

**POLLEN LIMITS SEED SET IN SMALL POPULATIONS OF FERAL *MISCANTHUS SINENSIS*, AN ORNAMENTAL GRASS WITH INVASIVE POTENTIAL**

Stephanie Verhoff  
verhoff.79@osu.edu  
Department of Horticulture and Crop Science  
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Faculty Co-Advisors  
Dr. Allison Snow  
Department of Evolution, Ecology, and Organismal Biology  
snow.1@osu.edu

Dr. Kristin Mercer  
Department of Horticulture and Crop Science  
mercerc.97@osu.edu

Abstract

*Miscanthus sinensis* (Chinese silvergrass) is a tall, perennial, C<sub>4</sub> grass native to Eastern Asia. Ornamental cultivars of *M. sinensis* are popular in Ohio and elsewhere, and may give rise to invasive populations. Naturalized (feral) populations already appear to be invasive in some areas of the southeastern US, with the potential to spread further, yet few studies have focused on the reproductive ecology of this non-clonal, self-incompatible species. In other self-incompatible species, Allee effects may have the potential to reduce fecundity due to a scarcity of pollen donors. The goal of the study was to determine whether seed production of feral *Miscanthus* populations is limited by available pollen from nearby individuals. In late October 2013, flowering shoots from nine populations of varying size and population density in Ohio and West Virginia were sampled. Population size ranged from 5 to >200 and 20 individuals were sampled per population whenever possible. Population density was quantified as the number of plants per

square meter in smaller populations, and as the average distance to the nearest three conspecific plants for each sampled individual in larger populations. Flowering shoots were x-rayed at the OSU Ornamental Germplasm Center to determine percent seed set for 300 florets per plant. Results show an increase in percent seed set as population size increases. Intriguingly, a small feral population in Columbus may have received pollen from a large, adjacent stand of ornamental plants, based on its relatively high seed set. In four large populations, individuals were divided into “center” and “edge” plants based on their locations in the population. Percent seed set was greater in “center” plants versus “edge” plants, which were farther apart on average. Overall, the results suggest that pollen-limited seed set was common. This finding supports the hypothesis that rates of invasion of *M. sinensis* may go through an initial “lag phase” until sufficient pollen is available to allow substantial seed production in newly established populations.

## **Introduction**

The ornamental plant industry may serve as a pathway for nonnative species to escape cultivation, become established, and potentially invade natural areas. Reichard and Campbell (1996) estimated that ~50% of invasive plants in the United States were introduced as ornamental plants. Furthermore, native species may become endangered or even extinct due to invasive species (e.g., Reichard and White, 2001). Thus, ornamental plants need to be assessed for their potential to escape and invade natural areas, especially since the traits commonly associated with invasion are also desirable to commercial nurseries and consumers (e.g., Li et al., 2004). For instance, successful ornamental plants typically require few inputs such as herbicides and fertilizers, tolerate a wide range of environmental conditions, and can both establish and reproduce easily. Increases in international trade also contribute to the introduction of nonnative, invasive species, and local demand, price, and availability should also be factored into a species' ability to escape and become established. Dehnen-Schmutz (2007) showed that there was a positive correlation between both the price and the number of catalogue listings and a species' invasion success in Britain. An example of an ornamental grass with high propagule pressure due to its commercial success is pampas grass (*Cortaderia selloana*). Microsatellite markers have proven that local landscape plantings have served as sources for the invasive pampas grass in California (Okada et al., 2007). Lastly, multiple ornamental plantings may be nurturing future invaders and building propagule pressure (Richardson and Pysek, 2012). Repeated small plantings at different locations are more likely to lead to naturalization and possibly invasion than one large introduction (Mack et al., 2000). Since the ornamental industry is a large vector for nonnative invasive species, factors contributing to the naturalization and invasion of these plants must be further studied.

It is vital to understand the stages of invasion in order to prevent economic and environmental costs due to the management of invasive species and the displacement of native species. Many studies are focused on current invasive species, ignoring the introduction and naturalization steps that occur before invasion. Often there is a 'lag phase' between initial escape and establishment. This lag phase is the amount of time required for a population to transition from initial introduction to rapid growth and expansion (Mack et al., 1985; Sakai et al., 2001). Possible reasons for this lag phase include changes in climatic conditions, increased availability of suitable habitats, hybridization, local adaptation, and species-specific requirements (Sakai et al., 2001). The entire introduction-naturalization-invasion continuum should be addressed, so that the factors causing the progression from escape to invasiveness may be better understood (Richardson and Pysek, 2012). By encompassing the entire continuum, predictions of invasiveness may be made and improved management strategies may be implemented. *Miscanthus sinensis* is an example of an ornamental grass that has escaped cultivation and is naturalized in certain areas while invasive in others. *M. sinensis* may have the potential to spread further and factors mediating both naturalization and invasion should be assessed.

*Miscanthus sinensis* is a C<sub>4</sub> perennial, bunchgrass native to Eastern Asia and was first introduced to Washington, D.C., as an ornamental plant in the late 1800's (Quinn et al., 2010; Figure 1). *M. sinensis* is both self-incompatible and wind-pollinated, and is considered a pioneering species in its native habitat in Japan, where it occurs in open grasslands (Stewart et al., 2009). In the USA, it is a widely used fall and winter ornamental plant with 100 cultivars commercially available (Dougherty et al., 2014), and is one of the most popular ornamental grasses (Quinn et al., 2010). *M. sinensis* flowers from August to October and produces viable seeds in USDA hardiness zones 4, 5, 6, and 7 (Meyer and Tchida, 1999). Additionally, seeds

may be dispersed at least 400 meters (Quinn et. al, 2011). Stewart et al. (2009) studied native populations of *M. sinensis* in Japan and determined that the average seed weight is .96 mg. and with a mean seed production of 1,051 seeds per plant. The study also concluded that the percent seed set ranged from 3-94% in native populations. In its native range, Stewart et al. (2009) also observed higher seed set in individuals with conspecific plants less than four meters away. *M. sinensis* requires few inputs and tolerates a wide range of environmental conditions, adding to its commercial value as an ornamental plant (Quinn et al., 2010). However, these same traits also contribute to its ability to naturalize and its potential invasiveness.

During the past century, *Miscanthus sinensis* has naturalized in 25 states in the USA, is designated as invasive or problematic in 13 of these states, and may have the potential to spread further and pose a greater threat to native species (Quinn et al. 2010, EddMaps; Figure 2). Naturalized or feral populations currently exist in Ohio, and *M. sinensis* is currently listed on the Ohio Invasive Watch List (Wilson and Knox, 2006). Feral populations occur in disturbed habitats, roadsides, forest edges, and occasionally the understory of forests (Dougherty et al., 2014). However, Matlaga et al. (2012) did not observe higher shade tolerance in introduced populations when compared to native populations in Japan. A high correlation between genetic diversity and population size has been found in naturalized populations, indicating that high levels of diversity would likely facilitate further spread through an increase in survivorship and local adaptation (Quinn et al., 2012).

Because *Miscanthus sinensis* has already shown the ability to survive and spread outside of ornamental plantings, factors mediating the progression from naturalization to invasion must be addressed. However, few studies have focused on the reproductive ecology of this self-incompatible species. Furthermore, an increase in propagule pressure with the species' rising

ornamental use and the development of novel cultivars will likely facilitate further spread (Quinn et al., 2010). Each year, \$125 billion is spent on the management of nearly 50,000 nonindigenous species (Allendorf and Linn, 2003). Thus, a rise in invasive populations of *M. sinensis*, may pose an economic risk and mitigation strategies must be developed. Surprisingly, Knight et al. (2011) found that the implementation of less fecund cultivars does not dramatically decrease population growth for perennials. Thus, the use of less fecund cultivars as a mitigation strategy may not decrease invasiveness in perennial plants. Nonetheless, higher fecundity is likely to confer a higher ability to spread and become invasive, since higher seed production increases the likelihood of reaching possible colonization sites.

The objective of this study was to assess percent seed set (percentage of florets setting seed) in feral or naturalized populations of *M. sinensis* and to determine the significance of population size, population density, and the location of an individual relative to the population on seed production. My hypothesis was that seed production of feral populations would be correlated with the availability of pollen from nearby individuals.

## **Materials and Methods**

Nine feral populations in both West Virginia and Ohio were sampled from in late October 2013 (Table 1). Small populations had  $\leq 35$  individuals and included populations labeled one through five. Large populations had  $> 100$  individuals and included populations labeled six through nine. Populations 1-5 were located in Central Ohio and Population 6 was located in southeastern Ohio. Populations 7-9 were located in northwest Virginia (Figure 3). Three panicles were collected from each sampled plant and 20 plants were sampled when possible. For populations with less than 20 individuals, all plants were sampled. In each large population, ten

“edge” plants and ten “center” plants were sampled. Edge plants were located on the perimeter of the population and at least 50 meters from the center of the population.

Percent seed set was determined for each plant by x-raying seeds with the Faxitron Specimen X-ray at the Ohio State University Ornamental Germplasm Center (Figure 4). One hundred florets were x-rayed from each panicle collected, totaling 300 florets for each sampled individual. Percent seed set was then calculated by counting the number of seeds for each individual x-ray image and taking the average of the three images for each plant.

Population density was measured in the small populations by estimating the area ( $m^2$ ) of the population using measurements derived from GPS coordinates and dividing the number of individuals by the total area. Population density in the large populations was calculated by measuring the distance between the sampled plant and the closest three conspecific plants, and taking the average of the three measurements.

## **Results**

### **Population Size:**

Population size ranged from five to greater than 200 individuals among populations. Populations 1-5 had  $\geq 35$  individuals, and Populations 6-9 having greater than 100 individuals (Table 1). A one-way ANOVA was performed to compare the mean percent seed sets of all populations and the percent seed sets of large populations were statistically higher than small populations. A Tukey Test was also performed in order to further compare the difference of mean percent seed set across populations. Percent seed set was much lower in small populations and presumed pollen limitation decreased as population size increased (Figure 5). However, it should be noted that population size and location (state) were confounded. Larger populations also tended to have plants that were much closer together (personal observation). Interestingly,

Population 1, the smallest population (N = 5), had a relatively high percent seed set, which may be due to the adjacent large stand of ornamental *M. sinensis*.

### Population Density

In edge plants in the larger populations, a simple linear regression of the effect of population density (average distance to 3 nearest conspecific plants) on percent seed set found no correlation between the two ( $R^2 = 1.5\%$ ;  $P = 0.46$ ; Figure 6). No correlation was found between percent seed set in edge plants and distance to the closest conspecific either (data not shown). However, a non-linear regression may reveal a stronger correlation.

### Center vs. Edge Plants

Sampling of flowering shoots was divided into ten center and ten edge plants for each larger population (6-9) in order to compare the effect of the position of an individual plant relative to the rest of the population on percent seed set. Individual T-tests were performed for each of the four sites. In three out of the four populations, center plants had a significantly higher percent seed set when compared to edge plants ( $P < 0.05$ ; Figure 7). Population 6 was the only large population where center and edge plants did not significantly differ in seed production.

### Discussion

Greater attention should be directed to small, founding populations which may undergo critical periods of expansion. These populations may have the potential to expand and become dense, established populations. Our results indicate that Allee effects may be influencing the spread of small, naturalized populations of *M. sinensis*. Allee effects are the reduction in

individual fitness components as population size decreases (Allee, 1949). Pollen limitation and Allee effects may play a significant role in the prediction of the behavior of an introduced population. In this study, population size was positively correlated with percent seed set. However, population size and the location of populations were confounded. Further sampling of smaller populations in West Virginia is needed to better assess pollen limitation in feral populations of *M. sinensis*. (No large feral populations were found in Ohio for this study). Our findings align with those of Firestone and Jasieniuk (2012), who found that when genetic diversity is low, the rate of seed set increased by 80% with increasing population size for the self-incompatible, wind-pollinated invasive ryegrass (*Lolium multiflorum*). Fecundity-reducing Allee effects may prolong the initial lag phase from initial escape to invasion (Taylor and Hastings, 2005).

A greater understanding of the reproduction ecology of *M. sinensis* is needed to form proper management strategies and prevent further spread. Allee effects must be considered when forming management strategies for invasive species. In pollen limited populations, a critical density threshold may arise (Taylor and Hastings, 2005). When population size falls below this threshold it will fail to further establish and spread into new areas. Thus, management efforts would only need to keep feral populations below the threshold to delay invasion. This would likely be a more economical strategy than complete eradication plans.

Some studies have shown that Allee effects can be complex in plant species with self-incompatibility. Self-incompatibility is a genetic mechanism which prevents self-fertilization. S alleles or self-sterility genes prevent self-fertilization by controlling the growth of the pollen tube, causing male sterility. Levin et al. (2009) concluded that self-incompatibility systems increase the magnitude of Allee effects by modeling the population dynamics of newly

established populations using cellular automation models. The correlation between population size and the availability of suitable pollen donors or receptors may influence the amount of time needed for a population to progress from naturalization to invasion. When compared to plant species without any self-incompatibility, species with S-alleles had lower growth rates. Moreover, naturalization may create a bottleneck since there is a reduced level of S-alleles present and consequently fewer potential mates. This has been observed in *Brassica insularis*. A high number of S-alleles present in the population may offset this effect though in initial populations. Quinn et al. (2012) found no genetic bottlenecks in six introduced populations of *M. sinensis* when compared to five native populations in Japan; however, S-alleles were not specifically studied. Research studying the number of S-alleles in feral populations of *M. sinensis* is still needed in order to understand its effect on population growth rate.

Theoretically, more dense populations would have sufficient levels of pollen from nearby individuals and ultimately higher seed production than less dense populations according to Allee effects. No correlation between population density and percent seed set was found in this study. However, alternative sampling methods may indicate otherwise. In the four large populations of *M. sinensis* sampled from, a regression analysis showed no significant relationship between the average distance to the nearest neighbor and percent seed set. Sampling more plants greater than 10 meters from other individuals, particularly in large populations may show a stronger relationship between density and percent seed set. Also an experimental plot designed to study the independent effect of population density on seed production may yield different results compared to the feral populations of varying sizes and densities used in this study. Though population density did not significantly impact seed production in the case, the position of individual plants relative to the population did.

Individuals on the leading edge of the population were generally more pollen limited than plants in the center of the population. This was observed in three out of the four large populations, using individual t-tests to compare percent seed sets of center and edge plants in each population. Even if *M. sinensis* can disperse seeds at least 400 meters, pollen must also be available for these potential colonists (Quinn et al., 2011). Both seed and pollen dispersal affect the long term viability of a population (Levin et al., 2004). This has been demonstrated in the self-incompatible, wind-pollinated species *Spartina alterniflora* (Davis et al., 2004). Contrary to the assumption that wind-pollinated plants' reproduction is not limited by pollen limitation, *S. alterniflora* individuals at the edge of the population were pollen limited. Instead of sampling flowering shoots and calculating percent seed set, Davis et al. (2004) utilized pollen supplementation and exclusion experiments to show the decrease in pollen deposition from the center to the edge of the population. This further indicates that Allee effects slow the invasion rate, or in the case of *M. sinensis* populations in Ohio and West Virginia, the progression from naturalization to invasion.

### **Acknowledgements**

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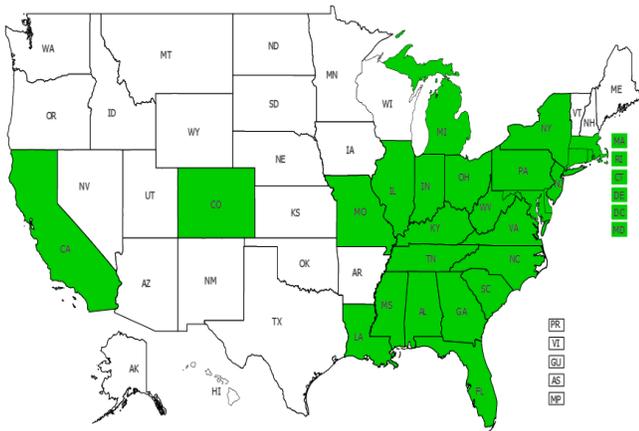
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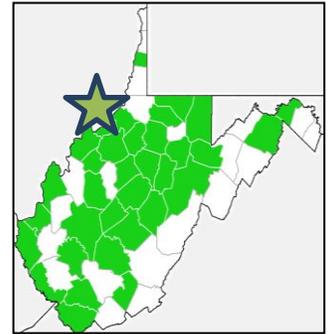
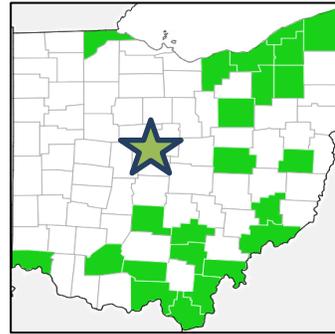
## Tables and Figures



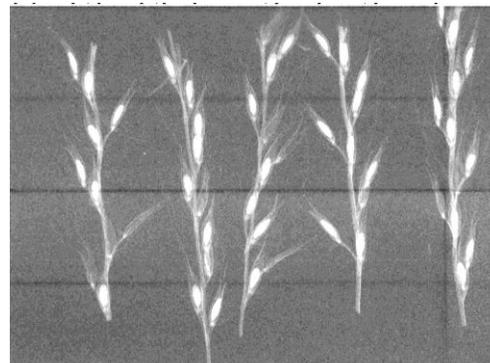
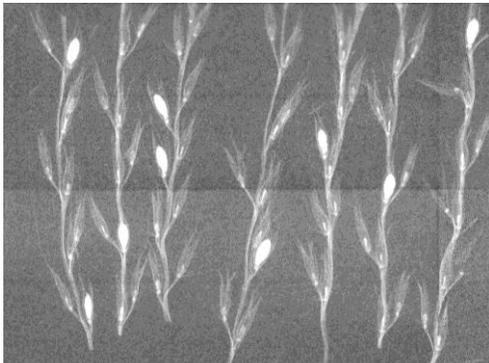
**Figure 1:** Comparison of small feral population on left (Population #5) and large feral population on the right (Population #8).



**Figure 2:** Distribution of naturalized *M. sinensis* in the U.S. ([www.eddmaps.org](http://www.eddmaps.org)).



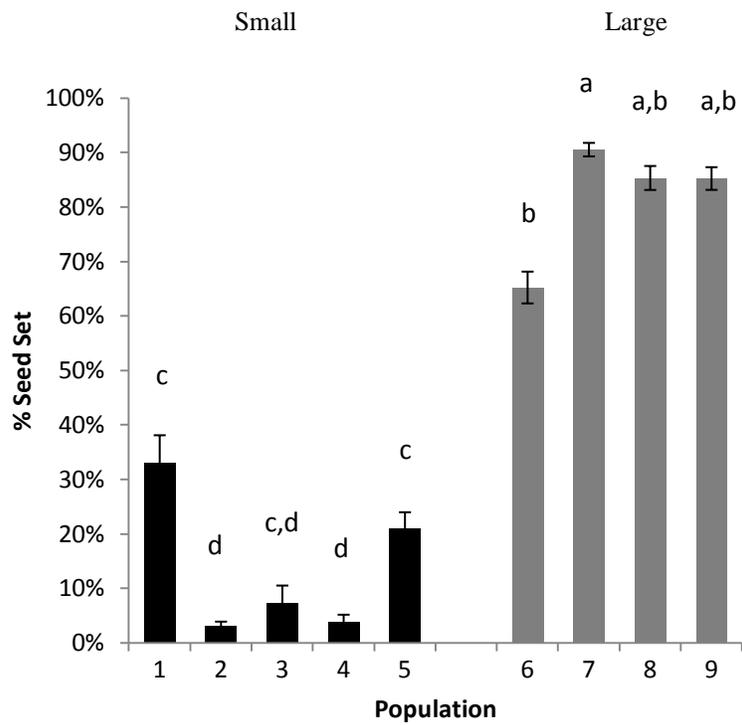
**Figure 3:** Reported occurrence of *M. sinensis* in OH and WV. Stars represent areas of sample collections in each state and green counties represent current locations of feral *Miscanthus sinensis* ([www.eddmaps.com/distribution](http://www.eddmaps.com/distribution)).



**Figure 4:** Seed X-ray images (Left: Population #6; Right: Population #9).

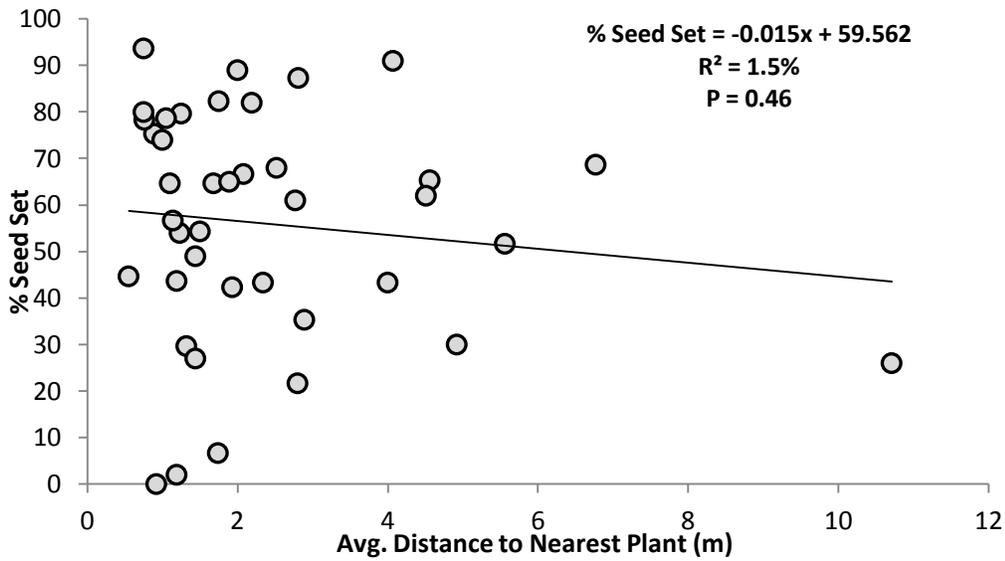
Population	1	2	3	4	5	6	7	8	9
Number of Individuals	5	7	11	12	35	101-200	101-200	>200	>200
GPS Coordinates	39.991662' N 83.110213' W	40.118205' N 82.871140' W	40.199185' N 82.895153' W	40.266698' N 82.942900' W	40.171033' N 82.916782' W	39.247355' N 81.359379' W	39.477387' N 81.301071' W	39.181974' N 81.367925' W	39.397334' N 81.436468
State	OH	OH	OH	OH	OH	WV	OH	WV	WV
N	5	7	11	12	20	20	20	20	20
Density	0.026/m <sup>2</sup>	0.010/m <sup>2</sup>	0.067/m <sup>2</sup>	0.006/m <sup>2</sup>	0.001/m <sup>2</sup>	96 cm. (281 cm.)	81 cm. (118 cm.)	101 cm. (151 cm.)	119 cm. (408 cm.)
Notes	Empty lot	Empty lot	Side of road	Empty lot	Empty lot	Side of road; state park	Side of road	Side of road	Open pasture area

**Table 1:** Population size, location, N (no. of individuals sampled), and population density. In Populations 1-5, density was measured by estimating that total area and dividing by the total number of individuals. In Populations 6-10 the average distance to the three closest conspecific plants was measured. Data was pooled from center plants to represent majority of populations; measurements in parentheses are the average distance to the three closest conspecific plants for edge plants.

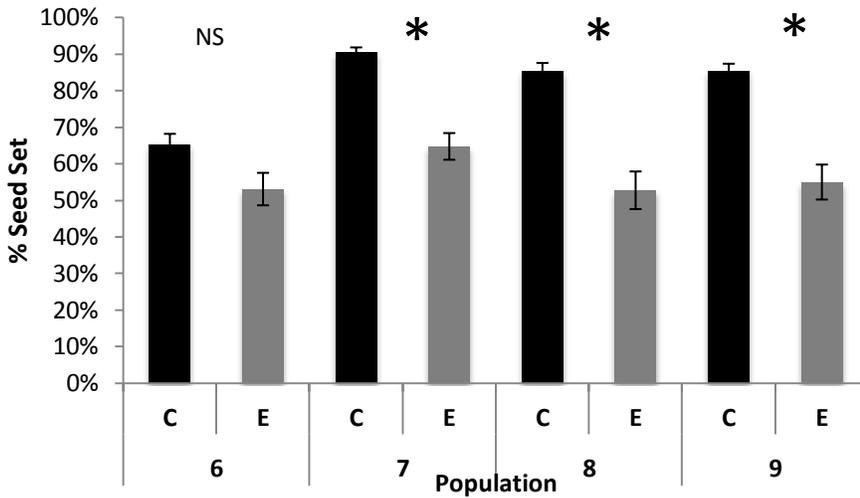


1	2	3	4	5	6	7	8	9
N = 5	N = 7	N = 11	N = 12	N = 35	100 < N < 200	100 < N < 200	N > 200	N > 200

**Figure 5: Mean seed set of all populations.** 95% confidence interval;  $R^2 = 86.78\%$ ; Tukey test; mean  $\pm$  1 SE for up to 20 plants. N = 10 for Populations 6-9.



**Figure 6:** Simple linear regression of population density's effect on % seed set in 40 edge plants, in large populations. Date was pooled from Populations 6-9.



**Figure 7:** Comparison of % seed set between center (C) and edge (E) plants at 4 sites. NS = not significant; \* = significant at  $P < 0.05$ ; t-test; mean  $\pm 1$  SE,  $N = 10$ .

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