in an experiment, see Fowler and Clay 1995). The plot in the open will receive direct sunlight at least two-thirds of the time between sunrise and sunset. The plot at the edge will receive direct sunlight approximately 40% of the time. The plot in the shade will receive no direct sunlight other than light flecks. To the extent possible, one transect will be located in each cardinal direction. Each plot will be at least 5 m apart from all other plots in the site. There will be 12 plots per site (4 transects x 3 plots/transect). Four transplants will be planted in each plot (48 transplants/site). A set of 20 seeds will also be sown in each plot.

Two sites will be located over dolomite just above the base of the Edwards Formation and two sites will be located near them over Walnut Formation limestone, based on information in City of Austin geological maps and records, in consultation with the city geologists. The Edwards Formation lies directly above the Walnut Formation (Young 1977). The Edwards is usually dolomite, the Walnut very rarely so. However, rock composition in all sites will be verified before planting. Identifying the specific factors responsible for differences in plant performance over the two types of bedrock is beyond the scope of this proposal; Mg content and seepage are the most likely.

Fresh greenhouse-grown seed will be obtained from plants grown by Fowler and Pepper. Some of this seed will be sown in the experimental plots. Some of it will be used to produce the plants to be transplanted into the experimental plots. Wild-collected seed is too scarce to use it, or plants grown from wild-collected seed, for experiments until we know how to do successful re-introductions.

Seeds will be sown in October, in time for normal fall germination. Because these seeds may not provide a sufficient sample size of plants to measure treatment effects on growth and fecundity, transplants grown from the same seed batch will be transplanted into the plot in December, using methods Fowler and her students have used successfully in other transplant experiments (Zippin 1997, Fowler 2002, Batchelor 2004).

Plants, including seedlings, will be marked so they can be monitored individually. Seeds will not be marked individually, but the edges of the subplots where they are sown will be marked to facilitate finding seedlings. Plants will be monitored regularly and their size and fecundity measured non-destructively (Zippin 1997). Some of the seeds will be collected to count seeds per infructescence. Others will be allowed to disperse naturally, in hopes of establishing an *S. bracteatus* population in the site (which will not be near any existing population). The effects of treatments will be compared with ANOVAs (size, fecundity, lifespan), G-tests (germination rates, survival rates) and logistic regression (mortality rates over time).

All experiment plants will be fenced to exclude deer. Zippin (1997) showed conclusively that deer herbivory has an important negative effect upon populations of *S. bracteatus*. The protection that woody plants, especially *Juniperus ashei*, provide from deer browsing may be the reason that in some sites *S. bracteatus* is more common under woody plants than in the open (Zippin 1997, Russell and Fowler 2004). An alternative explanation is that woody plant cover has increased and the understory plants are relicts of previous habitat conditions, a common condition of populations of endangered plants. Most of the populations on public land are already deer-fenced.

Experimental plants will also be protected from mollusks and insects (flat hoops sunk in the ground and coated with Tanglefoot®; a bio-degradable insecticide such as Neem as needed). Zippin (1997) found that invertebrate herbivory does not have a major effect on this species but does occur. Wild and cultivated plants are frequently heavily infected with powdery mildew (D. Price, pers. obs; N. Fowler, pers. obs.). Infected plants will be treated as needed with a sulfur preparation as needed. Our objective in preventing high levels of herbivore and disease damage is to isolate and measure the effects of the two factors of interest (woody cover and substrate) in an experiment that is both of manageable size and has a high likelihood of providing useful results.

II. Significant Deviations

Change in timeline

By the time funds were available to the PI (February 2008) it was too late to do the project. The project was therefore postponed one year, and carried out in 2008-2009.

Change in location

The proposal stated that four sites would be used, each with transects extending from 'woodland' to 'open' to provide the desired gradient in cover. I spent a great deal of time visiting potential sites and discussing them with biologists and land managers from the Nature Conservancy, the City of Austin Balcones Canyonlands Preserve (BCP), the City of Austin Water Quality Protection Lands (WQPL), Brackenridge Field Laboratory (BFL), and Bright Leaf Preserve. I discovered that the original plan of using four sites was unrealistic. Only Vireo Preserve had a suitable open area that was also potential *S. bracteatus* habitat; this small area was open because oak wilt had killed the canopy trees. Open areas in the other sites had vegetation already known to be unsuitable habitat for *S. bracteatus* (Zippin 1997), such as dense stands of grass or bare rock outcrops. Therefore, instead of a design based on variation in natural light levels, a design based on manipulating light levels was used. (This scarcity of non-grassland sites with relative low woody cover may be part of the reason that *S. bracteatus* is so rare; see the Discussion.)

Vireo Preserve was also suitable for another reason: we were permitted to reduce woody cover in plots there. In most of the other sites, golden-cheeked warbler management and/or a reluctance to sanction any manipulations of woody plants prevented any experimental reductions of cover. Even at Vireo Preserve, we were only allowed to prune back certain understory woody species. We located the treated plots in the oak wilt area and under natural canopy gaps containing the understory species we could prune, so as to provide as much light as possible to the treated plots. Nevertheless, the range of light levels was not as large as originally planned.

Significant changes in methods

As just described, variation in light levels was obtained by pruning of understory woody plants and by taking advantage of canopy gaps and of an area affected by oak wilt, rather than by using transects running out from woodland patches.

A further change in the methods was the deletion of seed additions. Instead, we used only transplants. The extremely low rainfall in the summer and fall of 2008 left the soil too dry for germination.

Because the drought continued during the winter and spring of 2009, we had to add protection from small climbing mammals (a poultry-wire cage around each transplant) and supplementary watering to our care of transplants. On the other hand, we did not need to use Tanglefoot.

Probably because of the drought, small mammal herbivory was so intense that two-thirds of our first batch of transplants were killed, and we had to add a second batch. This is the first report of small mammal herbivory on *S. bracteatus*, and was likely due to the absence of other sources of food and water.

We added direct measurements of woody cover to the design. This was done in two ways, first with a densiometer and then by hemispherical photographs shot from the height of a *Streptanthus bracteatus* plant. These photographs were analyzed with Gap Image Analyzer©. We took similar photographs from two sites with natural *S. bracteatus* populations for comparison with our experimental plots..

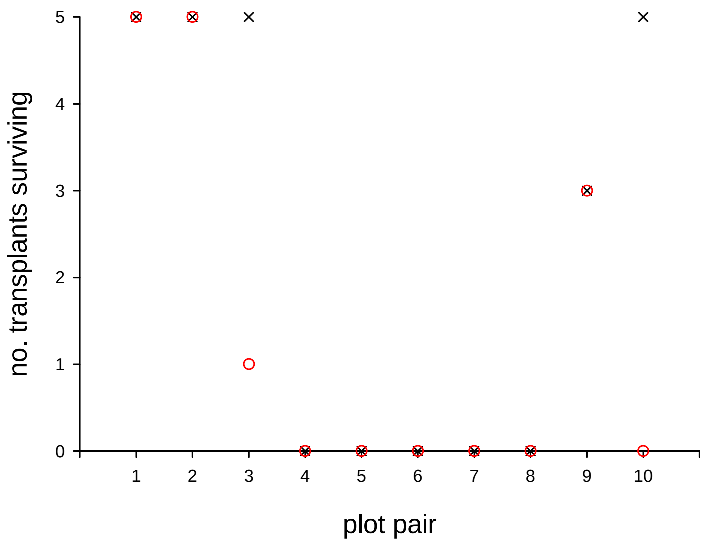


Figure 1. Number of survivors per plot (out of 5). Original 100 transplants only.

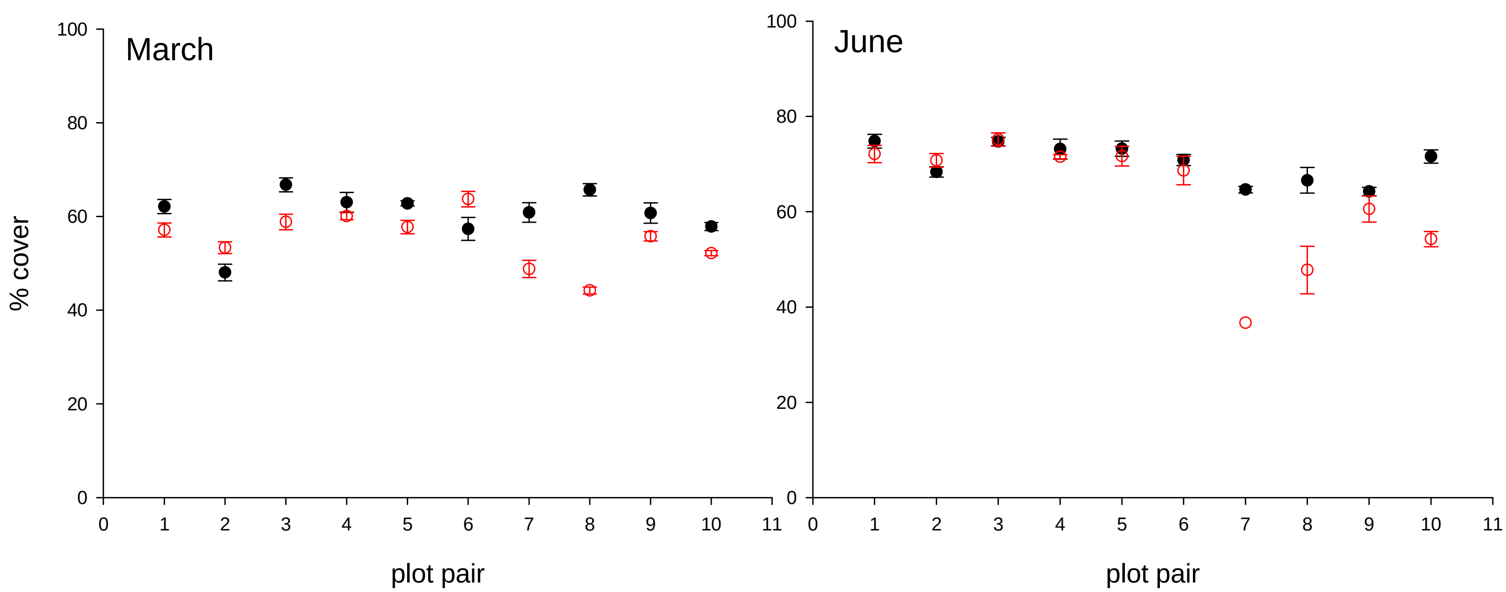
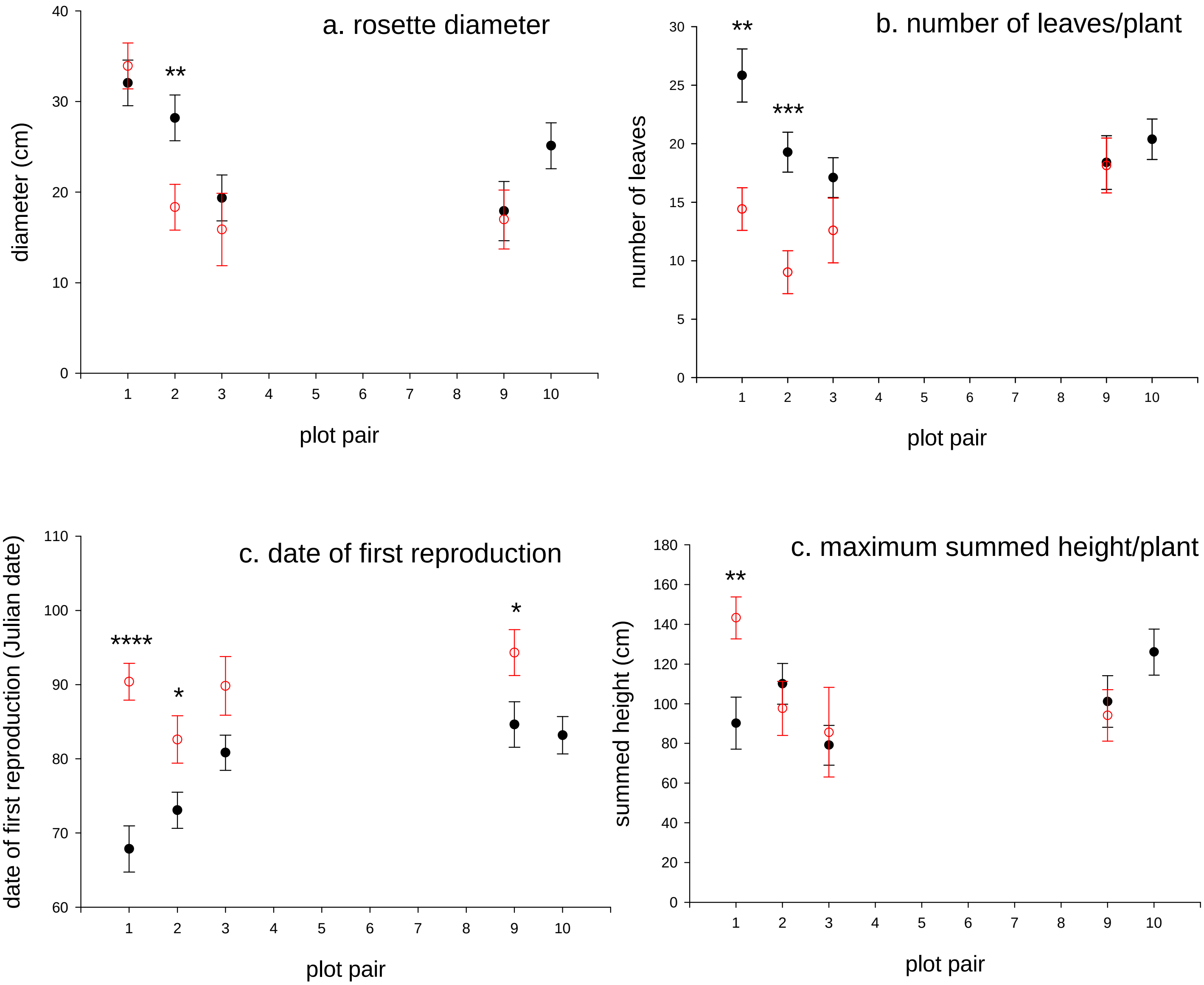


Figure 2. Average cover in March and June. Calculated from 5 hemispherical photographs per plot per date. Open red circles: thinned plots. Filled black circles: control plots. Bars represent 1 se.



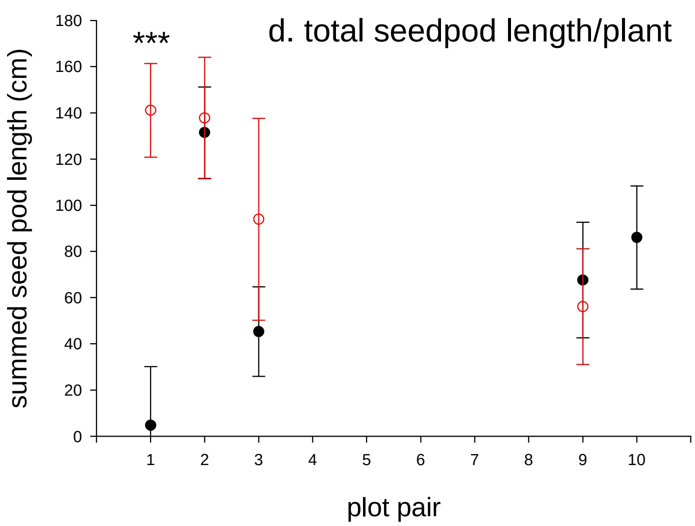


Fig 3. Means and standard errors of batch 1 plants, adjusted for any covariates used in calculating contrasts. Date of first reproduction is the date when a flowering stalk was first visible. Asterisks indicate significant contrasts between treatments. Open red circles: thinned plots. Filled black circles: control plots. *, P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001

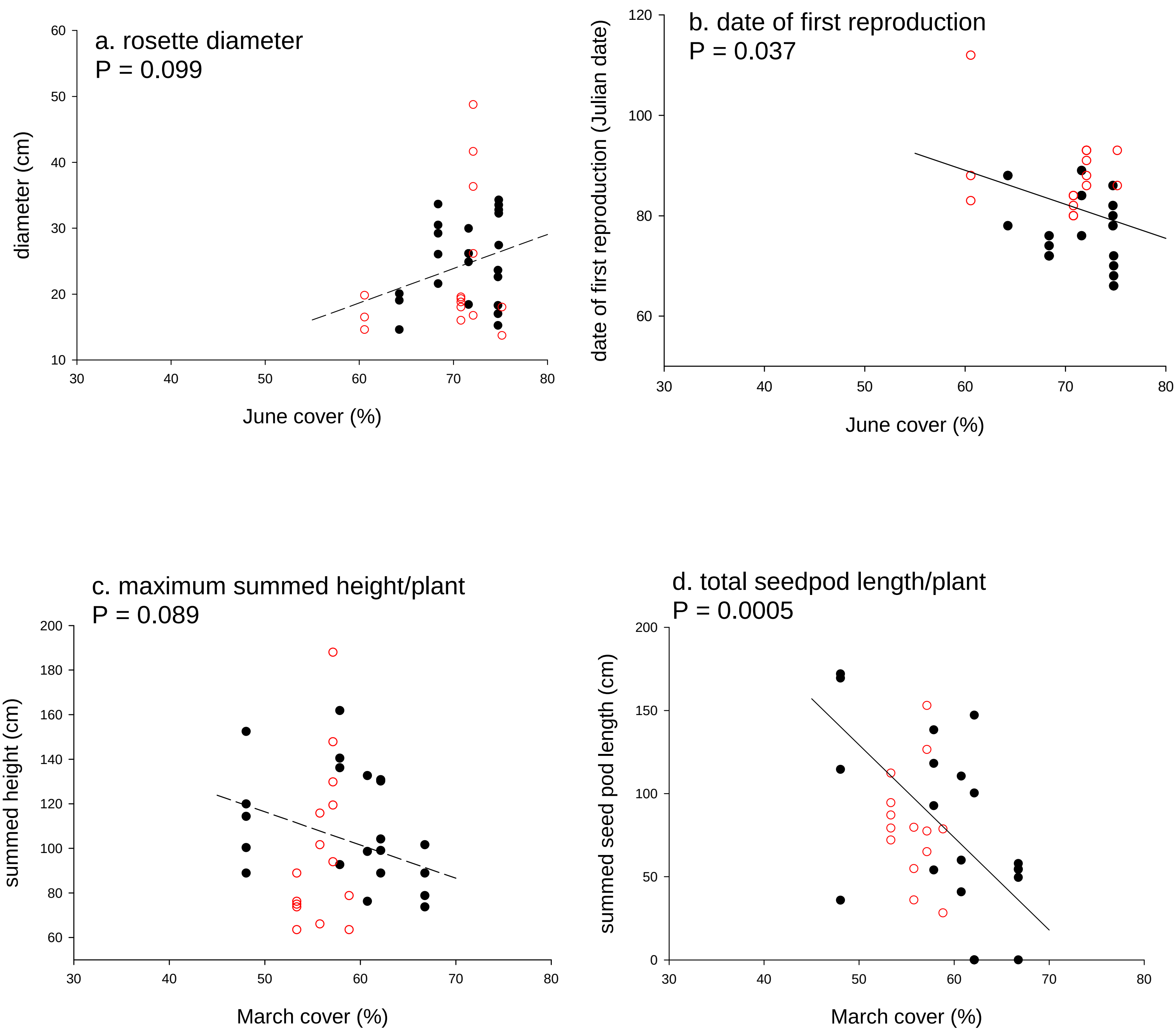


Figure 4. Regressions of batch 1 plant performance variables against cover..
Date of first reproduction is the date when a flowering stalk was first visible. Open red circles: thinned plots. Filled black circles: control plots.

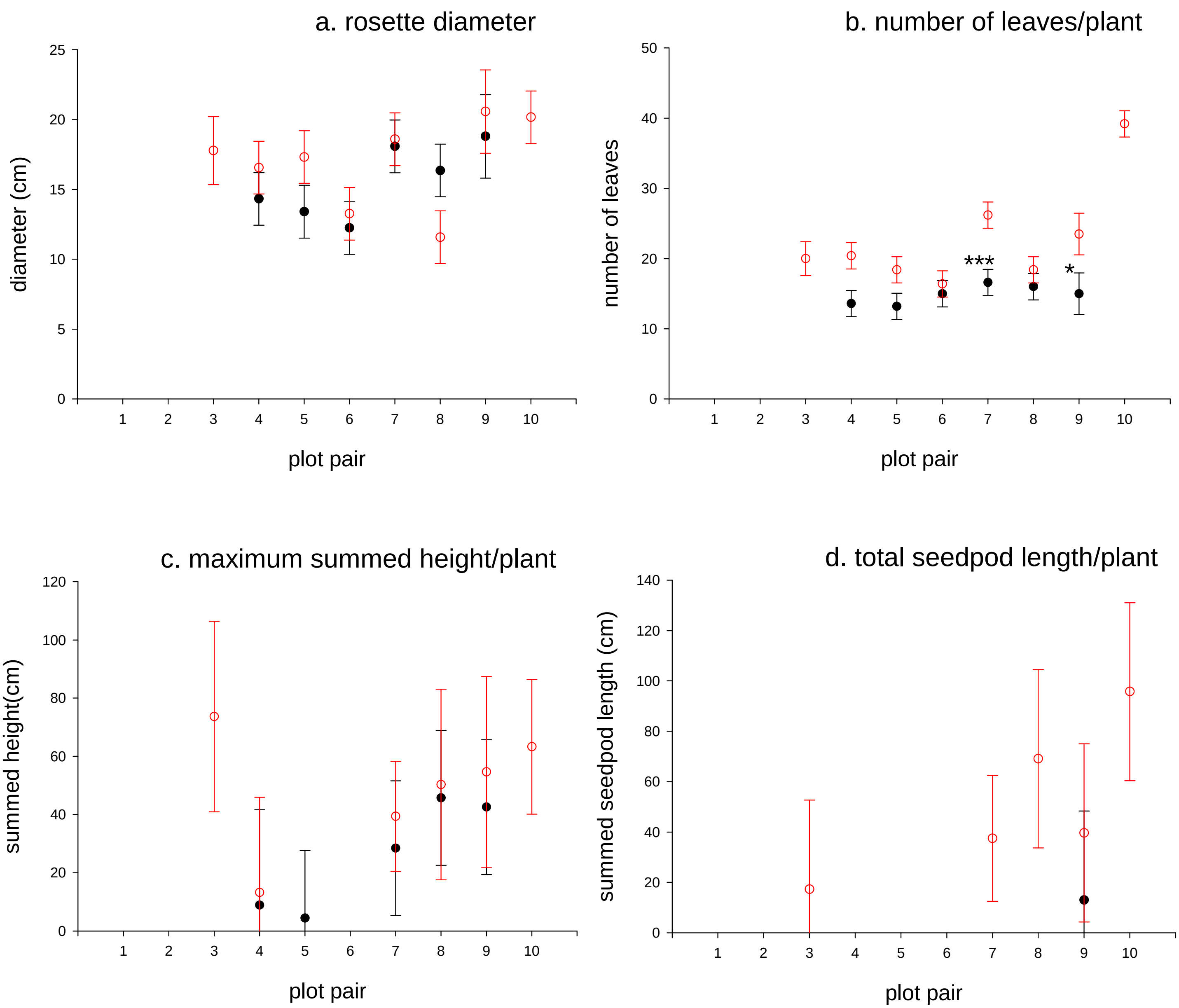


Fig 5. Means and standard errors of batch 2 plants. Asterisks indicate significant contrasts between treatments. Open red circles: thinned plots. Filled black circles: control plots. *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

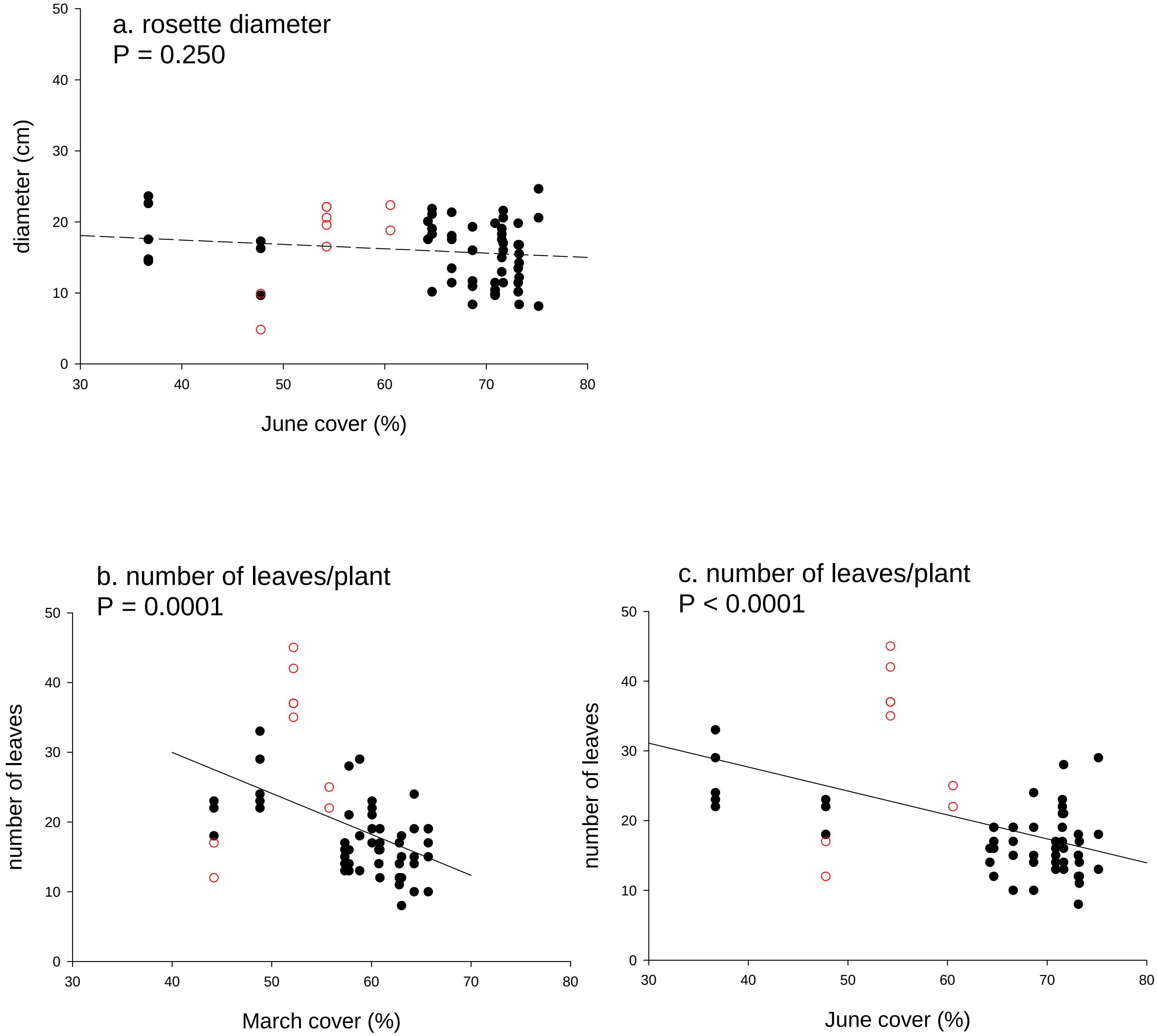


Figure 6. Regressions of batch 2 plant performance variables against cover.
Open red circles: thinned plots. Filled black circles: control plots.

Figure M1. Transplants in styrofoam pots just before transplanting.

Figure M2. Plot showing deer fencing, with access ‘gate’ open.

Fig. M3. Part of the drip irrigation system. Red gas can held water.

Fig. M4. Part of the drip irrigation system. This piece provided water to plant #3.78.

Figure M5. Preparing to take a canopy photograph.

Fig. M6. Canopy photograph taken 4 March 2009. Flagging and gas can visible on left.

Figure 7. *Streptanthus bracteatus* growing at Cat Mountain (average cover 64%).

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Fig. 8. *Streptanthus bracteatus* growing at Mt. Bonnell (average cover 60%).

Map 1. Location of experiment (red markers). The undeveloped land in the center of the image, east of Capitol of Texas Highway, is Vireo Preserve (north) and Wild Basin (south).

Map 2. Locations of pairs of experimental plots (red markers) at Vireo Preserve. The upper three markers are plot pairs 7-10 (plot pairs 9 and 10 cannot be distinguished at this scale), located in the oak wilt area.