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FACTORS CONSTRAINING THE REPRODUCTIVE OUTPUT OF
BAPTISIA ALBA MACROPHYLLA

Ashley M. Hembrough

40 Pages

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Baptisia alba macrophylla, a native, herbaceous perennial legume, produces inflorescences with a large number of flowers, yet matures relatively few fruits. I hypothesized that the number of seeds matured by *B. alba macrophylla* is maximized based on resource availability at each stage of development, but this optimal number is further compromised by extrinsic factors including pollen limitation and pre-dispersal seed predation by the weevils *Apion rostrum* and *Tychius sordidus*. To test this hypothesis, I conducted a field experiment at the John English Prairie, located in NW Hudson, IL, and also the Sugar Grove Nature Center and Funks Grove, located in SW McLean County, IL. Whole ramets were randomly assigned to one of two fertilizer treatments (no fertilizer, application of a granular fertilizer prior to first flowering and again post-flowering) and one of two seed predation treatments (no insect barrier, application of Tanglefoot® insect barrier). Individual flowers were then assigned to one of two pollination treatments (open pollination, open pollination supplemented with hand pollinated outcrossing) in an alternating manner.

At Sugar Grove Nature Center and Funks Grove, flowers that were supplemented

with hand pollination initiated significantly more fruits per flower than flowers that were not supplemented with hand pollination, but this did not translate to increased fruit maturation or an increased number of seeds matured per fruit. Nutrient treatment did not have significant effects on fruit initiation, fruit maturation, or seed maturation at this site. Application of Tanglefoot® significantly increased the number of initiated fruits that reached maturity. Likewise, plants treated with Tanglefoot® matured significantly more seeds per fruit than control plants. Further, although pollen supplementation significantly increased fruit initiation, predation treatment was the only treatment that had a significant effect on the number of initiated fruits that matured and the number of seeds matured per fruit. When the data were analyzed by ramet, rather than individual flowers or fruits, Tanglefoot®-treated ramets matured more fruits per ramet and more seeds per ramet than control ramets at this site.

At the John English Prairie, flowers that were supplemented with hand pollination initiated significantly more fruits per flower than flowers that were not supplemented with hand pollination, but neither nutrient treatment nor predation treatment affected fruit initiation at this site. There were no significant effects of nutrient treatment, pollination treatment, or predation treatment on fruit maturation at the John English Prairie. No statistical analysis was conducted on the number of seeds matured per fruit at this site because all but three fruits experienced complete reproductive failure (=zero seeds) due to pre-dispersal seed predation by *T. sordidus*. Further, it can be concluded that pre-dispersal seed predation has an overwhelming and devastating effect on seed maturation in this population.

I conclude that reproductive output of *B. alba macrophylla* is serially adjusted during offspring development, but there is no data indicating that this adjustment is caused by nutrient availability at each stage. Pollen supplementation increased fruit initiation at both sites, but these populations are not *pollen limited* because this effect did not translate to increased fruit maturation or an increased number of seeds per fruit. Pre-dispersal seed predation by weevils severely reduced the number of fruits matured per initiated fruit, the number of seeds matured per fruit, the number of fruits matured per ramet, and the number of seeds matured per ramet. Further, this study indicates that seed predation is a driving force shaping reproduction of this native legume, but neither nutrient limitation nor pollen limitation are limiting reproductive output in these populations of *B. alba macrophylla*.

FACTORS CONSTRAINING THE REPRODUCTIVE OUTPUT OF
BAPTISIA ALBA MACROPHYLLA

ASHLEY M. HEMBROUGH

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Fulfillment of the Requirements
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FACTORS CONSTRAINING THE REPRODUCTIVE OUTPUT OF
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CHAPTER I
INTRODUCTION

Limitations to Reproductive Output—Much research has been devoted to understanding what limits reproduction in flowering plants (Stephenson, 1981; Burd, 1994). Although the maximum number of fruits and seeds that can be produced by a plant is ideally limited by the number of female flowers and the number of ovules, respectively, a number of other factors can reduce the number of fruits and seeds that ultimately mature (Stephenson, 1981). These factors include, but are not limited to, fruit and seed predation (Boucher and Sork, 1979; Haddock and Chaplin, 1982; Mundahl, 2014), limited resource availability (Willson and Price, 1980), limited pollination and/or poor ovule fertilization (Piña et al., 2007), and can reduce fruit and seed maturation via flower and/or fruit abortion.

Many angiosperm species produce far greater numbers of flowers than fruits, and thus experience high levels of flower and/or fruit abortion (Willson and Price, 1977; Stephenson, 1979; Stephenson, 1981; Bawa and Webb, 1984; Montalvo and Ackerman, 1987), and Stephenson (1981) reports that these species are exceptionally diverse in both taxonomy and ecology. Investing nutrients in the production of a flower or fruit that is later aborted might *seem* like an unproductive strategy yet, as Darwin (1876) states, "...when we behold our orchard-trees covered with a white sheet of bloom in the spring, we should not falsely accuse nature of wasteful expenditure, though

comparatively little fruit is produced in the autumn." Hypotheses explaining the production of *excess* flowers, i.e., those that do not yield fruit, are numerous and include the following: these flowers are not in excess and instead they are produced for the male function of pollen dispersal (Willson and Price, 1977); the production of excess flowers is a strategy to attract pollinators (Stephenson, 1979); the production of excess reproductive units is a strategy that allows the plant some selection among offspring (Lloyd, 1980; Kozlowski and Stearns, 1989); and lastly, excess flowers can provide insurance against losses (Darwin, 1876; Groeneveld et al., 2010).

According to Lloyd's (1980) serial adjustment hypothesis, levels of maternal investment change throughout various stages of offspring development in a way that maximizes maternal fitness. At each developmental stage, a plant must resolve whether the continuity of resources into a given reproductive unit will maximize its maternal fitness and will then either continue or terminate investment into that unit. Using internal cues, such as the number of seeds in a fruit, and external cues, such as resource availability and pre-dispersal seed predation, a plant can adaptively and selectively abort offspring (Meyer et al., 2014), thus allowing the plant to invest in fruits with the greatest potential to survive and contribute viable seeds to the next generation. The following sections outline how pollen limitation, nutrient limitation, and pre-dispersal seed predation can limit reproduction, with specific reference to potential effects on reproductive output of *Baptisia alba* (L.) Vent. var. *macrophylla* (Larisey) Isely (Fabales: Fabaceae).

Pollen Limitation—Pollen limitation is a phenomenon that results in fewer fruits and/or seeds than could be produced if sufficient or higher quality pollen was

received (Ashman et al., 2004; Knight et al., 2005), thus compromising reproductive output. In their recent review of pollen supplementation experiments, Knight et al. (2005) report that 63% of the 482 records (1 record=1 population for a single year) were pollen limited in terms of fruit set, suggesting that pollen limitation is, in fact, a widespread occurrence in angiosperm species. Similarly, Burd (1994), using published data on 258 angiosperm species, found that 62% of the species were pollen limited at some time or site during experimentation. The high frequency of pollen limitation reported in angiosperm species is not surprising given that many generalist pollinators, notably several *Bombus* species (Hymenoptera: Apidae), have experienced precipitous declines in both abundance and geographic range in recent years (Cameron et al., 2011). Approximately 87.5% of all angiosperm species rely on animals for pollination (Ollerton et al., 2011), and these animal-pollinated species are at further risk of pollen limitation when found in low densities (Amarasekare, 2004).

Pollen limitation can be caused by a number of factors including insufficient visits by pollinators, insufficient pollen availability, unsuccessful pollen transfer, poor pollen-tube survival and/or zygote death (Harder and Aizen, 2010). In pollen-limited populations, various adaptations can reduce fitness losses caused by pollen limitation. For example, a number of strategies can increase visits by pollinators, including increased floral lifespan which can increase attractiveness to pollinators (Ashman, 2004) and increased plant size (Valido et al., 2002). Large plants are often preferentially visited by pollinators compared with smaller neighboring plants (Valido et al., 2002), which is not surprising given that increased floral density can increase visits by pollinators seeking to reduce energetic costs of searching (Hegland and Boeke, 2006;

Seifan et al., 2014). Additionally, adaptations that minimize interspecific pollen transfer, and thus encourage intraspecific pollen transfer, can ensure that available pollen is successfully transferred to appropriate flowers (Harder and Aizen, 2010). For example, pollinators of *B. alba macrophylla* move from basal, pollen-receiving flowers to distal, pollen-donating flowers two and a half times more frequently than in the reverse direction (Haddock and Chaplin, 1982). Pollinators that move distally on the racemes of *B. alba macrophylla* are rewarded by the increased nectar contained within the basal, pistillate-phase flowers, a mechanism that enhances outcrossing (Haddock and Chaplin, 1982). Further, if *B. alba macrophylla* is pollen limited, high floral density and the spatial arrangement of flowers may increase visits by pollinators and may encourage outcrossing, respectively.

Nutrient Limitation—While the number of female flowers should ideally limit the number of fruits produced by a plant, maternal resources often determine the ultimate number of flowers that set fruit (Stephenson, 1981), as demonstrated by experiments in which levels of fruit set are much smaller than the number of female flowers even when all female flowers are appropriately pollinated (Stephenson, 1979).

Given that maternal resources are often finite, the probability that an individual reproductive unit will acquire sufficient nutrients depends on the number of reproductive units already initiated (Stephenson, 1981). Seeds within developing fruits produce growth hormones that draw limited maternal resources into developing fruits (Biale, 1978). As resources are allocated to early-setting fruits, the total amount of available resources decreases, thus decreasing the probability that later-setting fruits will acquire sufficient nutrients for maturation. Interestingly, when early-setting fruits are

artificially removed, rates of abortion in later-setting fruits decrease, demonstrating that fruits do compete for limited resources (Tamas et al., 1979). Additionally, fertilizer application can decrease fruit abortion by increasing the availability of nutrients for fruit maturation (Willson and Price, 1980; Shi et al., 2010). When fruits are unable to acquire sufficient nutrients for growth via hormonal signaling, the quantity of growth hormones produced by the seeds decreases, and the fruits begin to release growth inhibitors (Tamas et al., 1979), thus prompting the abscission of juvenile fruits. This clearly demonstrates that fruit abortion can result from limited maternal resources.

A threshold exists in which maturation of a fruit with few seeds is disadvantageous because the amount of resources invested exceeds the reproductive potential of that investment (Stephenson, 1981; Bertin, 1982). When fruits with few seeds are aborted, resources that would have been invested in the continued maturation of these fruits can instead be allocated to fruits containing more seeds or to future reproduction and/or vegetative growth (Stephenson, 1981). Thus, fruit abortion may be an adaptive mechanism which ensures that the maximum number of offspring reach independence through the reallocation of limited maternal resources (Lloyd, 1980).

Pre-dispersal Seed Predation—Pre-dispersal seed predation can result in selective fruit abortion in which aborted fruits are more frequently occupied by seed predators than are non-damaged fruits (Fernandes and Whitham, 1989). Selective abortion of damaged fruits is advantageous because it allows a plant to cease resource investment in fruits that have few or no seeds that will reach maturation, and it can increase larval mortality of destructive seed predators (Fernandes and Whitham, 1989), thus minimizing future seed predation.

When predation pressure is high, various traits can decrease fitness losses caused by pre-dispersal seed predation, including the production of less showy, or smaller, inflorescences (Molau et al., 1989), the production of deterrent chemicals (Guimarães et al., 2003), and even the adoption of off-peak flowering phenologies to escape predation (Albrechtsen, 2000). *B. alba macrophylla* exhibits several traits consistent with selection by seed predators. For example, *B. alba macrophylla* produces defensive alkaloids (Gibbons et al., 1990), but despite these compounds, insects, including *Apion rostrum* Say (Coleoptera: Apionidae) and *Tychius sordidus* LeConte (Coleoptera: Curculionidae), are known to cause significant damage to the developing seeds (Haddock and Chaplin, 1982).

Individuals can also escape seed predation as a result of their location relative to other individuals. In a study by Haddock and Chaplin (1982), 49 of the mere 52 seeds produced by *B. alba macrophylla* at a single site in 1978 were produced by a single, hidden plant in thickets. Although a plant's location is not a response to predation pressure by weevils, it can minimize losses caused by seed predation in some cases. Seeds produced by hidden plants may be of special importance for populations experiencing significant losses in seed yield, but the significance of these plants requires additional research (Haddock and Chaplin, 1982).

Objective and Hypotheses—*B. alba macrophylla*, commonly named white wild indigo, is an herbaceous perennial legume that produces large inflorescences, yet matures a limited number of fruits and seeds (Petersen and Sleboda, 1994). High levels of flower and fruit abortion make *B. alba macrophylla* an ideal model species for testing hypotheses regarding the reproductive ecology of angiosperm species.

The objective of this one-year field study was to identify proximate factors that limit reproductive output of *B. alba macrophylla*. Specifically, I postulated that the number of seeds in which *B. alba macrophylla* invests is serially adjusted based on resource availability at each stage of development, but that the final number matured is further compromised by extrinsic factors. I tested the following hypotheses:

1. Pollen limitation constrains reproductive output of *B. alba macrophylla*. I predicted that pollen supplementation would increase the number of fruits initiated, the number of fruits matured, and the number of seeds matured.
2. Nutrient availability limits reproductive output of *B. alba macrophylla*. I predicted that addition of fertilizer would increase the number of fruits initiated, the number of fruits matured, and the number of seeds matured.
3. Pre-dispersal seed predation by *Apion rostrum* and *Tychius sordidus* further limits reproductive output of *B. alba macrophylla*. I predicted that application of an insect barrier would increase the number of fruits and seeds matured.

Baptisia alba macrophylla—*B. alba macrophylla* is native to the central and southeastern United States where it is found growing in prairies, woods, open fields, and along floodplains and roadsides (Woods and Diamond, 2014). *B. alba macrophylla* leaves are trifoliolate with each leaflet being up to 2-6 cm long (Woods and Diamond, 2014). The shoots of *B. alba macrophylla*, sometimes called white false indigo, often have a dark purple tint. Native Americans used this species as a substitute for the true indigo (*Indigofera*) when creating indigo dyes, as suggested by the aforementioned common name (Missouri Botanical Gardens, n.d.). These shoots, which resemble

asparagus (*Asparagus officinalis*) when emerging from the soil, can yield up to 14 erect racemes per ramet (Petersen and Sleboda, 1994). The number of flowers produced per raceme varies tremendously among raceme, ramet, and site. In the present study, primary racemes produced an average of 53.21 ± 41.02 flowers (averaged across site), and secondary racemes produced an average of 22.66 ± 17.86 flowers (averaged across site).

Flowering phenology varies substantially within this species. In central Illinois, early emerging shoots can flower as early as May, but late emerging shoots can flower well into August (*pers. obs.*). Petersen et al. (2010a) report that early flowering plants have a longer flowering period and a greater number of racemes per ramet than later flowering plants in northeastern Illinois. The inflorescences of *B. alba macrophylla* are indeterminate racemes whose youngest flowers are in the staminate phase and are distal to the older flowers in the pistillate phase (Haddock and Chaplin, 1982). The inflorescences of *B. alba macrophylla* can produce a myriad of flowers; in fact, a single plant can yield more than 500 flowers in a single season (*pers. obs.*). If pollination and fertilization are successful, each flower can yield an inflated fruit ranging 2 to 5 cm in length (Woods and Diamond, 2014), each of which has the potential to produce between 30 and 38 seeds (Petersen and Sleboda, 1994).

Interactions of B. alba macrophylla with Other Organisms—

Reproductive success of angiosperm species, including *B. alba macrophylla*, is dependent on mutualistic relationships with pollinators but is constrained by antagonistic relationships with herbivores and pre-dispersal seed predators (Horn and Hanula, 2004). The following section will examine the numerous interspecific relationships of *B. alba*

macrophylla that have both direct and indirect effects on the reproductive output of *B. alba macrophylla*.

Bombus fervidus, *B. bimaculatus*, *B. pennsylvanicus* and *B. nevadensis auricomis* are known pollinators of *B. alba macrophylla* (Haddock and Chaplin, 1982; Petersen and Sleboda, 1994; Petersen, 1996), but other organisms, including a single Ruby-throated Hummingbird (*Archilochus colubris*), have been observed visiting the flowers of *B. alba macrophylla* (*pers. obs.*). These natural pollinators are critical for the reproductive success of *B. alba macrophylla* because they are responsible for the transfer of the male gametes. In *B. alba macrophylla*, levels of fruit set are influenced by pollen source under different pollination treatments. While the transfer of pollen between flowers of the same plant (=geitonogamy) and between flowers of different plants (=xenogamy) yields 100% fruit set, the transfer of pollen within a single flower (=autogamy) results in very low rates of fruit set (~5%) in *B. alba macrophylla* (Haddock and Chaplin, 1982). These differences in fruit set suggest that although *B. alba macrophylla* is self-compatible, autogamy is unlikely to be a primary means of pollination.

A. rostrum is a well-documented pre-dispersal seed predator of *B. alba macrophylla* (Haddock and Chaplin, 1982; Petersen, 1989; Petersen and Sleboda, 1994; Petersen et al., 2006; Petersen et al., 2010a, Petersen et al., 2010b). Females oviposit within developing *B. alba macrophylla* fruits by making a hole at the base of the fruit and pushing their eggs inside of the fruit (Haddock and Chaplin, 1982). The weevil larvae emerge within a few days of oviposition and feed exclusively on the developing seeds of the fruit (Petersen, 1989). The effect of seed predation by *A. rostrum* can be remarkably

substantial; in fact, a single fruit can experience complete reproductive failure if occupied by several developing weevils. The weevil larvae then pupate within the developing fruit, and adults emerge from the fruit upon dehiscence which can occur in early fall or the following spring (Haddock and Chaplin, 1982). In the latter case, the adults overwinter within the fruits of *B. alba macrophylla* (Haddock and Chaplin, 1982).

T. sordidus is a known herbivore and pre-dispersal seed predator of several *Baptisia* species, including *B. alba macrophylla* (Clark and Burke, 1977; Haddock and Chaplin, 1982). *T. sordidus* is found in the eastern and midwestern United States and in the eastern part of Texas (Clark and Burke, 1977). The range of *T. sordidus* appears to coincide with the range of its host species, including *B. leucantha* (= *B. alba* var *macrophylla*), *B. bracteata*, *B. cuneata*, and *B. villosa* (Clark, 1971; Clark and Burke, 1977). According to Clark (1971), *T. sordidus* is not restricted to *Baptisia* hosts alone and has been recorded on species of both *Acerates* (Gentianales: Apocynaceae) and *Croton* (Malpighiales: Euphorbiaceae).

Adults of *T. sordidus* emerge from pupal cases underground in early spring when *Baptisia* host plants are emerging from the soil (Clark and Burke, 1977). Emerging adults feed on *Baptisia* stamens and leaves despite the presence of glycosides and alkaloids, specifically quinolizidines, which are found in all parts of the plant (Gibbons et al., 1990) and are characteristic of *Baptisia* species (Clark and Burke, 1977). In addition to herbivory, *T. sordidus* further impacts *B. alba macrophylla* via pre-dispersal seed predation. After mating, which occurs on the flowers of *Baptisia*, the females chew a hole through the side of the calyx and then deposit an egg inside the immature ovary (Clark and Burke, 1977). The resulting larvae will then feed on the

developing seeds of the fruit. Rather than consuming one seed at a time, the larvae move within the developing fruit and feed on several seeds at a time, so that even very young larvae can damage all of the seeds within a fruit (Clark and Burke, 1977). Up to thirteen eggs have been reported within a single *Baptisia* fruit, and sometimes the larger larvae will consume the smaller larvae (Clark and Burke, 1977). After consuming all of the seeds within a single fruit, larvae chew an emergence hole through the wall of the fruit. The larvae then burrow in the soil beneath their host plant where they pupate inside a pupal cell made from a secretion obtained from the anal lobes (Clark and Burke, 1977). Adults overwinter in pupal cells beneath the soil, and emerge in the spring when host plants begin to emerge from the soil (Clark and Burke, 1977).

CHAPTER II

MATERIALS AND METHODS

Study Sites—The field experiment was conducted at the John English Prairie, a restored tallgrass prairie located in Comlara Park, NW McLean County, Illinois, USA and the Sugar Grove Nature Center and Funks Grove (hereinafter referred to as Funks Grove), a restored tallgrass prairie located in SW McLean County, Illinois, USA. Directly surrounding both prairies are forested areas and some rural roads. Both prairies are nested within the larger agricultural matrix which covers much of central Illinois. The John English Prairie was last burned in the spring of 2014, and Funks Grove was last burned in the spring of 2013.

Experimental Design—*B. alba macrophylla* ramets were selected for experimentation beginning on 8 June 2014 based on the following criteria: ramet recently emerged from soil (all ramets <92 cm tall), emerging ramet not yet branching, and ramet located more than two meters from other experimental ramets. Twenty-four ramets were initially selected for experimentation at the John English Prairie and thirty-five ramets were initially selected at Funks Grove. One of the twenty-four experimental ramets at the John English Prairie was damaged due to human error and was thus excluded from the analysis. Four additional ramets were not included in the statistical analysis because they failed to produce flowers during the 2014 flowering season. Four of the experimental

ramets at Funks Grove died during flowering and were thus excluded from the experiment. Because two of these ramets died relatively early in the flowering period, I substituted two additional ramets that met the above criteria. As a result, a total of thirty-three ramets from Funks Grove were included in the experiment. Between the two sites, fifty-two ramets were included in the analysis. I found that that a sample size of fifty-two was sufficiently large due to the time-intensive nature of this hand-pollination study. Although I sampled only a small proportion of plants at both sites, the experimental data appear to be representative of each site based on personal field observations.

Experimental ramets were randomly assigned one of two fertilizer treatments: (i) no fertilizer application, or (ii) application of 70 grams of Ideal™ All-Purpose 10-10-10 NPK granular fertilizer (Eau Claire Coop Oil Company, Eau Claire, Wisconsin, USA) to the 1 m² area surrounding the base of the ramet prior to first flowering and again post-flowering. Experimental ramets were then randomly assigned one of two insect barrier treatments: (i) application of Tanglefoot® insect barrier (Contech Enterprises, Inc., Victoria, British Columbia, Canada) on a plastic drinking straw that was slit open lengthwise and wrapped around the base of the emerging ramet, or (ii) placement of a bare drinking straw around the base of the emerging ramet. The straws varied in length because they were cut to fit the base of each emerging ramet. Tanglefoot® was not applied directly to the ramet of *B. alba macrophylla* to ensure that the experimental treatment did not interfere with photosynthetic activity. Tanglefoot® was re-applied as needed throughout the season.

The first flower to bloom on each raceme was randomly assigned one of two pollination treatments: (i) open pollination supplemented with hand pollinated outcrossing, or (ii) open pollination. The next flower to bloom on each raceme was assigned the remaining pollination treatment, and all subsequent flowers on that same raceme were assigned a pollination treatment following this alternating pattern. Hand pollination was performed using homemade bee sticks that were constructed by pasting a toothpick to the abdomen of a dead bee (Carolina Biological Supply Company©, Burlington, North Carolina, USA). After assigning individual flowers to a pollination treatment, a small piece of colored drinking straw was placed around the pedicel to indicate the pollination treatment. Each flower continued to receive its assigned pollination treatment daily until it set fruit, was aborted, or otherwise lost from the plant. On average, the lifespan of a single *B. alba macrophylla* flower is 3-4 days (Haddock and Chaplin, 1982), and thus hand pollinated flowers were supplemented for approximately 3 or 4 days depending on the lifespan of each individual flower. Not all flowers assigned to the hand pollination treatment were hand pollinated on 21 June 2014 due to extended rain showers. Given that each hand pollinated flower was supplemented for several days, it is unlikely that lack of hand pollination on this date adversely affected the study.

Fruits from both prairies matured in the field, and were collected prior to dehiscence. Collection from the John English Prairie occurred on the 29th of July, 2014, and collection from Funks Grove occurred on the 28th and 29th of August, 2014. Most of the fruits were placed in a freezer at -10 °C, and were dissected between 25 September 2014 and 13 February 2015. During dissection of each fruit, I quantified the following: the number of undamaged seeds, the number of *A. rostrum* and *T. sordidus* at each stage

of development, the number of other organisms, and the length of the fruit. A random subset of the fruits was not placed directly in the freezer because these fruits were used for a germination experiment which is not included in this document. These pods were placed in a refrigerator at 5 °C and were dissected via microscopy between 11 September 2014 and 24 September 2014.

Statistical Analysis—Data were analyzed using SAS 9.3 software (SAS Institute Inc., Cary, North Carolina, USA). The first analysis I conducted examined the probability that an individual flower will initiate fruit, that an initiated fruit will reach maturity, and that an individual flower will eventually produce seeds. Data from the John English Prairie and Funks Grove were analyzed independently due to differences in site, including differences in seed predators, differences in burning patterns, and differences in plant traits, including length of flowering period, number of racemes per ramet and size of ramet.

At the John English Prairie, the effects of pollination treatment, predation treatment, and nutrient treatment on the probability of fruit initiation were analyzed with a generalized linear model using a binomial error distribution and logit link function. At Funks Grove, the effect of each treatment on the probability of fruit initiation was analyzed with a generalized linear model using a Gaussian error distribution and logit link function. At both sites, each flower was treated as a replicate of pollination treatment within whole plants given a single nutrient-predation treatment. At both the John English Prairie and Funks Grove, a similar analysis utilizing a binomial error distribution was performed on the probability that an individual initiated fruit would be

retained through maturation. The number of seeds that matured within pods at Funks Grove was analyzed using a zero inflated Poisson error distribution.

The second analysis examined reproduction of entire ramets rather than individual flowers or fruits. Reproduction was analyzed by MANOVA using site, nutrient treatment, and predation treatment as main effects and log-transformed flowers, matured fruits, and total seeds per ramet as response variables. Differences among means for significant interactions were tested using Tukey-Kramer adjustment for multiple comparisons. Pollen treatment was not examined in this analysis because pollen treatment was assigned to individual flowers, not entire ramets.

CHAPTER III

RESULTS

Analysis by Flower and Fruit—The first analysis I conducted examined the probability that an individual flower will initiate fruit, that an initiated fruit will be retained through maturation, and that an individual flower will eventually produce seeds. The results of this analysis are listed below by site.

Funks Grove—At Funks Grove, the mean number of flower-producing racemes/ramet was 4.15 ± 2.83 (mean \pm SD). All ramets at this site produced flowers during the 2014 flowering season. The mean flowering period, defined as the time span between the date of first flowering and the last date a new flower opened on an individual ramet, was 15.36 days \pm 5.62 days. Although I did not record these data, the ramets produced at Funks Grove appeared much taller than the ramets produced at the John English Prairie (*pers. obs.*).

The mean number of flowers that initiated fruit was significantly affected by pollen treatment ($F_{1,4771} = 4.75$, $P = 0.0294$) but not nutrient treatment, predation treatment, or any interactions ($P > 0.05$ for all). Flowers that were supplemented with hand pollination were significantly more likely to initiate fruit than were flowers that were not supplemented with hand pollination (Fig. 1). Only predation treatment affected the probability that initiated fruits were retained through maturation ($F_{1,29} = 10.41$, $P =$

0.0031). Ramets treated with Tanglefoot® matured significantly more initiated fruits than did ramets receiving the control treatment (Fig. 2). Likewise, the mean number of seeds matured per fruit was significantly affected by predation treatment ($F_{1,29} = 10.93$, $P = 0.0025$). Ramets treated with Tanglefoot® matured significantly more seeds per fruit than did ramets receiving the control treatment (Fig. 3), but nutrient treatment, pollen treatment, or their interactions were not significant factors ($P > 0.05$ for all).

John English Prairie—At the John English Prairie, four of the twenty-three experimental ramets (17.39%) failed to produce any flowers during the 2014 flowering season and were thus excluded from statistical analysis. The mean number of flower-producing racemes/ramet was 1.05 ± 0.23 racemes (mean \pm SD), which is approximately one-fourth the mean number of racemes/ramet at Funks Grove. Only one ramet produced multiple flower-producing racemes at this site. The mean flowering period was 5.89 ± 2.66 days. The mean flower period at the John English Prairie was approximately two-fifths the mean flowering period at Funks Grove.

Pollen treatment significantly affected fruit initiation ($F_{1,289} = 4.23$, $P = 0.0405$). Flowers that were supplemented with hand pollination were significantly more likely to initiate a fruit than were flowers not supplemented with hand pollination (Fig. 4). Although pollen supplementation significantly increased fruit initiation, nutrient treatment, predation treatment, and their interactions were not significant factors ($P > 0.05$ for all). The probability that initiated fruits subsequently matured was not significantly affected by pollination treatment, nutrient treatment, or predation treatment, and none of the interactions were significant ($P > 0.05$ for all). Statistical analysis was

not conducted on the number of seeds matured per fruit, because only three of the sixty-seven mature fruits produced one or more mature seeds. It is worth noting that the three, seed-producing fruits were not from plants assigned to the same treatments. The nutrient treatment, predation treatment, and pollen treatment for the first fruit which initiated a single seed were as follows: application of fertilizer, application of Tanglefoot®, and open pollination supplemented with hand pollinated outcrossing. The treatments for the second fruit which initiated two seeds were as follows: no fertilizer application, no Tanglefoot® application, and open pollination. The treatments for the third fruit which initiated a single seed were as follows: no fertilizer application, Tanglefoot® application, and open pollination.

Analysis by Ramet—The second analysis I conducted examined reproduction of whole ramets rather than individual flowers or fruits. There was a significant predation by site interaction (Pillai's Trace: $F_{3,42} = 3.99$, $P = 0.0137$). The effect of Tanglefoot® insect barrier on flower number was significant at Funks Grove ($P = 0.0152$), but not at the John English Prairie ($P = 0.9186$). At Funks Grove, Tanglefoot®-treated ramets initiated more flowers than control ramets (Fig. 5). It is possible that ramets assigned the Tanglefoot® treatment initiated more flowers by chance. The effect of Tanglefoot® insect barrier on fruit maturation was significant at Funks Grove ($P < 0.0001$), but not at the John English Prairie ($P = 0.9992$). At Funks Grove, Tanglefoot®-treated ramets matured more fruits than control ramets (Fig. 6). The effect of Tanglefoot® insect barrier on seed number was also significant at Funks Grove ($P < 0.0001$), but not at the John English Prairie ($P = 0.9994$). At Funks Grove, Tanglefoot®-treated ramets matured more seeds than control ramets (Fig. 7). There was

no effect of nutrient treatment on flower number, fruit number, or seed number ($P > 0.05$ for all).

CHAPTER IV

DISCUSSION

The objective of this one-year field study was to identify proximate factors that limit reproductive output of *B. alba macrophylla*. Specifically, I hypothesized that the number of seeds matured by *B. alba macrophylla* is serially adjusted based on nutrient availability at each stage of development, but that the final number matured is further compromised by extrinsic factors such as pollen limitation and pre-dispersal seed predation by the weevils *A. rostrum* and *T. sordidus*. My data provide equivocal support for the hypothesis. While reproductive output of *B. alba macrophylla* is serially adjusted during each stage of development, there are no data indicating that this adjustment is caused by nutrient availability at each stage. Pollen supplementation was found to increase fruit set, although this did not translate to greater fruit and/or seed maturation. Pre-dispersal seed predation reduces the number of fruits matured per initiated fruit, the number of seeds matured per fruit, the number of fruits matured per ramet, and the number of seeds matured per ramet.

Pollen Limitation—The hypothesis that pollen limitation reduces reproductive output of *B. alba macrophylla* during all stages of offspring development was not supported by the data. Flowers that were supplemented with hand pollination initiated slightly, but significantly, more fruits than flowers not supplemented with hand

pollination at both the John English Prairie and Funks Grove, yet only a small proportion of initiated fruits subsequently matured, and pollen treatment did not significantly affect the number of initiated fruits that matured or the number of seeds matured per fruit. As a result, these populations are likely not *pollen limited*, which is a specific term referring to the production of fewer fruits and seeds than could be produced if sufficient or higher quality pollen was received (Ashman et al., 2004; Knight et al., 2005).

Rates of pollen limitation can be elevated in pollen supplementation experiments if less than all of the flowers on an individual plant are supplemented by hand pollination (Zimmerman and Pyke, 1988; Knight et al., 2005). In such experiments, flowers supplemented with hand pollination may be able to yield a greater number of fruits and/or seeds than non-supplemented flowers, but that does not mean that the entire plant would be able to yield more fruits and/or seeds if all flowers were supplemented (Zimmerman and Pyke, 1988; Knight et al., 2005). Although I observed an effect of pollen treatment during the fruit initiation stage, this effect was not significant in terms of fruits and/or seeds matured, and it is possible that this effect would not be observed if a different experimental design were utilized, such as assigning whole racemes or ramets to a single pollination treatment.

Nutrient Limitation—Although the number of fruits and seeds produced by a plant is ideally determined by the number of female flowers and ovules, respectively (Stephenson, 1981), the availability of maternal resources during each stage of offspring development can sequentially decrease the number of reproductive units that are ultimately produced (Lloyd, 1980). The hypothesis that nutrient limitation sequentially

reduces reproductive output of *B. alba macrophylla* during fruit initiation, fruit maturation, and seed maturation was not supported by the experimental data. Absence of a fertilizer effect during all stages of offspring development suggests that the *B. alba macrophylla* populations at the John English Prairie and Funks Grove are not nutrient limited. However, it is possible that I may have observed a significant effect of nutrient treatment if the effect of predation was not so overwhelmingly large even on Tanglefoot®-treated plants.

It is worth noting that the application of granular fertilizer to this perennial species may not have increased the availability of nutrients for reproduction in the current season. Instead, these resources may be used for growth and/or reproduction in subsequent years. A multi-year study would yield a more comprehensive understanding of resource availability and allocation in *B. alba macrophylla*.

Pre-dispersal Seed Predation—The hypothesis that pre-dispersal seed predation by weevils constrains reproductive output of *B. alba macrophylla* was strongly supported by our data. Pre-dispersal seed predation by *T. sordidus* at the John English Prairie and *A. rostrum* at Funks Grove reduced reproductive output during fruit maturation and seed maturation, but there was no indication that weevils had a significant effect during the fruit initiation stage. At the John English Prairie, only 4.5% of all mature fruits escaped some degree of damage by seed predators (n = 67). At Funks Grove, 58.8% of mature fruits escaped some degree of damage by seed predators (n = 690). While these losses seem great in magnitude, even greater losses in seed production by *B. alba macrophylla* were observed by Haddock and Chaplin (1982) who report that

only 1.3% and 5.9% of all fruits produced any seeds in 1978 and 1979, respectively, mostly due to pre-dispersal seed predation by insects.

When the data were analyzed by ramet, rather than individual flowers or fruits, there was a significant effect of predation treatment on the number of fruits and seeds matured at Funks Grove, though this effect was not observed at the John English Prairie. This site difference is likely due to differences in the species of seed predators found at the two field sites and/or the effectiveness of the Tanglefoot® treatment at each site. *A. rostrum* was the only species of weevil observed on *B. alba macrophylla* at Funks Grove, whereas *T. sordidus* was the only species of weevil observed on *B. alba macrophylla* at the John English Prairie. Only 3 ramets at the John English Prairie produced *any* seeds during the 2014 flowering season which suggests that the Tanglefoot® treatment was less effective at this site possibly due to the species of seed predator found here.

It is worth noting that Tanglefoot® treatment did not completely eliminate seed predators from Tanglefoot®-treated plants. Although Tanglefoot® application prevented weevils from advancing up the base of the plant, ovipositing weevils were observed moving between plants that were in physical contact with one another. To minimize the occurrence of such movement, I regularly trimmed neighboring vegetation that surrounded all experimental plants. Nonetheless, some weevils were capable of accessing the Tanglefoot®-treated plants in this manner. Additionally, *A. rostrum* adults were observed moving between plants via weak flight. Although it does not appear that this species has strong flight abilities (Peter Brabant, *pers. comm.*; May 2014), it certainly

has the ability to fly, or rather glide, short distances between plants (*pers. obs.*). Further, although the Tanglefoot® treatment was effective at reducing predation by weevil larvae, this treatment was not effective in completely eliminating seed predators from plants.

Conclusions and Implications—These data provide equivocal support for the hypothesis that reproductive output of *B. alba macrophylla* is serially adjusted during each stage of offspring development. At the John English Prairie, 324 flowers were initiated, yet only 270 of these flowers initiated fruit. A mere 67 of these initiated fruits reached maturity, and only 3 fruits matured *any* seeds. At Funks Grove, 4,821 flowers were initiated, yet only 4,268 of these flowers initiated fruit. A mere 690 of these initiated fruits reached maturity, and only 406 mature fruits contained *any* seeds. It is quite evident that there is a sequential reduction in reproductive units during offspring development, but there is no evidence to suggest that this adjustment is based on resource availability at each stage of development.

Although flowers supplemented with hand pollination initiated significantly more fruits than control flowers, this difference did not translate into a greater probability of fruit maturation or a greater number of seeds per fruit, and I conclude that our populations of *B. alba macrophylla* are likely not *pollen limited*.

I conclude that seed predation by weevil larvae, specifically *A. rostrum* and *T. sordidus*, has significant and negative effects on the realized reproductive output of *B. alba macrophylla*, as measured by the number of seeds matured per fruit and per ramet. These data are in accordance with previous work by Petersen (1989) who reports that Tanglefoot®-treated ramets yielded greater grand mean numbers of fruits/ramet and

seeds/fruit than control ramets. Petersen (1989) also reports that Tanglefoot®-treated ramets had smaller grand mean numbers of *A. rostrum*/fruit. In conclusion, this study indicates that seed predation is a driving force shaping reproduction of this native legume, but neither nutrient limitation nor pollen limitation are limiting reproductive output in these populations of *B. alba macrophylla*.

Recommendations for Future Research—Although this study indicates that pre-dispersal seed predation by weevils significantly reduces reproductive output of *B. alba macrophylla*, further research should be conducted to determine the long-term consequences of pre-dispersal predation on *B. alba macrophylla* at the population level. Weevil larvae cause almost complete reproductive failure on some sites, and thus it is important to investigate the consequences of predation at the population level.

Additionally, further research should be conducted to determine the reproductive potential of *B. alba macrophylla* in the absence of weevils. As outlined above, Tanglefoot® insect barrier was effective in minimizing predation pressure by weevils in the present study and in a study by Petersen (1989), yet this treatment did not completely eliminate weevils from experimental plants. In this study and others (Haddock and Chaplin, 1982), seed predation by weevils causes very high levels of seed destruction. A study in which weevils are completely eliminated from *B. alba macrophylla* would determine if the fitness losses caused by pre-dispersal seed predation are additive or compensatory.

CHAPTER V

SIGNIFICANCE

Determining the factors that limit reproductive output of *B. alba macrophylla* is imperative for (i) understanding the reproductive ecology of angiosperm species and (ii) making informed decisions regarding the conservation and restoration of tallgrass prairies in North America. It is estimated that angiosperm species account for approximately 89% of all extant plant species (Crepet and Niklas, 2009), and thus research examining the reproductive ecology of angiosperm species is essential.

Understanding the life history of all organisms is important, but this is especially true for *B. alba macrophylla*, a native, leguminous component species of the tallgrass prairie ecosystem. In the "Prairie State" of Illinois where this study took place, it is estimated that the tallgrass prairie occupied 8.9 million hectares of the Illinois landscape during the 1820's (Petersen, 1996). Over the past two centuries, the tallgrass prairie ecosystem has been degraded to a mere 0.01% of its historic range in Illinois (Samson and Knopf, 1994). These disheartening data certainly demonstrate the need for research on what remains of this rare and fragmented ecosystem.

Although possessing membership in the tallgrass prairie ecosystem is enough to justify the necessity of research, *B. alba macrophylla* is a legume and, like other legumes, plays a critical role in nutrient cycling of the tallgrass prairie ecosystem

(Mundahl, 2014). When legumes are not present in prairie habitats and restorations, prairie development and diversity can be compromised (Graham et al., 2008). Further, this research and any research investigating factors which limit reproduction in leguminous, tallgrass prairie component species is critical for the restoration and preservation of what remains of this beautiful ecosystem.

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FIGURES

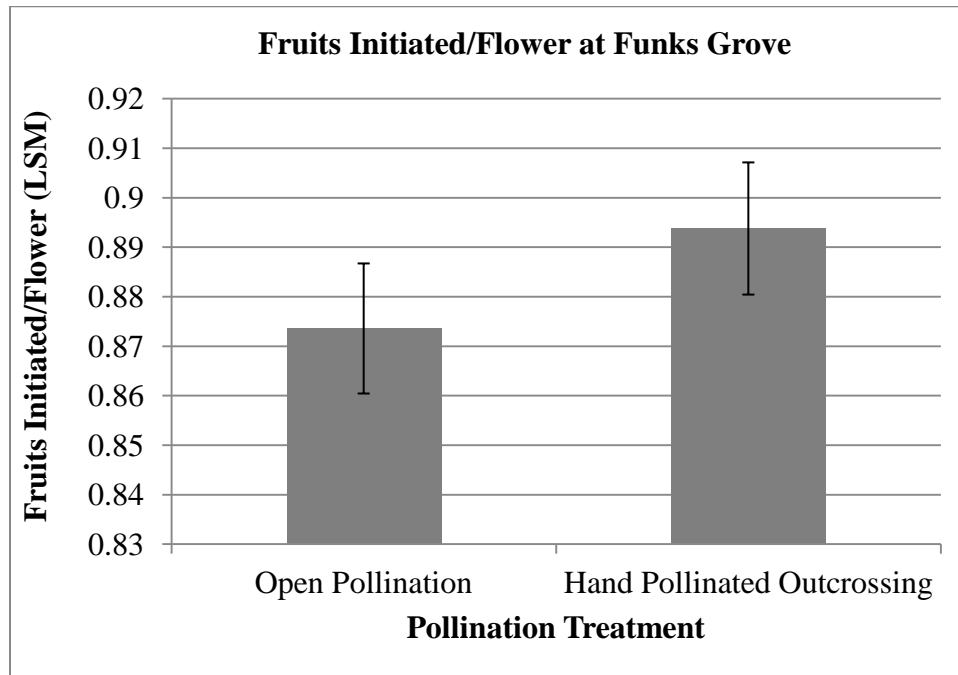


Fig. 1. Fruits Initiated/Flower (Least Squares Means) at Funks Grove. Flowers supplemented with hand pollination initiated significantly more fruits per flower than open pollinated flowers.

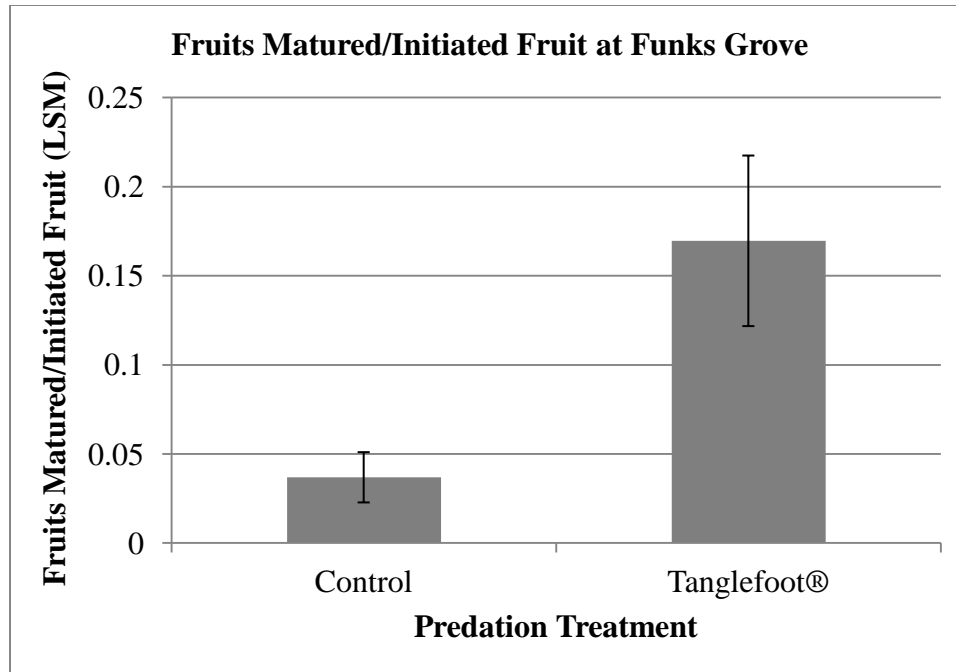


Fig. 2. Fruits Matured/Initiated Fruit (Least Squares Means) at Funks Grove. Ramets treated with Tanglefoot® matured significantly more initiated fruits than control ramets.

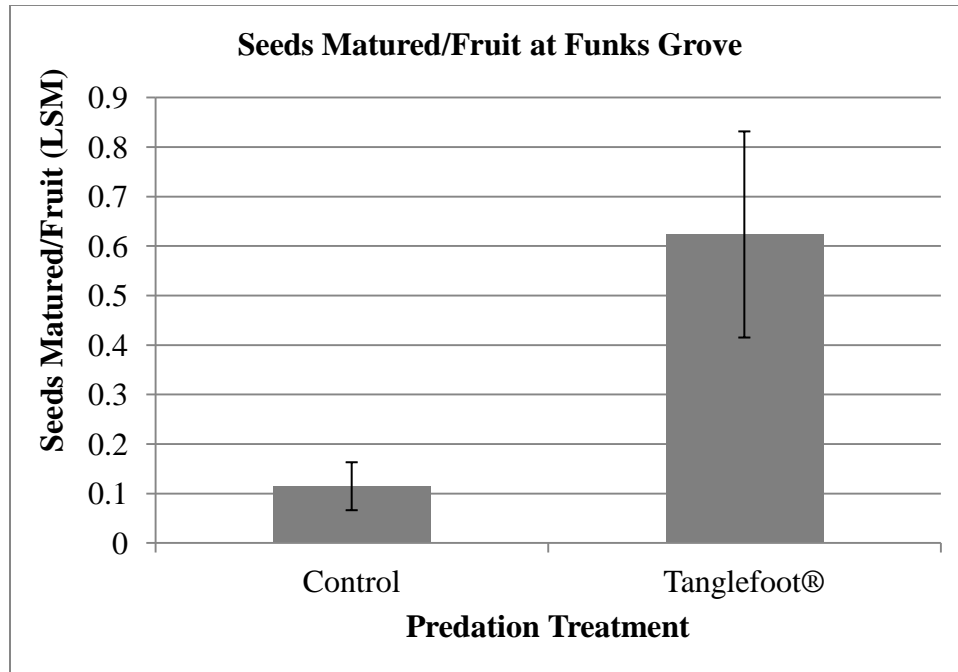


Fig. 3. Seeds Matured/Fruit (Least Squares Means) at Funks Grove. Ramets treated with Tanglefoot® matured significantly more seeds per fruit than control ramets.

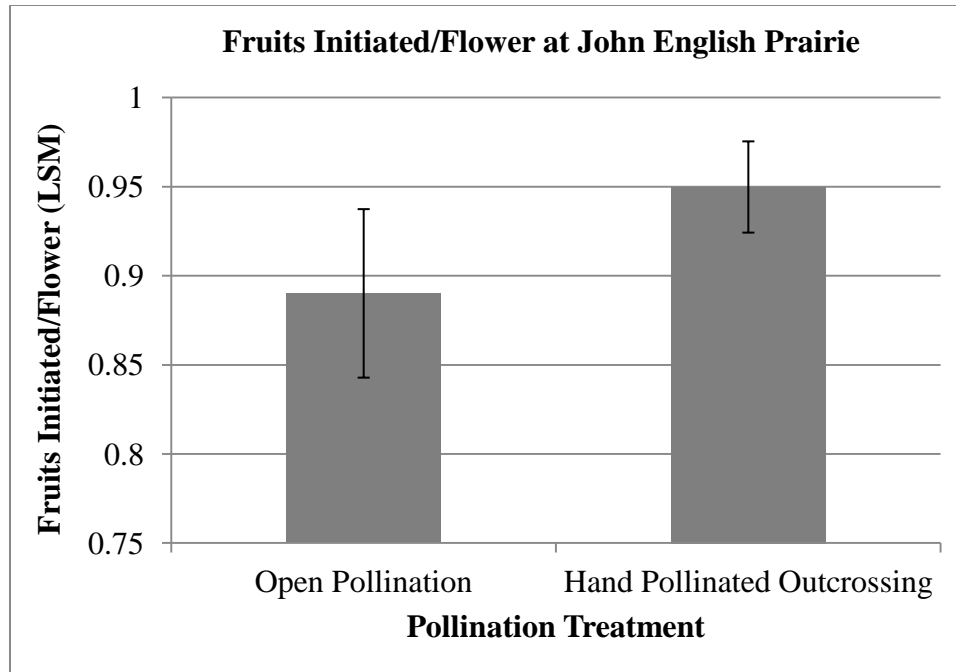


Fig. 4. Fruits Initiated/Flower (Least Squares Means) at the John English Prairie. Flowers supplemented with hand pollination initiated significantly more fruits than open pollinated flowers.

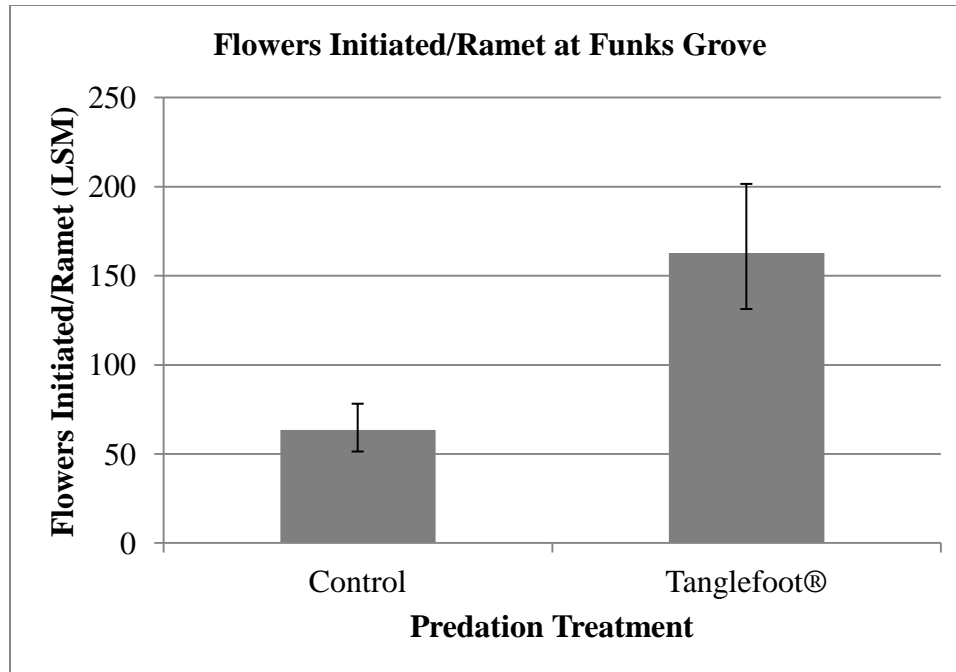


Fig. 5. Flowers Initiated/Ramet (Least Squares Means) at Funks Grove. Tanglefoot®-treated ramets initiated significantly more flowers than control ramets.

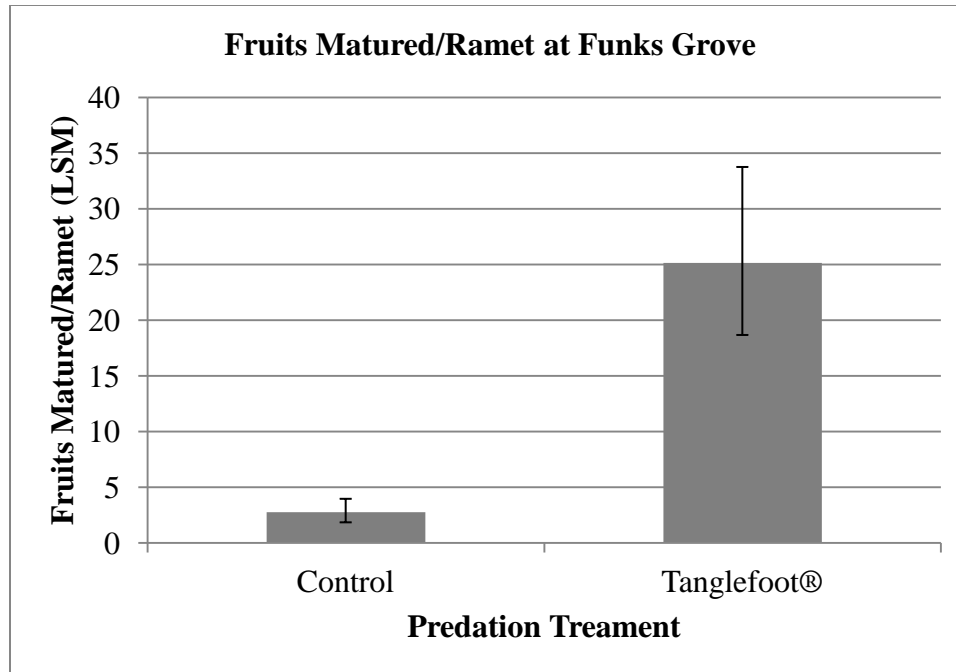


Fig. 6. Fruits Matured/Ramet (Least Squares Means) at Funks Grove. Tanglefoot®-treated ramets initiated significantly more fruits than control ramets.

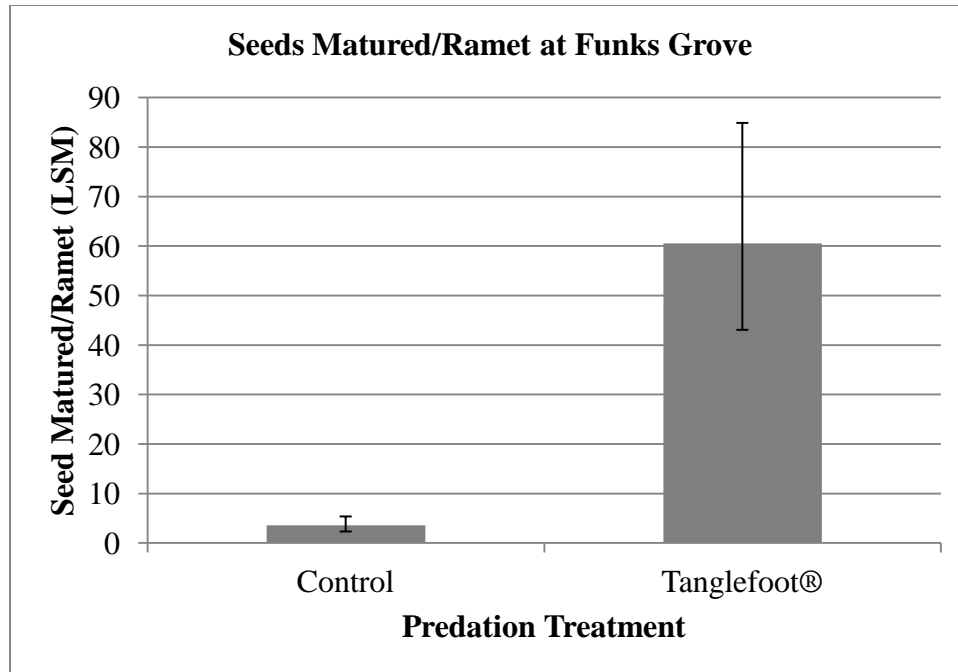


Fig. 7. Seeds Matured/Ramet (Least Squares Means) at Funks Grove. Tanglefoot®-treated ramets matured significantly more seeds than control ramets.