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# Seed Production With Insect Herbivory and Fungal Occurrence for the Rare *Penstemon haydenii*

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We quantified seed production and viability, floral herbivory and fungal infection on blowout penstemon (*Penstemon haydenii* S. Watson), an endangered species of the Nebraska Sandhills, USA, in order to determine the potential for perpetuation of this, and possibly other short-lived, rare perennials of fragmented habitats. Over three years, the number of seeds per infructescence averaged 518 (SE 29.01). Plants produced an average of 1398 seeds. Seed viability of 38% reduced reproductive potential to 531 viable seeds per plant. Plants in multiple blowout sites in two counties were assigned to one of four treatments: insecticide, fungicide, both, or neither (control). Insect herbivore damage varied between site-year combinations, but was not successfully reduced by treatments. For example, grasshoppers (Orthoptera, Suborder Caelifera) caused high levels of herbivory at the Hooker County sites in 2007. All three non-control treatments reduced visible signs of fungal occurrence, except in Hooker County in 2006. However, total seed number per plant, seed weight, and seed viability were similar among treatments, suggesting fungal attack did not reduce fecundity. The main difference observed was significant variation in each of these parameters of plant performance among site-year combinations. Consequently, we conclude that site-specific conditions are important to blowout penstemon regeneration.

## Introduction

The rare blowout penstemon (*Penstemon haydenii* S. Watson) is a short-lived perennial forb that is native to the shifting sand of open disturbances called blowouts in the Sandhills of northcentral Nebraska and the northeastern Great Divide Basin, Wyoming (Fritz et al. 1992, Great Plains Flora Association 1986, Heidel 2005, 2006, Kaul et al. 2006). It was classified as endangered in 1987 (USFWS 1987). Blowout penstemon is a short-lived perennial, estimated to live about six to eight years (Stubbendieck et al. 1997). Since monocarpic or short-lived perennials often depend on seed input for persistence, their dynamics often are vulnerable to seed losses (Louda and Potvin 1995). Yet, little research has been conducted to date on the effects of natural enemies on blowout penstemon seed production. Plant fecundity often is size-specific, with seed production proportional to shoot mass (Rees and Crawley 1989). Yet, fecundity among individuals of the same genotype may vary by four orders of magnitude, depending upon the growing conditions (Crawley 1990); and it may reflect variation in the magnitude of biotic interactions. Extensive variability in fecundity was recorded among local populations of blowout penstemon (Kottas 2008).

We estimated ambient seed production and viability. Based on observations of damage, our aim was to evaluate whether insect herbivory or fungal pathogens negatively influenced blowout penstemon seed production or viability, reducing seed available for seed banks and seedling recruitment. The underlying goal was to improve the knowledge of ambient seed production and the contribution of biotic interactions to the variation observed in the

seed production rates of this rare species across locations and years. Thus, our first objective was to accurately estimate seed production. The second objective was to quantify the impact of insect herbivory and fungal disease on seed production.

## Methods

### Natural History

Blowout penstemon plants are restricted to disturbances in sand prairie (Fritz et al. 1992; Stubbendieck and Kottas 2007). Flowering occurs in early June in Nebraska populations. The inflorescence of blowout penstemon is a thyrse characterized by a series of pairs of opposite leafy bracts subtending individual cymes, called verticillasters, of two to eight flowers (Great Plains Flora Association 1986). Each lavender flower produces a capsule bearing 25 to 35 discoid brown to black seeds (Fritz et al. 1992). Stubbendieck et al. (1982b) reported seed weight averaged 3.6 mg/seed. Range management, fire reduction, and natural succession have increased plant cover and reduced areas of shifting sand in the Nebraska Sandhills, thereby further fragmenting areas of blowing sand habitat (Stubbendieck et al. 1982a). Such fragmentation provides the context within which natural enemies, herbivores and pathogens, may impact relatively isolated populations of endangered plants.

We observed several foliage, floral, and seed feeding insects feeding on blowout penstemon in 2004 and 2005. The majority of these insects were phytophagous members of the stink bug family (Pentatomidae), and included stink bugs (*Euschistus vaiolarius* Palisot and *Thyanta* sp.), the spotted

stinkbug (*Holcostethus limbolarius* Stål), and the blackbug (*Corimelaena pulcaria* Germar).

Additional frequent visitors to blowout penstemon flowers, thought to be the common pollinators of *Penstemon*, include four species of megachilid bees (*Hoplitis pilosifrons* Cresson, *Osmia cyaneonitens* Cockerell, *O. distincta* Cresson, and *O. integra* Cresson) (Lawson et al. 1989), and two species of halictid bees (*Lasioglossum* spp., also known as *Dialictus* spp.) (Tepedino et al. 2006).

We also identified *Fusarium*, *Rhizoctonia*, *Alternaria*, and *Cercospora* fungal pathogens from field-collected blowout penstemon. Over 500 species of *Cercospora* spp. cause leaf spot and blight diseases on many plant species (Daub & Ehrenshaft 2000, Farr et al. 1989). *Cercospora* infection was widespread among blowout penstemon plants in 2005, especially among inflorescences compared to vegetative shoots (Kottas 2008). Many plants exhibiting *Cercospora* symptoms had aborted fruiting structures, comparable to the effect of *Cercospora kikuchii* on soybeans (*Glycine max*) at full flower or young pod stages. (Chen et al. 1979).

### Study Sites

We applied treatments to manipulate floral herbivory and plant pathogen infection in multiple blowouts at sites in two Nebraska Sandhills counties (Hooker County: 42° 01' N, 101° 16' W; and, Cherry County: 42° 22' N, 101° 19' W). The experimental plants came from blowout penstemon seedlings that were started in the greenhouse and then planted and established at the Hooker County sites in 1991 and at the Cherry County sites in 1996.

Average yearly precipitation (1980 through 2008) was 444 mm at Gudmundsen, Nebraska, less than 30 km from the research sites (High Plains Regional Climate Center 2008). Yearly precipitation was above normal in 2005 and 2007 and below normal in 2006.

### Insect Herbivore and Fungal Exclusion Treatments

An issue in the many insect exclusion studies (Crawley 1989, 1990, Edwards and Crawley 1999) has been about which insecticides are least harmful to insect pollinators (Diaz & McLeod 2005, Maus et al. 2003, Suchail et al. 2000). Imidacloprid, an insecticide with a long residual effect (Sarkar et al. 2001), is reported to be effective against sucking insects (Yamamoto et al. 1998), to have low mammalian toxicity (Gupta 2007, Moriya et al. 1992), and to show no phytotoxicity (Weichel & Nauen 2004). However, nectar can become contaminated when imidacloprid is applied as a foliar spray (Suchail et al. 2000). Based on these findings, we applied imidacloprid granules to the soil for the insect exclusion treatment.

For the fungal exclusion treatment, we used GreenCure®, a potassium bicarbonate compound that is used to reduce fungal infection in food and ornamental crops (Yeager 2006). Since this fungicide does not have a long residual effect, it was applied at approximately 3-week intervals during June, July, and August. We used both insecticide and fungicide for a third treatment. Control plants were not treated.

In 2006, we marked 20 plants per block for three blocks, in each of five blowout sites within both Cherry and Hooker County locations (N = 300 plants total). In 2007, we used only four blowout sites in each of the two county locations (N = 240 plants total), since additional accessible blowouts with sufficient numbers of previously untreated plants were not available. Five plants in each block were randomly assigned to one of four treatments: insecticide, foliar fungicide, insecticide + fungicide, or no treated (control).

We applied the systemic insecticide imidacloprid in the form of granules (GrubEX®, active ingredient imidacloprid 0.2%) in each growing season. In 2006, the insecticide was applied somewhat late, the first week in July, after permission was granted. In 2007, the insecticide was applied in the first week in June. The insecticide granules were placed in a trench 5 to 7 cm deep at the base of each plant. We applied water as recommended (Zalom et al. 2003) and covered the insecticide granules with sand. The amount applied varied with the size of the plant, averaging 3.5 g of granular insecticide per plant.

We applied fungicide treatment (GreenCure®, active ingredient potassium bicarbonate 85%) every 3 weeks starting the first week in June, with three applications before seed harvest. After adding 28 g of fungicide to 7.6 l water, we sprayed to cover both the upper and lower surface of the leaves as well as the stems. Control plants received no treatment, since humidity is conducive to fungal infection (Harveson 2007).

### Quantification of Ambient Seed Production

In order to quantify average ambient seed production, we began collecting randomly selected infructescences (fruiting stalks) in August 2005. In August 2006 and 2007, since the experiment reduced the number of available untreated plants, we quantified seed production with the control plants from the experiment. We randomly selected and harvested one infructescence per plant each of the five blowouts in each of the two county locations for seed count, weight, and viability. In order to reduce the impact of seed collection on these populations, we used the control treatment to quantify ambient

seed production (2006: total N = 59; 2007: total N = 60). We placed bags over the infructescence immediately before harvest to retain all of the seeds. We air dried infructescences for 1 week. We estimated plant seed production from infructescence characters, following other studies (e.g., Schulz et al. 2004, Zimmerman and Gross 1984). We quantified: 1) the number of infructescences per plant; 2) numbers of verticillasters per infructescence, capsules per verticillaster, and mature and aborted seeds per capsule, 3) seed weight; 4) seed viability; and, 5) seed production in relation to infructescence stem height. Aborted capsules were defined as ovaries that did not swell and were assumed to be unfertilized or aborted by insects, weather, or another factor.

We counted all of the seeds from each capsule, without regard to size, damage, or apparent viability, and recorded the numbers of mature versus aborted seeds in each capsule. Aborted seeds were those which were thin and not fully filled or formed. In 2005, we quantified seed weight per plant in each blowout. In 2006 and 2007, we calculated seed production per plant by weighing all the seeds per infructescence and multiplying the number of seeds per infructescence by the average number of infructescences produced per plant over a 14-year monitoring period across Nebraska sites (Stubbendieck et al. 2007).

#### Seed Viability

We tested seed viability using tetrazolium (2,3,5-triphenyl tetrazolium hydrochloride) on 400 randomly selected seeds from each treatment in each blowout following standard procedures (Peters 2000). Viable seed absorbed the tetrazolium solution throughout the seed embryo and surrounding endosperm. We categorized as non-respiring those seeds with unstained sections, stripes, or no embryo.

#### Quantification of Insect Herbivory and Fungal Attack

Insect damage on stalks removed from treated sites in August of 2006 and 2007 was rated by the amount of plant tissue removed or damaged by insects. We scored plants on a scale of 0 to 5 for level of insect damage. A score of 0 indicated no damage, while a score of 1 indicated that up to 20% of the plant showed signs of damage, a score of 2 indicated from 21% to 40% of the plant showed signs of damage and so on. Fungal occurrence, evidenced by dark circles 2 to 30 mm diameter on vegetative parts of the plants, was rated on the basis of the amount of the plant tissue covered with lesions. Again, we scored plants 0 to 5 for fungal occurrence. A score of 0 indicated no damage, while a score of 1 indicated that up to 20%

of the plant showed signs of damage, a score of 2 indicated from 21% to 40% of the plant showed signs of damage and so on.

#### Statistical Analyses

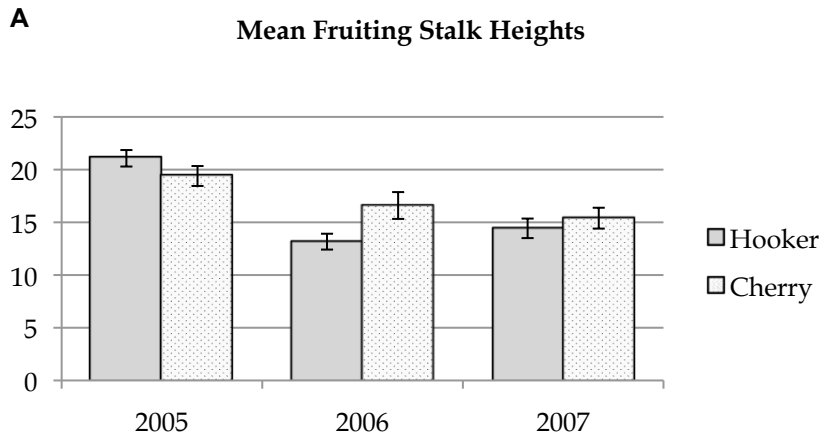
We used Analysis of Variance (ANOVA) to assess seed productivity (Proc Mixed, SAS Institute 2003). This experiment was a randomized complete block design with three replicate blocks. Factors in the ANOVA included treatment, county, and year as fixed variables. We treated site, block, and plant as random variables. We assessed normality and homogeneity of variances (SAS Institute 2003). We transformed data that were not normally distributed prior to analysis, using natural log transformations. When presenting means, we back-transformed natural log-transformed results to the original scale. We used  $\alpha = 0.05$  for all statistical comparisons. Also, we analyzed correlations between seed counts or seed weight and stem length (Proc Corr: SAS Institute 2003).

#### Results

##### Ambient Seed Production by Untreated Plants

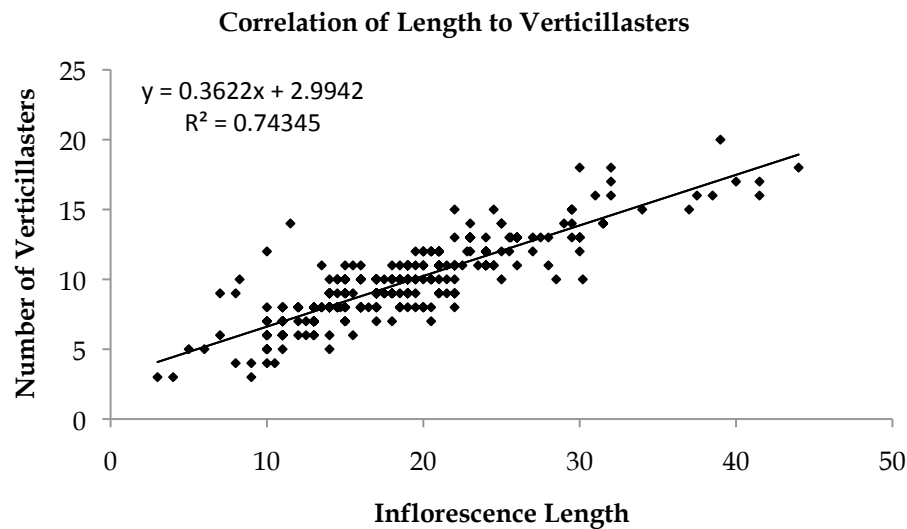
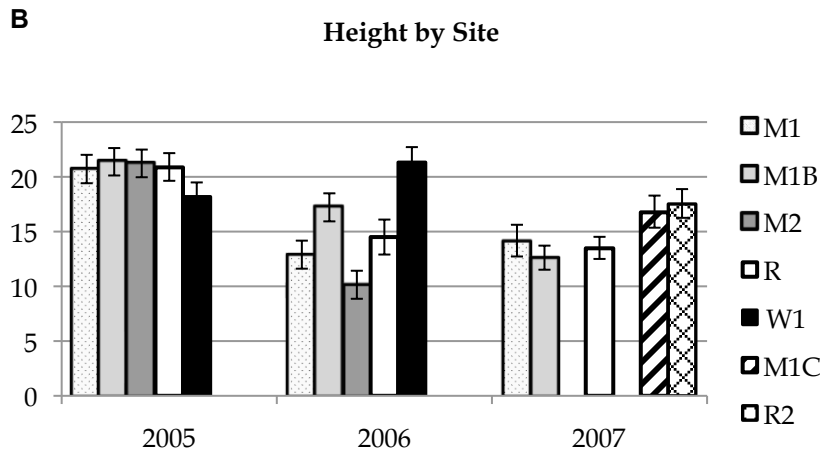
Infructescence size varied among plants. Infructescences from both Hooker and Cherry County locations were significantly taller in 2005 than in 2006 or 2007 (both  $P < 0.0001$ ) (Fig. 1a). Infructescence length averaged 21.7 cm ( $SE = 0.54$ ) in 2005, but only 15.5 cm ( $SE = 0.85$ ) in 2006 and 15.6 cm ( $SE = 0.84$ ) in 2007. Differences between blowout sites in Hooker versus those in Cherry County were not significant within year; however, differences among individual blowout sites were highly significant ( $P < 0.0001$ ) (Fig. 1b). Thus, infructescence length varied among years and among blowout sites under ambient conditions, when disease and herbivory were not excluded.

The number of verticillasters increased as the length of the infructescence stalk increased (Fig. 2;  $N = 261$ ,  $P < 0.0001$ ). This establishes a way of estimating seed production without destructive sampling. The mean number of verticillasters/cm height was 0.55 ( $SE = 0.01$ ,  $P < 0.0001$ ). Overall, year and county location interacted in determining the number of verticillasters per infructescence ( $P = 0.02$ ). Taller infructescences in 2005 produced more verticillasters, but no differences occurred in the number of verticillasters per stalk among blowout sites ( $P = 0.16$ ) (Kottas, 2008). However, height differences were significant in 2006 ( $P < 0.001$ ) and 2007 ( $P = 0.003$ ). Stalk length and number of verticillasters per infructescence were specific to blowout site rather than to county location of the blowouts.



**Figure 1. Fruiting Stalk Heights.**

(A) Fruiting stalk heights by county. (B) Fruiting stalk heights by site. Least-square mean height of blowout penstemon infructescences for research sites in each county in each year and for each site. Height is given in centimeters. Differences between the counties were not significant within years. Error bars represent standard error. Sites M1, M1B, M2 and M1C were in Hooker County. Sites R, R2 and W1 were in Cherry County.



**Figure 2. Correlation of Stalk Length to Verticillasters.**

The correlation of blowout penstemon verticillasters to stalk length. Length is presented in centimeters. There is a positive correlation of height to number of verticillasters [ $R^2 = 0.74$ ,  $N = 261$ ,  $P < 0.0001$ ; verticillasters =  $0.3622$  (height) +  $2.99$ ].

The mean number of mature capsules per verticillaster varied from 2.5 in Hooker County in 2006 to 5.5 in Hooker County in 2005. Total capsules per infructescence and inflorescence height were correlated [ $R^2 = 0.65$ ,  $P < 0.0001$ ; total capsules = 4.38 (height) - 7.20]. County and year interacted to explain the number of total capsules per infructescence ( $P < 0.0001$ ); and, the differences were significant among sites in 2005 ( $P < 0.001$ ) and 2006 ( $P = 0.002$ ), but not in 2007 ( $P = 0.09$ ).

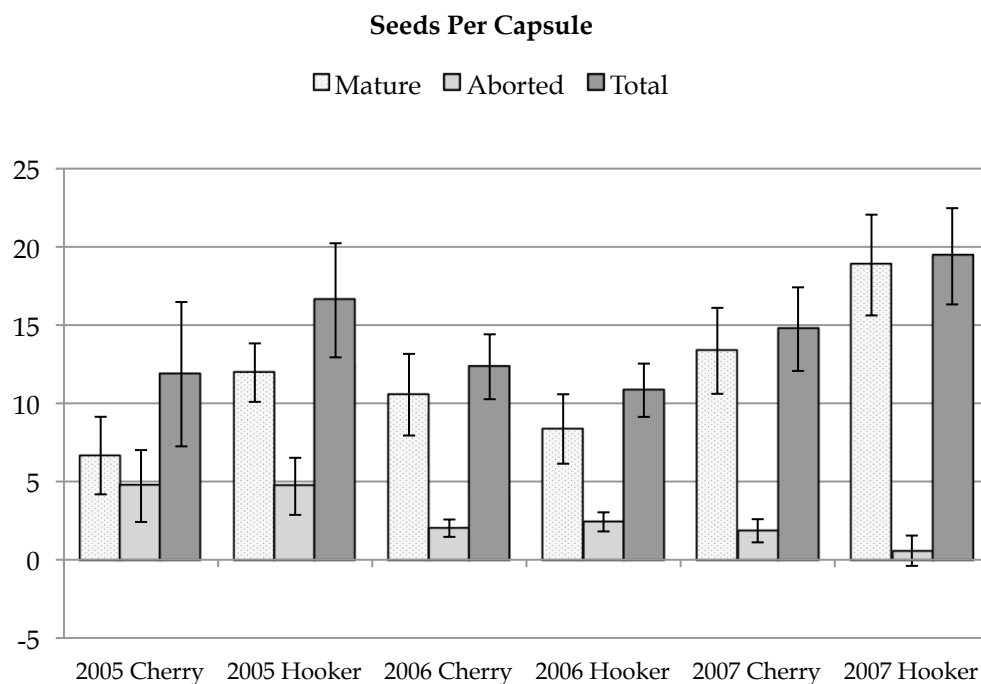
The total number of seeds per capsule in the control treatment did not differ significantly among years or between counties (Fig. 3). The number of mature seeds per capsule averaged 8.9 in 2005 ( $SE = 1.09$ ), 9.2 in 2006 ( $SE = 1.27$ ), and 16 in 2007 ( $SE = 2.27$ ), which was explained by a significant year  $\times$  county interaction ( $N = 795$ ,  $P < 0.0001$ ) (Kottas 2008). Thus, seeds per capsule for the control plants varied significantly, primarily among sites in some years.

The numbers of vegetative plants, flowering plants, and infructescences have been monitored in Nebraska blowout populations since 1993. Using these data, we calculated that the number of inflorescences per flowering plant over eight Sandhills counties over the last 15 years averaged 3.5 ( $SE = 0.12$ ,  $N=563$  blowouts or blowout complexes). This is consistent with the results for total flowering inflorescences per plant in a parallel three-year demographic study, including those lost to herbivory (3.8 infructescences per plant ( $SE = 1.7$ ) (Kottas 2008). Of these, 17% were lost to herbivory at the time of

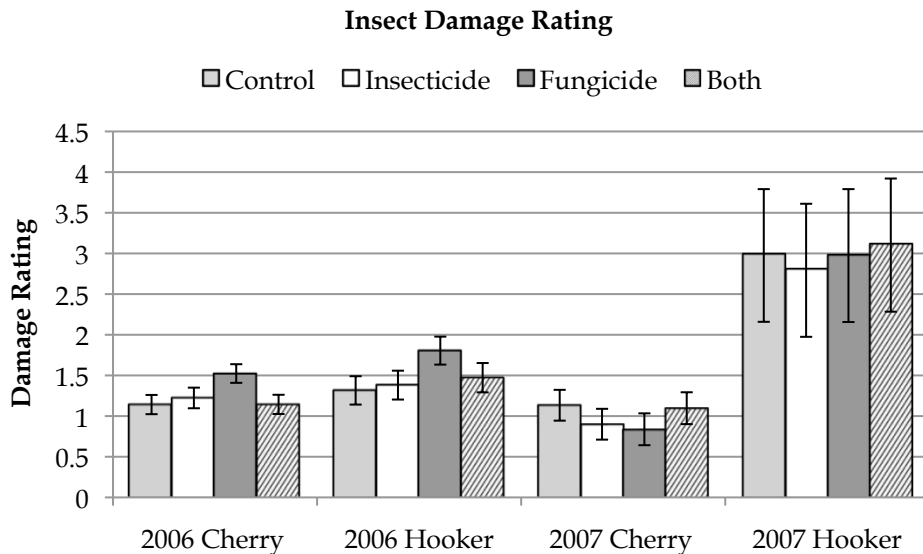
census (Kottas 2008). Thus, flowering plants generally average 3.5 - 3.8 infructescences.

**Evidence of Treatment Effectiveness**

Neither insecticide nor fungicide treatments were uniformly effective in reducing intensities of herbivory and fungal attack. First, insecticide treatment did not reduce the damage score based on visible insect damage (Fig. 4). However, in 2006, there were significant differences in insect damage scores among treatments ( $P = 0.009$ ). Fungicide treated plants had a higher mean insect damage score (1.7,  $SE = 0.13$ ) than control plants (1.2,  $SE = 0.13$ ), insecticide-treated plants (1.3,  $SE = 0.14$ ), or insecticide + fungicide- treated plants (1.3,  $SE = 0.13$ ). In 2007, although there was no treatment effect (mean score = 2.6,  $P = 0.45$ ), insect damage score was significantly higher in Hooker County (mean score = 4.28,  $SE = 0.15$ ) than it was in Cherry County independent of treatment (mean score = 0.99,  $SE = 0.16$ ) ( $P < 0.0001$ ). The two treatments with insecticide had a 0.20 lower insect damage score in 2007, when contrasted with fungicide treated plants ( $SE = 0.09$ ,  $P = 0.03$ ). The significant year  $\times$  county interaction ( $P < 0.0001$ ) reflected the higher levels of insect damage in Hooker County in 2007, independent of treatment (Fig. 4). All treatments had significantly greater insect damage than in the other county or year combinations (Fig. 4). Overall, the insecticide exclusion was not effective, but herbivory varied significantly among blowout sites in 2007.

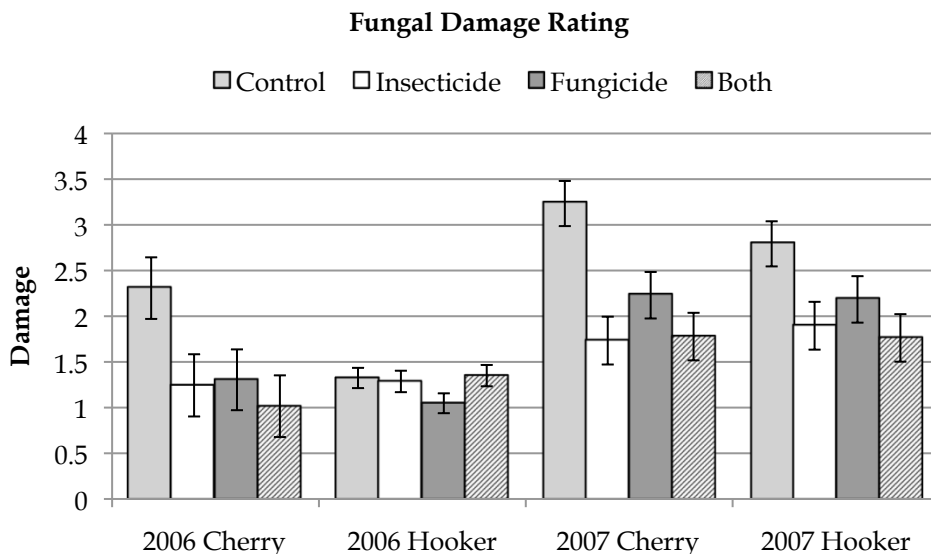


**Figure 3. Ambient Seed Production.** Seeds per capsule. Mean number of mature aborted and total seeds per capsule in blowout penstemon. Total seed was not significantly different among years or site. Closed capsules in 2007 control plants were too few to establish a significant difference from other years.



**Figure 4. Insect Herbivore Damage Rating.**

All blowout penstemon plants were scored on a scale of 0 to 5 for insect damage, with 5 representing the greatest amount of damage. Only Hooker County in 2006 showed significant differences among treatments in which plants treated with fungicide showed significantly more insect damage than any other treatment (Grade = 1.8,  $P = 0.003$ ,  $N = 152$ ). Grasshopper infestation may account for the increased damage in the fall of 2007 in Hooker County.



**Figure 5. Fungal Damage Rating.**

All blowout penstemon plants were scored on a scale of 0 to 5 for fungal occurrence, with 5 representing the greatest amount of damage. There was less insect damage among all treatments than in control plants except in 2006 in Hooker County.

Second, the fungicide treatment reduced fungal occurrence score overall, reflecting significant decreases in each year and location except in 2006 in Hooker County (Fig. 5). These results led to a significant interaction between county location and treatment ( $P < 0.0001$ ). Plants in the control treatment had significantly more fungal occurrence than did plants in any other treatment in Cherry County in both 2006 (mean score = 2.3,  $SE = 0.32$ ,  $N = 83$ ,  $P = 0.0007$ ) and 2007 (3.2,  $SE = 0.22$ ,  $N = 114$ ,  $P < 0.001$ ) and in Hooker County in 2007 (2.8,  $SE = 0.29$ ,  $N = 208$ ,  $P < 0.004$ ). Given the county  $\times$  treatment interaction, the contrast between the control treatment and the other three treatments in mean fungal occurrence score was significant only in Cherry County (mean difference -1.22,  $SE = 0.19$ ,  $P <$

0.0001). Also, comparison of the two treatments that included fungicide (fungicide, fungicide + insecticide) with the two treatments that did not include fungicide (insecticide, control) showed a mean difference (0.55) with lower fungal occurrence in Hooker County than Cherry County ( $SE = 0.17$ ,  $P < 0.002$ ). Overall, while the insecticide had little effect on herbivory, the treatments showed significantly lower fungal occurrence, compared to the control treatment, in three of the four location-year combinations examined.

**Seed Production by Treated Plants**

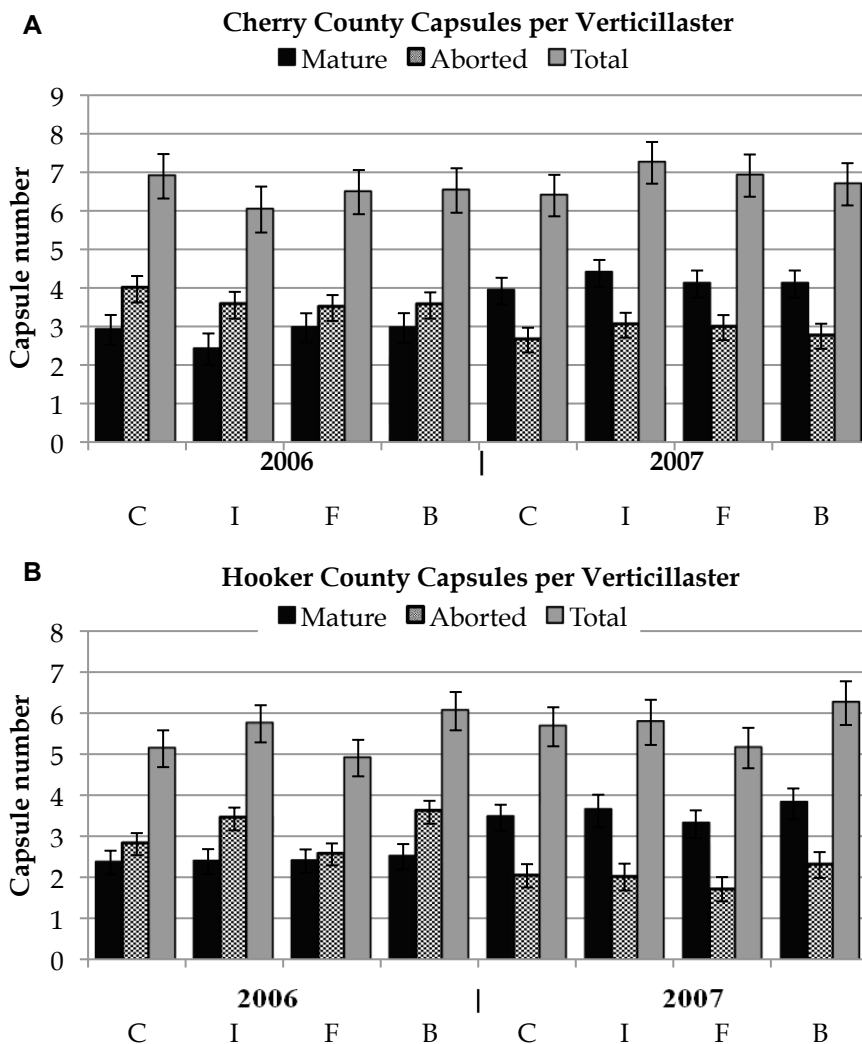
Infructescence length did not differ among treatments, either among years or between sites in the two county locations ( $P < 0.001$ ). Treatment did not alter infructescence length (contrast control vs. other

treatments:  $P = 0.69$ ). The number of verticillasters per infructescence also did not differ significantly among treatments. The average number of verticillasters per infructescence was 8.3 ( $SE = 0.32$ ,  $N = 41$ ). Thus, we found no difference in infructescence height related to differences in fungal occurrence scores.

The mean number of total capsules per verticillaster (6.1,  $SE = 0.21$ ,  $N = 441$ ) also was not different among treatments across years. The numbers of mature (mean 3.22,  $SE = 0.11$ ) and aborted (mean 2.89,  $SE = 0.15$ ) seed capsules per verticillaster did not differ significantly among treatments ( $P = 0.85$  and  $P = 0.12$ , respectively). However, both varied significantly between years ( $P = 0.0016$  and  $P < 0.0001$ , 2006 and 2007 respectively). The overall mean number of mature capsules per verticillaster across all treatments and locations was greater in 2007 (3.8,  $SE = 0.16$ ) than in 2006 (2.6,  $SE = 0.16$ ) ( $P = 0.0016$ ). Significant differences occurred in

the number of aborted capsules per verticillaster among treatments in Hooker County in 2006 ( $P = 0.006$ ) (Fig. 6). Unexpectedly, in 2006, the insecticide treated plants in Hooker County had an additional 0.55 ( $SE = 0.17$ ) aborted capsules per verticillaster than plants in the other two treatments (insecticide alone: 3.4,  $SE = 0.29$ , and insecticide + fungicide: 3.6,  $SE = 0.29$  versus control: 2.8,  $SE = 0.28$ ), and fungicide-only: 2.6,  $SE = 0.28$ ]. Overall no differences among treatments were found in the total number of capsules per verticillaster with the exception of aborted capsules in insecticide-treated plants were increased in one county in one year.

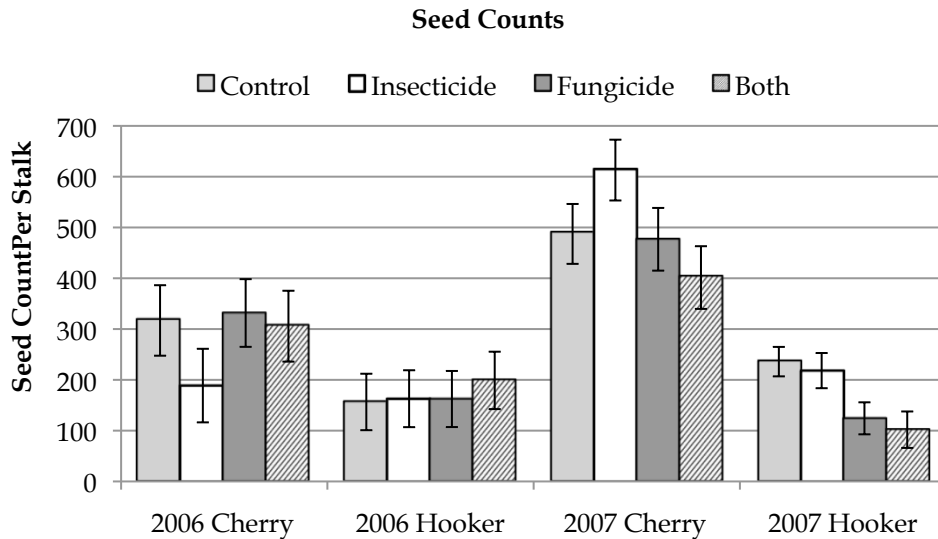
The average number of seeds per infructescence stalk varied significantly between the two county locations ( $P = 0.01$ ), with Cherry County levels higher than in Hooker County each year, especially in 2007 (Fig. 7). Seed per infructescence did not vary significantly among treatments or consistently between years (Fig. 7). A significant treatment x year



**Figure 6. Capsules per Verticillaster in Cherry and in Hooker County.**

(A) Cherry County capsules per verticillaster. (B) Hooker County capsules per verticillaster. The number of capsules per verticillaster on blowout penstemon plants are shown for research sites in Cherry and Hooker County. Treatments were control (C) insecticide (I), fungicide (F), and both insecticide and fungicide (B).





**Figure 7. Seed Counts.**

Seed numbers of blowout penstemon fruiting stalks are presented here. Error bars represent standard error. Seed numbers among treatments varied between counties and by year, but were not significantly different among treatments at each site.

interaction reflected higher seed production in 2007 by Hooker County plants that were not treated with fungicide ( $P = 0.003$ ). No other differences were significant, either by county or year. Thus, we found no predictable differences in seed production related to treatment, and none of the treatments produced consistent results among sites or years.

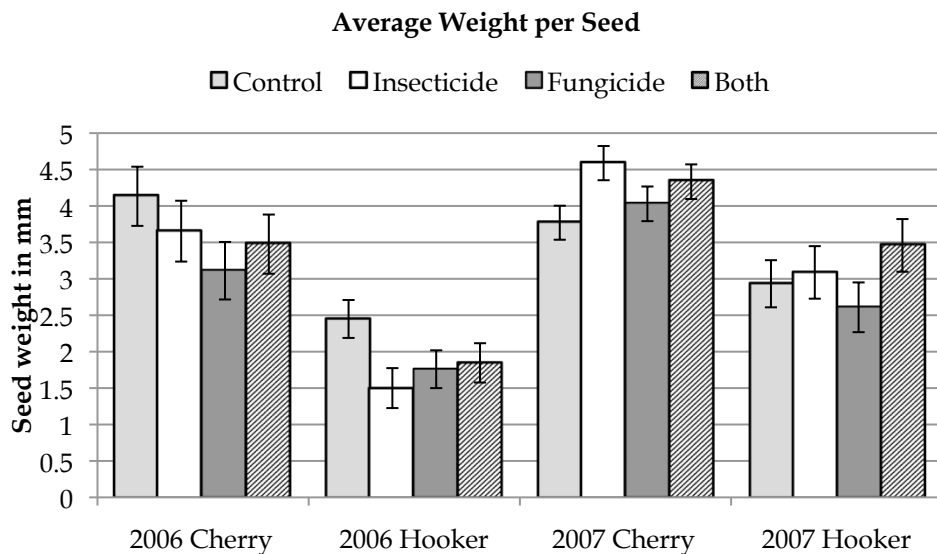
In 2007, when the treatments were started in early season, the contrast between the two treatments with fungicide versus the two treatments without fungicide showed that fungicide-treated plants actually averaged 110 fewer seeds ( $SE = 30.92$ ) than did plants without fungicide treatment (control, insecticide-only) ( $P = 0.0004$ ), especially in Hooker County (Fig. 7). Thus, opposite of expectation, fungicide treatment reduced, rather than increased the number of seeds produced.

Treatment did not consistently affect the weight of individual seeds. In 2006, mean seed weight was 3.6 mg per seed ( $SE = 1.20$ ) in Cherry County and 1.93 mg per seed ( $SE = 0.98$ ) in Hooker County. In 2007, mean seed weight was 4.18 mg/seed ( $SE = 0.94$ ) in Cherry County, and 3.02 mg/seed ( $SE = 1.14$ ) in Hooker County. The low seed weight in 2006 in Hooker County led to a significant treatment  $\times$  year interaction ( $P < 0.0001$ ). Both county and treatment affected mean seed weight significantly in both years (2006: county  $P < 0.0001$ , treatment  $P = 0.004$ ; 2007: county  $P = 0.003$ , treatment  $P = 0.05$ ) (Fig. 8). Seed weight varied significantly by location and treatment, but not consistently between years or among treatments.

Seed viability varied overall between years and locations, but not with treatment (Fig. 9). Seed

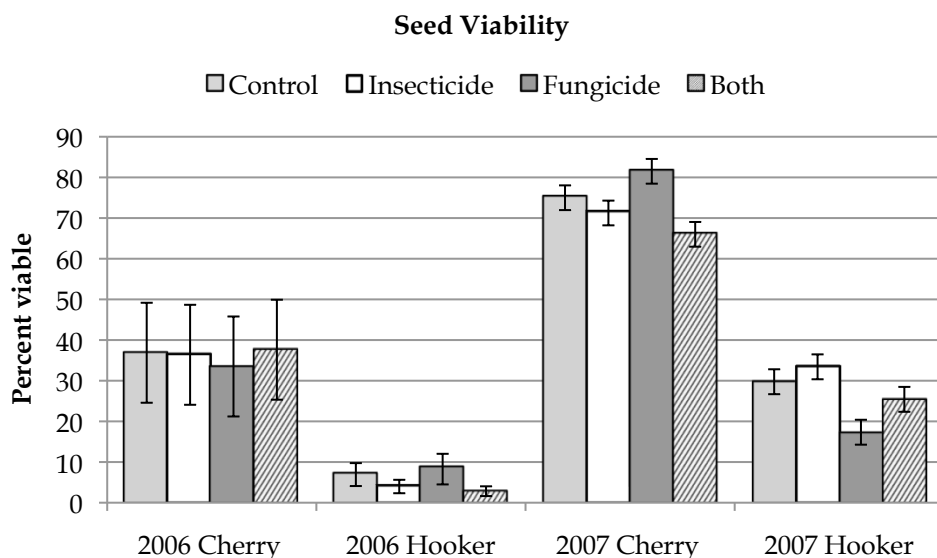
viability in Cherry County was 36% and 73% in 2006 and 2007, respectively, compared to 8% and 25% in Hooker County in 2006 and 2007, respectively. So, the percent seed viability was significantly higher in Cherry than in Hooker County in both years ( $P = 0.01$ ). Interactions were important in explaining the variation in seed viability: county  $\times$  year  $\times$  treatment interaction, ( $P < 0.0001$ ); and county  $\times$  treatment interaction ( $P = 0.07$ ). Also, the year  $\times$  treatment interaction was significant for both Hooker County ( $P = 0.0002$ ) and for Cherry County ( $P = 0.02$ ) locations. For example, in 2007 fungicide-treated plants had reduced seed viability in Hooker County, compared to plants in other treatments, but higher seed viability in Cherry County (Fig. 9). In summary, seed viability was not affected consistently by treatment, but varied significantly in interactions between year, location, and fungicide treatment.

Thus, the treatments were effective in reducing fungal occurrence (control versus other treatments) but not evidence of insect herbivory. However, the three spray treatments did not increase parameters of seed reproduction, relative to the control treatment: mature capsules per verticillaster (Fig. 1a and b), number of seeds (Fig. 7), seed weight (Fig. 8), or viability (Fig. 9). Fungicide unexpectedly reduced seed production. In fact, insecticide was more effective in reducing fungal damage than was fungicide (Fig. 5). The most striking result was the variability between locations and years in parameters of seed reproduction, independent of treatment.



**Figure 8. Weight per Seed.**

Treatments did not affect individual seed weights of blowout penstemon consistently. Both county and treatment effects were significant in 2006 (county  $P < 0.0001$ , treatment  $P = 0.0042$ ) and in 2007 (county  $P = 0.0039$ , treatment  $P = 0.0019$ ).



**Figure 9. Seed Viability.**

Seed viability of blowout penstemon was based on year and location and was independent of treatment. Viability over all sites in Cherry County was 36% and 73% in 2006 and 2007, respectively. Viability over all sites in Hooker County was 8% and 25% in 2006 and 2007, respectively.

**Discussion**

**Ambient Levels of Seed Production**

We found a correlation between the total number of capsules produced and inflorescence length ( $P < 0.0001$ ). Our results are similar to those reported by Tepedino et al. (2006) based on the relationship of the tallest inflorescence and the inflorescence height; however the method used to measure inflorescence height was not described. It may have been taken from the soil surface, which is not stable. Many cm of soil can be deposited or removed from the base of the penstemon plant as the wind continually reworks the surface of the blowout. Our measurements taken from the pair of leaves just below the first fruit bearing verticillaster avoids the variable of

determining the base of the plant. In any case, there is a strong, predictive relationship between stalk length and the number of verticillasters, which can be used to estimate seed production and reduce the need for destructive seed counts.

Our estimate of average seed production by blowout penstemon is similar or lower than estimates in the literature. Recently, Tepedino et al. (2006) estimated seed production at 2,000 per plant. However, since they used the tallest inflorescence per plant as a basis this value may be an overestimate. If we use our averages, ignoring briefly the differences between site locations and between years, our estimate of potential seed production (on randomly chosen inflorescences) was 14 seeds per capsule,

similar to the 18 seeds per capsule reported by Tepedino et al. (2006), but much lower than the 25 to 35 seeds per capsule estimated by Fritz et al. (1992). In this study, of the 14 seeds, 12 were fully developed on average. Further, we found six capsules initiated per verticillaster but only 3.22 capsules maturing to produce seed. The number of verticillasters was 0.56 per cm of length or 10.5 verticillasters per infructescence. Mean height of infructescences from the 3-year study was 18.8 cm; and the long-term average number of infructescences per plant observed over 14 years was 3.5 (Kottas 2008).

However, inflorescences are lost to herbivory. For example, about one-third of inflorescences were lost to herbivory or frugivory in the study by Tepedino et al. (2006). At our sites, an average of 17% of infructescences was lost to herbivory by mid June each year (Kottas 2008). Additional loss of infructescences may occur from mid June through August before seeds ripen, therefore 33% may be a realistic estimate for such losses. Since the 14 years of counts also were made in June, they would not include some of the lost infructescences. However, if 17% of infructescences were lost by June, the estimate is four flowering infructescences initiated per flowering plant on average prior to stem loss to herbivory.

Assuming one-third of those four infructescences were removed before fall seed set, total seed production would match the Tepedino et al. (2006) estimate of 2,328 seeds per plant on average (14 seeds/capsule  $\times$  6 capsules  $\times$  10.5 verticillasters per infructescence  $\times$  4 infructescences per plant  $\times$  0.66). Further, if average seed viability of 38%, as found in this study, the estimated number of viable seeds produced per plant would be 884. However, in this study counts of seeds per infructescence over the three years, only showed an average of 518 seeds per infructescence; and, the number of seeds per plant ranged widely, from 0 to 3,140 seeds per infructescence (Kottas 2008). These numbers lead to an estimate of 1,398 (518  $\times$  4  $\times$  0.66) total seeds per plant, and 531 viable seeds per plant. Some of the differences in the calculations versus the actual counts may be due to the fact that we used only unopened capsules to count seed. These capsules may not have been representative of all capsules. In any case, it is clear that in using such estimates to make predictions, it is important to include seed viability along with total numbers produced.

### **Insect Herbivory**

We found no consistent difference in the qualitative scores of insect feeding among treatments, indicating that insecticide treatments were not effective in

reducing herbivory (Fig. 4). However, the three pesticide treatments had lower fungal occurrence than the control treatment (Fig. 5). Consequently, the lack of change in the number of seeds, seed weight, or seed viability among treatments reflects, at least, little effect of fungal occurrence on key parameters of seed reproduction. However, in 2007, we did find significant differences in mean seed viability, number of seeds, and insect damage ratings between plants at the Cherry County and Hooker County locations. Where grasshopper herbivory appeared high (Hooker County in 2007), seed production was reduced. Overall, while we were not able to reduce herbivory sufficiently to evaluate its effects on blowout penstemon, the spatial variation in the intensity and impact of some types of herbivory among blowout locations was clear.

We assumed that the most damage to developing seeds would be from stink bugs, but that was not directly measured in our experiment. Grasshoppers were relatively sparse in our blowouts in 2005, so they were not considered in the selection of the insecticide. Also, the fact that blowout penstemon is limited by the lack of bare sand and number of blowouts (Fritz et al. 1992; Stubbendieck and Kottas 2007) may contribute to the large impact of grasshoppers. Some subspecies of grasshoppers that are typically grass-feeders also feed on forbs (Joern 1983). Blowout penstemon may be susceptible to insect herbivores that follow the spread of grasses and forbs into the blowouts as well as by the encroaching grasses competing for resources. Beville et al. (1999) made a strong argument that insect herbivores can limit populations of rare species and that exclusion of such herbivores can be an effective management tool. Treatments arranged specifically for the measurement of grasshopper damage may be more appropriate than those for stink bugs. Other insect species were observed to be a problem for some populations of blowout penstemon prior to 2004. Determining which insects will have the greatest effect in any one blowout in a particular year is yet another difficulty for researchers. Further study of herbivory and potential control measures for herbivores, including vertebrate consumers, will be valuable to facilitate management objectives.

### **Fungal Occurrence**

The evidence of fungal occurrence was greater on control plants than on plants in the other three treatments, indicating that both fungicide and insecticide significantly reduced fungal infection. The fact that the control had higher fungal occurrence in three of the four county-by-year comparisons suggests that the insecticide was as effective as the

fungicide in reducing fungal attack. One hypothesis to explain this result is that insecticide reduces the effectiveness of insect vectors of plant disease.

### Management Implications

Measurements of infructescences from the leaf pair just below the lowest producing leaf pair provide a repeatable, effective estimate of seed production. Although these estimates are inflated when compared to actual counts, they were only slightly lower than estimates used in the recovery plan (Fritz et al. 1992). However, we found production of mature and viable seed to be lower than either estimates or actual seed counts, suggesting environmental factors, potentially including biotic interactions, can at times be important in limiting seed. The results of this study, along with studies of the potential for seed banks and seedling recruitment, will be invaluable in determining relative reproductive success of this species, and potentially other short-lived, rare perennial species in fragmented habitat.

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