ABSTRACT

GADD, LAURA ELIZABETH. Pollination biology of the federally endangered *Echinacea laevigata* (Boynton and Beadle) Blake, Smooth Coneflower, in small, isolated populations. (Under the direction of Dr. Jon M. Stucky)

Echinacea laevigata (Boynton and Beadle) Blake, a federally endangered species, occurs in several small, isolated populations and a single large population in the northern Piedmont of North Carolina. Currently, little is known of the reproductive biology of this species. Therefore, we sought to describe its flowering phenology, compatibility pattern, and which of its various flower visitors were the more effective pollinators, to inform conservation efforts. In addition, pollinator limitation can reduce seed number and seed quality in small, isolated plant populations. We conducted a study of insect flower visitation and seed production in these populations to test our hypothesis that plants in the small, isolated populations are visited by fewer insect taxa, receive fewer visits, and produce fewer and/or less fit seeds than do plants in the large population. Our data show that average insect visitor species richness was significantly greater in the large population than in small populations and all but one of the small populations had fewer pollinator visits per head during fifteen minute observations than the large population; however, plants in several small populations produced as many or more seeds per head than did plants in the large population. Therefore, our results were not consistent with expectations of pollinator limitation. However, results show that seeds from small populations produce seedlings that are less fit as those from the large population. We conclude that other factors not examined in this study are more threatening to small, isolated coneflower populations than is pollinator limitation.

POLLINATION BIOLOGY OF THE FEDERALLY ENDANGERED *ECHINACEA LAEVIGATA* (BOYNTON AND BEADLE) BLAKE, SMOOTH CONEFLOWER, IN SMALL, ISOLATED POPULATIONS

by

LAURA ELIZABETH GADD

A thesis submitted to the Graduate Faculty of
North Carolina State University
In partial fulfillment of the
Requirements for the degree of
Master of Science
In

BOTANY

Raleigh, North Carolina

2006

APPROVED BY:

Dr. John R. Meyer

Dr. Ted H. Shear

Dr. Jon M. Stucky
Chair of Advisory Committee

DEDICATION

This is dedicated to my parents, Charlie and Sally Gadd, whose love, patience, encouragement, guidance, and faith have opened the doors of opportunity for me.

BIOGRAPHY

I grew up in the once small town of Wake Forest, North Carolina. As a child, I freely roamed the woods all around our house and learned to appreciate and love nature. I was especially influenced by my Dad, a naturalist who, despite his lack of formal training, is thrilled that he can still teach me a thing or two about plants on our walks through the woods.

I attended Meredith College in Raleigh, NC, where I graduated with a degree in Biology and a concentration in Environmental Science in 2001. Dr. Janice C. Swab was the first professor to introduce me into the botanical world and was very influential in my decision to pursue an environmental career. I am grateful to my first boss and dear family friend, Larry Roper, who had enough confidence in me to give me my first job as a Floriculturist. Larry taught me not to be afraid of taking on big challenges---even to driving a 500 gallon water truck through rush hour traffic in downtown Raleigh. Following my college days, I was employed for two years with an environmental consulting firm in Raleigh, NC. I started as a graduate student at NCSU in 2003. One of the best things that happened to me at NCSU was meeting William and Mary Coker Joslin. It has been my delight to live and work in their beautiful garden for the past two years, and to be blessed by their passion for the environment, their knowledge of plants, and the gracious way they have opened their home and their hearts to me. Upon completion of graduate school, I plan to continue to work in plant conservation.

ACKNOWLEDGMENTS

I thank the members of my advisory committee of Dr. Jon Stucky, Dr. John Meyer, and Dr. Ted Shear for their guidance and support throughout the duration of this study. I especially thank Dr. Jon Stucky, the chair of my advisory committee, for his patience and enthusiasm in working with me, his availability and willingness to discuss any aspect of the project, and to answer questions at any given time. He has been an encouraging and inspiring teacher and friend during my time at NCSU.

The development of an effective experimental design and statistical analysis of data would not have been possible if were not for the patience and unflagging assistance of Dr. Cavell Brownie. I am also grateful to Dave Stephan for his entomological expertise, and his identification of all the insects.

I thank my tireless field helpers, Angela Richardson, Julie Roszko, and Jennifer Petitte. I also thank Caitlin Elam for her help in the greenhouse, and Mike Beddoe for his many hours spent counting pollen grains. I am especially grateful to Andy Walker for acting as a sounding board for ideas and for his help with practically every part of this project.

This research was funded by the NC Agriculture Research Service and the NCSU RISE program. Permits to do the work were granted by the NC Plant Conservation Program and the US Army Corps of Engineers. I also thank the Food Lion Distribution Center in Butner, NC for graciously allowing passage through their property every day to access Picture Creek.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

FORWARD

This master's thesis is not a traditional thesis with a literature review and numerous chapters. In comparison to a traditional thesis, the text is somewhat condensed and was written in the format for publication in a scientific journal.

Pollination biology of the federally endangered *Echinacea laevigata* (Boynton and Beadle) Blake, Smooth Coneflower, in small, isolated populations

Laura E. Gadd[1], Jon M. Stucky[1], and Cavell Brownie[2]

[1] Department of Botany, North Carolina State University

[2] Department of Statistics, North Carolina State University

ABSTRACT

Echinacea laevigata (Boynton and Beadle) Blake, a federally endangered species, occurs in several small, isolated populations and a single large population in the northern Piedmont of North Carolina. Currently, little is known of the reproductive biology of this species. Therefore, we sought to describe its flowering phenology, compatibility pattern, and which of its various flower visitors were the more effective pollinators, to inform conservation efforts. In addition, pollinator limitation can reduce seed number and seed quality in small, isolated plant populations. We conducted a study of insect flower visitation and seed production in these populations to test our hypothesis that plants in the small, isolated populations are visited by fewer insect taxa, receive fewer visits, and produce fewer and/or less fit seeds than do plants in the large population. Our data show that average insect visitor species richness was significantly greater in the large population than in small populations and all but one of the small populations had fewer pollinator visits per head during fifteen minute observations than the large population; however, plants in several small populations produced as many or more seeds per head than did plants in the large population. Therefore, our results were not consistent with expectations of pollinator limitation. However, results show that seeds from small populations produce seedlings that are less fit as those from the large population. We conclude that other factors not examined in this study are more threatening to small, isolated coneflower populations than is pollinator limitation.

INTRODUCTION

Echinacea laevigata (Boyton & Beadle) Blake (Asteraceae), smooth purple coneflower (coneflower), is a federally endangered (Murdock 1992) herbaceous, perennial that currently occurs on alfisols and mollisols in sites with reduced cover of woody vegetation in Virginia, North Carolina, South Carolina and Georgia (Smith 1986, Schafale & Weakley 1990, Gaddy 1991, Slapcinsky 1994, Evans *et al*. 2002). Coneflower was, most likely, a component of presettlement grasslands maintained by fire over large areas of the southeastern Piedmont (Juras 1997). Subsequent to European settlement, years of fire suppression caused the decline of Piedmont grasslands (Barden 1997, Juras 1997) and the current, general pattern of small, isolated coneflower populations.

Small, isolated populations, such as those of coneflower, may incur consequences of their reduced size. They might experience decreased pollinator diversity and flower visitation and, therefore, produce fewer seeds than do larger, more abundantly flowering populations (Jennersten 1988, Lamont *et al*. 1993, Pavlick *et al.* 1993, Rathke & Jules 1993, Aizen and Feinsinger 1994, Olesen & Jain 1994, Agren 1996, Buchmann & Nabhlan 1996, Kearns *et al.* 1998, Kwak *et al.* 1998, Bosch & Waser 1999, Cunningham 2000, Spira 2001, Goverde *et al.* 2002, Brys *et al*. 2004, Kolb 2005, Wagenius 2006), a phenomenon called pollinator limitation (Bierzychudek 1981, Sih & Baltus 1987, Moody-Weis & Heywood 2001). However, flower visitation frequency and seed production in some remnant populations have equaled or even exceeded those in large conspecific populations (Becker et al 1991, Aizen and Feinsinger 1994, Cane 2001, Donaldson *et al.* 2002, Yates and Ladd 2005). Clearly, it is not necessarily the case that small coneflower populations experience pollinator limitation. Small populations might also experience reduced seed germination and seedling fitness as a consequence of low genetic variation and inbreeding depression (Menges 1991, 1995, Fischer and Matthies 1998, Wagenius 2000, Bruna 2002, Donaldson et al. 2002, Hooftman *et al.* 2003, Kolb 2005).

There is considerable interest in conserving southeastern Piedmont grasslands (Barden 1997, Davis *et al.* 2002, Evans *et al*. 2002) and in understanding the viability of small coneflower populations. Previous studies of *E. laevigata* describing patterns of genetic variation (Apsit & Dixon 2001, Peters 2005), reintroduction methods (Alley and Affolter 2004), effects of disturbance and light on growth (Emanuel 1996, Alley *et al*. 2005) and monitoring methods designed to account for the species' moderate amount of vegetative reproduction (Philippi *et al.* 2001) and studies of other *Echinacea* species (McGregor 1968, Leuszler *et al.* 1996, Walck *et al*. 2002, Wagenius 2000, 2004, 2006) inform coneflower conservation efforts. Studies of coneflower seed production would be important because they would provide insight into the mechanism that reduces genetic load in this rare species (Paland and Lynch 2006, Nielsen 2006), generation of genetic variation as a hedge against a changing environment, and propagules that can found new populations (Boyle and Menges 2001, Cummings and Alexander 2002, Méndez *et al.* 2004). Since most coneflower populations are small, information regarding seed production in small, as well as large, populations would be pertinent to conservation.

We studied the reproductive biology of *Echinacea laevigata* to inform efforts to conserve it (Murdock 1995) and to evaluate the reproductive status of its small populations. Specific objectives of our coneflower study were to describe (1) its flowering phenology and compatibility pattern, and determine (2) which of its various flower visitors were the more effective pollinators, (3) if pollinator limitation was reducing the numbers of seeds produced in small populations, and (4) if the fitness of seeds and seedlings from small populations were less than the fitness of those from a large population.

METHODS AND MATERIALS

Study Sites

This study was conducted during 2004 and 2005 in five small coneflower populations of habitat fragments and one large population in a managed grassland, all in the northern piedmont of North Carolina (Table 1). The Knap of Reeds and Snow Hill Road populations were lost to the study when land managers burned them and the surrounding habitat during the spring of 2005. Three other remnant populations, Freudenberg, Briardale Road, and Lakeside Drive, were included in the study during 2005.

Flowering Phenology

During late May to early July, 2004, flowering individuals of each species in ten randomly distributed 2x10 m transects at Picture Creek were counted and the relative abundance of each in the flowering community determined approximately weekly. Flowering time niche breadth and niche overlap (Levins 1968) for the entire sampling period were determined for coneflower and all other sampled species. Niche breadth and niche overlap were determined as:

$$B = 1 / \sum_j^r (p^2_{ij}) \text{ and } O_{1,2} = \{\sum_j^r [(p_{1j})(p_{2j})]\} / B,$$

respectively, where B is niche breadth, $O_{1,2}$ is niche overlap of species 1 on species 2, p is the proportion of total flowers for a species over the entire sampling period that were observed in an individual sample, i is the i^{th} species, j is an individual sample, and r is the array of samples. We also recorded the blooming period for coneflower in each small population. Plant nomenclature followed the PLANTS Database (USDA, NRCS 2006).

Pollen Fertility

Pollen fertility was estimated during 2004 as pollen stainability in cotton blue in lactophenol (Kearns and Inuoye 1993). Numbers of heads sampled were eight for Picture Creek, three for Snow Hill Rd., five for Knap of Reeds, three for Briardale Rd., three for Lakeside, and three for Freudenberg. Anthers were macerated in a drop of stain on a microscope slide, pollen viewed at 100X, and fertile (dark blue) and non-fertile (light blue) grains counted.

Compatibility

Three head bagging treatments were used during June, 2004 in Picture Creek to evaluate coneflower reproductive compatibility and the ability to auto self-pollinate. The flowering head treatments were bagged; bagged and artificially self-pollinated; and unmanipulated. The unmanipulated heads were the same heads designated as the natural, open-pollinated treatment described below for testing pollinator limitation. Twenty-three heads were enclosed in nylon bags prior to disk flower opening. Disk flowers of ten bagged heads were artificially self-pollinated and rebagged daily for the flowering duration of the head. Ten other bagged heads were not artificially pollinated. After artificial self-pollination was finished, ten previously unbagged heads were bagged to prevent the loss of mature seeds. Contents of the 30 bagged heads were harvested in September and the numbers of plump and non-plump seeds in each head were determined. Additionally, stigmas of three one-day-old disk flowers were removed from each of the three remaining bagged heads and from three unmanipulated heads, individually placed in vials, immediately taken to the lab, examined at 50 X and coneflower pollen grains counted in a 0.3 mm wide belt transect across the middle of one stigma receptive surface.

Pollinator Effectiveness

During 2004 and 2005, we determined the relative pollinator effectiveness of coneflower flower visitors at Picture Creek based on a linear combination of four criteria: (1) insect visitation rates to flowering heads, (2) locations of pollen loads on insect bodies, (3) numbers of coneflower pollen grains in samples of insect pollen loads, and (4) percentage coneflower composition of individual insect pollen loads. Quantitative evaluations for each of the four criteria were ranked across insect visitor groups. Then the four criteria ranks for each group were summed. The relative pollinator effectiveness of each insect group was indicated by the rank order of its sum.

Visits by individual insect taxa to flowering heads in eight randomly distributed 2 x 2 m plots were counted during 41 15-minute intervals in 2004 and 36 intervals during 2005. All intervals occurred on sunny days, primarily between the hours of 0900 and 1300. 19 of these intervals were recorded between the hours of 1300 and 1600. Additionally, nocturnal flower head visitors at Picture Creek were identified during three observation hours in 2005.

Flower visitors were collected from coneflower heads and taken to the laboratory in small vials. Locations of pollen on the bodies of at least three individuals of each taxon were determined (Table 2). Pollen located on proboscides, legs, or the underside of the head, thorax or abdomen was considered more likely to be transferred to stigmas than pollen located elsewhere. A number of 0-4 was assigned to each insect based on the location of pollen grains on the insect body. Insects with all of its visible pollen located on the proboscis, legs, underside of the head, thorax, or abdomen or any combination of these parts received a rank of 4. Insects with most of its visible pollen located on the above mentioned parts but also some on the dorsal surface, wings, or in pollen sacs received a rank of 3.

Insects with little visible pollen in parts likely to transfer pollen, but mostly in unlikely places received a rank of 2. Insects with very few visible pollen grains anywhere on the body received a rank of 1. Insects with no visible pollen grains received a rank of 0. Three ml of distilled water and one drop of detergent were added to each vial containing an insect and shaken vigorously until all pollen appeared to be removed from the insect and suspended in solution. Pollen sacs on the legs of Hymenopterans were removed prior to adding the solution because pollen in them was unavailable to stigmas. 0.05 ml of the solution was placed on a microscope slide, observed at 100X, and the coneflower and non-coneflower pollen grains were counted.

Pollinator Limitation

Pollinator limitation would be indicated by a combination of reduced pollinator visits and/or pollinator species richness and fewer seeds produced by open-pollinated heads than by heads receiving open-pollination augmented with artificial cross-pollination. To determine if pollinator limitation was affecting small coneflower populations but not that at Picture Creek, we monitored flowering head visitation rates and seed production in all of the populations. At Picture Creek, insect visits to flowering heads and insect taxa visiting heads were counted in the eight randomly distributed 2 x 2 m plots during each of 41 15-min observation periods in 2004 and 36 periods in 2005. Multiple plots were not used in small populations because they were composed of so few plants. Instead, in these small populations, visits to flowering heads that could be seen from one vantage point were observed. Five observation periods were sampled at Snow Hill Rd., seven and Knap of Reeds, twelve at Briardale Rd., six at Lakeside Dr., and six at Freudenberg. A mixed model ANOVA, with site fixed, and days in site as random, along with the least significant difference procedure (lsd) were used on both square-root transformed visitation data and untransformed species richness data to test the null hypotheses of no differences among populations within each year (SAS Institute 2000).

During early June in 2004 and 2005, pollination studies were conducted to determine percentages of viable seeds produced by two pollination treatments, natural open-pollination and open-pollination augmented with artificial cross-pollination. During both years at Picture Creek, 10 randomly selected heads were artificially cross-pollinated daily for the flowering duration of each head and left unbagged to receive natural open-pollination (Table 4). Fewer than 10 flowering heads were artificially cross-pollinated in each of the small populations because they included limited numbers of flowering heads (Table 4). The remaining flowering heads in each population were unmanipulated and available for natural pollination. In each population, all open-pollinated plus artificially cross-pollinated heads and at least six open-pollinated heads were bagged in mid-July to retain seeds. Some artificially pollinated heads did not survive due to roadside mowing and insect predation (Table 4). Two cross-pollinated heads at Picture Creek in 2005 were lost to the study because they began to drop mature seeds before heads were bagged prior to seed harvest. In early September, plump and shriveled seeds were counted in each surviving head. A preliminary test showed that tissue of all 65 plump seeds in a sample became pink when exposed to a 0.1% solution of 2,3,5 triphenyltetrazolium chloride in water, indicating viability (Kearns & Inouye 1993), and that no shriveled seeds became pink. We ran a two-way ANOVA testing site, treatment, and interaction effects on seed production. The null hypothesis of no difference between pollination treatments was tested within individual populations using individual contrasts. Also, a one-way ANOVA was used to test the null hypothesis of no seed production differences in open-pollinated heads among populations.

Pollinator Visitation Experiments

During mid-June, 2005 at Picture Creek, we conducted a controlled experiment to estimate the number of bee (*Bombus*, *Psithyrus*, *Xylocopa*, or *Megachile*) visits to individual heads required to produce as many seeds as produced by each open-pollinated head. We bagged 60 heads before their disk flowers opened. Twelve heads were allocated to a control (0 visits) and each of four treatments: ½ visit per day (1 visit every 2 days), and one, three, and five visits per day. An additional 10 heads that remained unbagged throughout the study was a natural open-pollination treatment. These 10 unbagged heads were the same open-pollinated heads that were used in evaluating pollination limitation. The heads of the control and the ½ – 5 visit treatments were all located within a 25 m^2 area. The ten heads of the open-pollination treatment were located within 20 meters of the other treatments. Each day for twelve consecutive days, each head of the one, three, and five visits per day treatments was unbagged until it was visited by a bee the designated number of times. It was then re-bagged until the next day. Each head of the ½ visit per day treatment was unbagged, visited once by a bee, and re-bagged every other day. Lepidopterans were not allowed to visit heads. All experimental heads were bagged after all pollination treatments had been completed. In early September, the numbers of plump and shriveled seeds in each head were counted. A one-way ANOVA was used to evaluate the null hypothesis of no seed production differences among treatments followed by the lsd procedure for means separation.

Seed Germination and Seedling Fitness

Plump seeds produced by open-pollination at Picture Creek and Snow Hill Road during 2004 were moist-stratified (4°C) for 50 days and planted in ten 4 x 2 meter blocks at Picture Creek during early March, 2005. Prior to planting, vegetation was removed from the blocks by applying herbicide and by clipping. Twenty seeds from each of the two populations were planted in two separate 0.5 x 0.5 meter plots within each block. An unplanted control plot was included in each block. Numbers of seed germinations and seedling deaths in each plot were recorded weekly from March to July. In late August, 2005, the total number of leaves, an indicator of fitness, was determined for each surviving seedling.

We applied a randomized block ANOVA to arc sine transformed data to test the null hypotheses of no differences for seed germination and seedling survivorship between the two populations. A mixed model ANOVA with fixed treatment and random block and block by treatment effects was applied to arc sine transformed measurements for individual plants to test the null hypotheses of no differences for seedling leaf numbers between populations.

On March 5, 2005, 10 plump seeds produced by open-pollination at Picture Creek were planted in each of 20 greenhouse pots. On the same date, ten seeds produced by open-pollination at Snow Hill Road were also planted in each of 20 greenhouse pots. Pots were lightly watered to maintain a moist surface. Temperatures in the greenhouse ranged from 75°F to 85°F. Numbers of seed germinations and seedling deaths were recorded for each pot weekly until no more germination occurred. Then, all seedlings but the largest in each pot were removed. In late August, 2005, the dry weight of the seedling in each pot was determined.

A mixed model ANOVA with site fixed and plant within site random was applied to arc sine transformed percentage data for individual pots to test the null hypotheses of no differences for seed germination and seedling survivorship between populations. The same analysis was used with non-transformed dry weight data to test the null hypothesis of no differences between populations.

RESULTS

Flowering phenology

During late May to early July, 2004, at Picture Creek, smooth coneflower composed 0.4 - 0.7 of the blooming plant community (Figure 1). The blooming periods of *Blephila ciliata* (L.) Benth., *Erigeron strigosus* Muhl., *Houstonia longifolia* Gaertn., *Penstemon australis* Small, and *Rosa carolina* L. overlapped that of coneflower. Coneflower flowering time niche breadth, 2.5, was near the middle of the range of niche breadths for the other species, 1.1 – 4.5. The average coneflower flowering time niche overlap with the other five species, 0.6, also was near the middle of the average overlaps for the other species, 0.2 – 1.0.

In 2004, coneflower flowered at Snow Hill Road and Knap of Reeds from June 4 to 26 and June 4 to 28, respectively. In 2005, flowering occurred at Briardale Road, Lakeside Drive, and Freudenberg from June 3 to 23, June 9 to 30, and June 6 to 27, respectively. Few other species shared this blooming period with coneflower at these sites.

Pollen Fertility

Percents of normally staining pollen in sampled heads ranged from 93% to 96% for Picture Creek, 98% to 99% for Snow Hill Road, 94% to 97% for Knap of Reeds, 98% to 100% for Briardale Rd., 97% to 98% for Lakeside Dr., and 99% to 100% for Freudenberg.

Compatibility

No viable seeds were produced by any head of either bagged treatment. Plump seed production by open-pollinated heads from Picture Creek ranged from 17 to 68%. Numbers of pollen grains in stigma receptive surface samples from bagged and open-pollinated heads ranged from 0 to 26 and 18 to 42, respectively.

Pollinator Effectiveness

Pollinator effectiveness was evaluated for five groups of Hymenopterans, *Apis mellifera* L., Halictidae, *Bombus* spp. + *Psithyrus citrinus* Smith, *Xylocopa virginica* L. and *Megachile* spp., two groups of Lepidopterans, Hesperiidae (skippers) and Nymphalidae (butterflies), and a Hemipteran, *Lygaeus kalmii* Stal (Table 2). *Psithyrus citrinus* was included in the *Bombus* spp. group because it is so similar to *Bombus* that it might have been mis-identified as such during field observations. Generally, the native bees were more effective than *Apis mellifera* and the Lepidopterans according to each evaluation criterion. An exception was that skippers visited flowering heads more frequently than all groups except *Bombus*. Based on a linear combination of all four criteria, the five most effective pollinator groups in order of their effectiveness were *Bombus* spp., *Xylocopa virginica*., *Megachile* spp., *Lygaeus kalmii*, and *Apis mellifera*. Additional species that were observed, but at such low frequencies that their effectiveness was not evaluated, included two Coleopterans, *Brachyloptura vegans* Olivier and *Typocerus zebra* Olivier (Cerambycidae), two Lepidopterans, *Eusarca confusaria* Hubner (Geometridae) and *Harrisina* sp. (Zygaenidae), three Dipterans, *Eristalis transverses* Wiedemann, *Milesia virgineinsis* Drury, and *Toxomerus* sp. (Syrphidae), and one Hymenopteran, *Ammophila* sp. probably *nigricans* Dahlbom (Sphecidae).

Pollinator Limitation

Insect flower visitation rates varied among populations ($P < 0.0001$; Table 3). In 2004, the large Picture Creek population had a higher rate than the two small populations.

However, during 2005, the small Freudenberg population had the highest visitation rate, but the rate in Picture Creek exceeded those in the two remaining small populations. Picture Creek had the highest pollinator species richness during both 2004 and 2005 ($P < 0.0001$ during both years; Table 3). Except for the high number of flower visits in Freudenberg during 2005, these results are consistent with expectations given pollinator limitation.

Open-pollination augmented with artificial cross-pollination did not produce more seeds than open-pollination in any population during either year (Table 4). These results are not consistent with expectations of pollinator limitation. However, during 2005 in Picture Creek, natural open-pollination produced more seeds, 56%, than did open-pollination augmented with artificial cross-pollination, 35 % (Table 4). During 2004, there were no seed production differences from open-pollination between Picture Creek and the small populations; however, during 2005, open-pollination produced more seeds in Picture Creek than in the small populations (Table 4).

Pollinator Visitation Experiments

No plump seeds were produced by the unvisited control heads. None of the treatments that received a specified number of bee visits produced as many seeds as did open-pollination, 56%; however, three visits per day produced nearly 2/3 as many seeds as did open-pollination ($P < 0.0001$; Table 5).

Seed Germination and Seedling Fitness

There were no differences in the field or greenhouse for seed germination or seedling survivorship between Snow Hill Road and Picture Creek, $P = 0.06$ and $P = 0.70$ for field germination and survivorship and $P = 0.51$ and $P = 0.34$ for greenhouse germination and survivorship (Table 6). On average, each field-raised Picture Creek seedling had one more leaf than did each Snow Hill Road seedling ($P = 0.018$). In the greenhouse, on average, each Picture Creek seedling weighed approximately 35% more than did each Snow Hill Road seedling ($P < 0.0001$).

DISCUSSION

The size of the coneflower population at Picture Creek suggests that it is vigorous and that its reproductive biology can be a reference against which that in other populations can be compared.

Although *Echinacea laevigata* auto self-pollinates to a limited extent, it is self-incompatible and is dependent on insect pollinators for cross-pollination, as are other *Echinacea* species (McGregor 1968, Leuszler *et al*. 1996). Consequently, coneflower conservation must consider pollinator relationships.

Coneflower flowering time was not separated noticeably from that of other species blooming during late-May to mid-July at Picture Creek. However, the large coneflower heads on tall stems were very conspicuous and were visited frequently by large Hymenopterans and skippers. Some common species that bloomed before and after coneflower, including *Baptisia australis* (L.) R. Br. ex Ait. f., *Liatris squarrosa* (L.) Michx., and *L. squarrulosa* Michx. produced showy flowers or inflorescences that attracted *Bombus, Xylocopa,* and *Megachile.* However, of the species that flowered with coneflower, only *Rosa carolina* and *Penstemon australis* attracted large bees, the most effective coneflower pollinators. Both of these plant species ceased flowering just as coneflower reached its blooming peak. No other species that regularly attracted bees were sympatric with the small coneflower populations.

The most effective coneflower pollinators were bees, so coneflower management plans should ensure suitable bee nest sites (Sipes and Tepedino 1995). Although skippers and butterflies were not relatively effective coneflower pollinators, they were frequent nectar foragers. Coneflower conservation will promote a large community of these foragers.

In the current study, in all but one instance, flower visitation and visitor species richness were lower in small populations than in Picture Creek. In 2005, the Freudenberg population had much higher visitation rates than Picture Creek; however the species richness for this site was very low. Essentially all of the pollinator visits at Freudenberg were by *Psithyrus citrinus.* Given the high visitor frequency of this one species, we speculate that the *Psithyrus* nest was very close to the coneflower population. Nevertheless, in the small coneflower populations, open-pollination did not produce fewer seeds than did open-pollination augmented with artificial cross- pollination. Therefore, pollinator limitation did not reduce seed production in these populations. Seed production from open-pollinated heads was significantly higher than that from open-pollinated heads augmented with cross pollinations in Picture Creek in 2005. Although there is no obvious explanation for this unexpected result, it does not suggest the occurrence of pollinator limitation at Picture Creek. Studies of *Verticordia fembrilepis* (Turcz) (Yates and Ladd 2005) and some species in renosterveld in South Africa (Donaldson *et al*. 2002) also reported a lack of pollinator limitation. The plants in these studies were visited by generalist pollinators, as was coneflower (Donaldson *et al*. 2002, Yates and Ladd 2005).

Results of our pollinator visitation experiment provided an explanation for our finding no pollinator limitation in small populations even though insect visits in these populations were few. In this experiment, three bee visits per day produced 35% viable seeds. Since the duration of pollinator activity observed in all populations was at least 7 hr / day, the seemingly low insect visitation rates in Snow Hill Road and Knap of Reeds, 0.3 and 0.5 times / 15 min, respectively, corresponded to 9 and 14 visits / day. Clearly, insects visited heads in these small populations three and five times as often as required for 35% seed production. Consequently, it is not surprising that seed production of open-pollinated heads in these small populations was approximately 50% and was not pollinator limited. Apparently, insect visits would have to be as few as 0.1 – 0.2 / 15 min. (approximately 3 – 5 / 7 hr pollinator day) for pollinator limitation to reduce seed production. After a coneflower head is visited a few times by bees, subsequent visits that day benefit the visitors without increasing seed production. Similarly, Silander and Primack (1978) reported that just a single bee visit to an *Oenothera fruiticosa* flower deposited enough pollen on the stigma to fertilize 70% of the ovules and that additional pollinator visits beyond three did not increase seed production.

The absence of pollinator limitation in small *Echinacea laevigata* populations does not correspond with reports of pollen limitation in small populations of *Echinacea angustifolia* DC. in the North American tall grass prairies (Wagenius 2006). It was speculated that the observed pollen limitation, as indicated by stigmas which did not wither, was caused by reduced pollinator abundance in fragmented habitats or by mate scarcity. Apparently, the prairie populations of *E. angustifolia* were more effectively isolated and/or smaller than were the *E. laevigata* populations in our current study. Another possible explanation of these different results could be that pollinator populations are more common in the eastern U.S. than in the Midwestern prairies.

During 2005, open-pollination produced more seeds per head in Picture Creek than in the small populations of that year. Our previous results indicated that the mechanism(s) limiting seed production in these small populations was not pollinator limitation. However, we noted a mechanism in the small populations that could explain reduced seed production in their open-pollinated heads. During several successive typically hot and sunny days, all disk flower anthers in these populations failed to dehisce. These unopened anthers contained very few pollen grains. This phenomenon, which precluded pollination for several days, was preceded and followed by periods of normal anther dehiscence and normal amounts of pollen. This mechanism was not observed in Picture Creek or in the small populations during 2004. Reduced coneflower pollen production could have been a symptom of inbreeding depression, as has been shown in *Mimulus guttatus* (Carr and Dudash 1995, 1997).

Seed production in small populations was reduced not only by abnormal anther development, but also by mechanisms that reduced the probability of heads surviving to maturity. In early June of both 2004 and 2005, we observed a long-horned beetle, *Hemierana marginata* Fabricus, chewing into and laying eggs in flowering stalks. On each damaged stalk, the tissue, including the flower head, died above the point of beetle damage. No heads died on undamaged flowering stalks. Beetle damage caused the death of 18 heads at Briardale Road and four at Lakeside Drive during 2005 and several heads at Snow Hill Road during 2004. Head survival in small roadside populations was also vulnerable to human-mediated actions. For example, ten heads involved in an experimental pollination treatment at Lakeside Drive were mowed down before seeds developed. Reduced seed production in small populations may not be an immediate threat to population persistence;

however, it does represent a reduced supply of propagules that could otherwise aid population expansion or the founding of new populations. It also represents a loss of novel progeny genotypes.

Road widening and roadside utility maintenance can eliminate entire roadside populations by destroying plants. Given these immediate threats and the consequences of limits to seed production, small roadside populations teeter on the brink of extirpation. Conservation of small coneflower populations need not be concerned with pollinator limitation of seed production; instead, it should be concerned with reducing long-horned beetle damage and human-mediated threats to head survival.

During 2004, coneflower seedlings from the large Picture Creek population were larger, possibly more fit, than those from the small Snow Hill Road population. Similarly, Wagenius (2000) reported that *E. angustifolia* seedlings from large populations were more vigorous than those from small populations and suggested that this reduced fitness may have been caused by inbreeding depression. Conservationists should consider raising seedlings from small populations and then transplanting them into neighbor populations, as has been suggested for the federally endangered *Lysimachia asperulifolia* (rough-leaf loosestrife) (Franklin *et al*. 2006). This method might increase genetic variation within individual populations and reduce inbreeding.

Although seeds were produced in all of the studied coneflower populations, we did not notice naturally occurring seedlings in any population. Others have commented on the lack of seedlings or their infrequent occurrence (Philippi *et al.* 2001, Franklin, pers. comm.). Even though our study proved that coneflower seeds can germinate and seedlings can survive in the field, appropriate conditions for seed germination and/or seedling survival, apparently, do not frequently occur in piedmont habitats. This recruitment failure means that novel genotypes are often wasted as dispersed seeds lose their viability. Since recruitment of novel genotypes contributes to the ability of a population to adapt to changing environments, it is important that future coneflower research address seedling recruitment requirements.

We offer the following speculation to stimulate future work. We suggest that coneflower seedling recruitment occurs infrequently, only when viable seeds are dispersed into recently disturbed sites with bare soil. This may have occurred when the powerline right-of-way at Picture Creek was installed through a dry oak forest that included glade openings supporting small coneflower populations. Given the increased light resulting from the construction, glade plants near the right-of-way may have produced seeds that were dispersed into the newly opened right-of-way and seedlings may have established on bare soil to initiate the large population that currently occurs at Picture Creek. Seedling recruitment may have continued for several growing seasons, until the bare soil became vegetated. If seedling recruitment is, in fact, tied to soil disturbance, land managers might be able to promote recruitment by creating local soil disturbances in flowering populations.

REFERENCES CITED

Agren J. 1996. Population size, pollinator limitation, and seed set in the self incompatible herb *Lythrum salicaria*. Ecology 77(6):1779-1790.

Aizen, M. A. and P. Feinsinger. 1994. Habitat Fragmentation, native insect pollinators and feral honeybees in Argentine Chaco Serrano. Ecological Applications 4(2): 378-392.

Alley, H. and J. Affolter. 2004. Experimental Comparison of Reintroduction Methods for the Endangered *Echinacea laevigata* (Boyton and Beadle) Blake. Natural Areas Journal 24 (4): 345-350.

Alley, H., Reiger, M. and J. Affolter. 2005. Effects of Development Light Level on Photosynthesis and Biomass Production in *Echinacea laevigata*, a Federally Listed Endangered Species. Natural Areas Journal 25 (2):117-122.

Apsit, V.J., & P. M. Dixon. 2001. Genetic Diversity and Population Structure in *Echinacea laevigata* (Boyton and Beadle) Blake, an Endangered Plant Species. Natural Areas Journal 21: 71-77.

Barden, L. 1997. Historic Prairies in the Piedmont of North and South Carolina, USA. Natural Areas Journal 17 (2): 149-152.

Becker, P., J.S. Moure, & F.J.A. Peralta. 1991. More about euglossine bees in Amazonian forest fragments. Biotropica 23: 586-591.

Bierzychudek, P. 1981. Pollinator Limitation of Plant Reproductive effort. American Naturalist 117 (5): 838-840.

Bosch, M., N.M. Waser. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum*. American Journal of Botany 86:871-879.

Boyle, O.D. and E.S. Menges. 2001. Pollinator visitation to *Hypericum cumulicola* (Hypericaceae), a rare Florida scrub endemic. Fla. Sci. 64: 107-117.

Bruna, E. 2002. Effects of forest fragmentation on *Heliconia acuminate* seedling recruitment in central Amazonia. Oecologia 132:235-243.

Brys, R. H. Jacquemyn, P. Endels, F. Van Rossum, M. Hermy, L. Triest, L. DeBruyn, and G. Blust. 2004. Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*. Journal of Ecology 92, 5-14.

Buchmann, S. L., and G. P. Nabhlan. 1996. The Forgotten Pollinators. Island Press, Washington D.C., USA. p. 103-116.

Cane, JH. 2001. Habitat fragmentation and native bees: a premature verdict? Conservation Ecology 5 (1):3. [online] URL: http://www.consecol.org/vol5/iss1/art3.

Carr, D.E. and M.R. Dudash. 1995. Inbreeding depression under a competitive regime in *Mimulus-guttatus*—consequences for potential male and female function. Heredity 75: 437-445.

Carr, D.E. and M.R. Dudash. 1997. The effects of five generations of enforced selfing on potential male and female function in *Mimulus guttatus*. Evolution 51 (6): 1797-1807.

Cummings, C. L and H.M Alexander. 2002. Population ecology of wild sunflowers: effects of seed density and post-dispersal vertebrate seed predation on numbers of plants per patch and seed production. Oecologia (Berl.) 130: 272-280.

Cunningham, S.A. 2000. Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. Conservation Biology 14:758-768.

Davis, J. E., C. McRae, B.L. Estep, L.S. Barden, J.F. Matthews 2002. Vascular Flora of Piedmont Prairies: Evidence from Several Prairie Remnants. Castanea 67:1-12.

Donaldson, J., I. Nanni, C. Zachariades, and J. Kemper. 2002. Effects of Habitat Fragmentation on Pollinator Diversity and Plant Reproductive Success in Renosterveld Shrublands of South Africa. Conservation Biology 16 (5): 1267-1276

Emanuel, C.M. 1996. Silvicultural options for recovering the endangered smooth coneflower, *Echinacea laevigata* (Boynton and Beadle) Blake. Masters Thesis. Clemson University.

Evans, R.E., M. Pyne, & S. Hiltner. 2002. Remnant Diabase Vegetation in the North-Central North Carolina Piedmont. Presented by NatureServe for the 3rd Eastern Native Grass Symposium, Chapel Hill, NC.

Fischer, M. and D. Matthies. 1998. Effects of population size on performance in the rare plant *Gentianella germanica*. Journal of Ecology 86, 195-204.

Franklin, M. A., J. M. Stucky, T. R. Wentworth, C. Brownie, and T. Roulston. 2006. Limitations to fruit and seed production by *Lysimachia asperulifolia* Poir. (Primulaceae), a rare plant species of the Carolinas. In Press. Journal of the Torry Botanical Society.

Gaddy, L.L. 1991. The status of *Echinacea laevigata* (Boynton & Beadle) Blake. Unpublished report to the U.S. Fish and Wildlife Service. Asheville, NC 24 pp. + appendices and maps.

Goverde, M., K. Schweizer, B. Baur, and A. Erhardt. 2002. Small-scale habitat fragmentation effects on pollinator behaviour experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. Biological Conservation 104: 293-299.

Hooftman, D.A.P., M. van Kleunen, M. Deimer. 2003. Effects of habitat fragmentation on the fitness of two common wetland species, *Carex davalliana* and *Succisa pratensis*. Oecologia 134:350-359.

Jennerston, O. 1988. Pollination in *Dianthus deltoids* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2 (4): 359-366.

Juras, P. 1997. The presettlement Piedmont savanna: A model for landscape design and management. Masters thesis. University of Georgia. http://philipjuras.com/thesis/. accessed February 4, 2006.

Kearns, C.A. and D.W. Inuoye. 1993. Techniques for Pollination Biologists. Niwot, Colorado: University Press of Colorado.

Kearns, C.A., D.W. Inouye, and N.M. Waser. 1998. Endangered Mutualisms: The conservation of plant-pollinator interactions. Annu. Rev. Ecological Systems. 29: 83-112.

Kolb, A. 2005. Reduced reproductive success and offspring survival in fragmented populations of the forest herb *Phyteuma spicatum*. Journal of Ecology 93:1226-1237.

Kwak, MM., O. Velterop, J. van Andel. 1998. Pollen and gene flow in fragmented habitats. Applied Vegetation Science 1:37-54.

Lamont, B. B., P.G.L. Klinkhamer, E.T.F. Witkowski. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodie*- a demonstration of the Allee effect. Oecologia 94: 446-450.

Levins, R. 1968. Evolution in Changing Environments: Some theoretical explorations. Princeton Univ. Press, Princeton, NJ

Leuszler, H. K., V.J. Tepedino, and D.G. Alston. 1996. Reproductive Biology of Purple Coneflower in Southwestern North Dakota. The Prairie Naturalist 28(2).

McGregor, R.L. 1968. The taxonomy of the genus *Echinacea* (Compositae). University of Kansas Science Bulletin 48: 113-142.

Mendez, M. D. Rafael, I. Olmsted, and K. Oyama. 2004. Population dynamics of *Pterocereus gaumeria*, a rare and endemic columnar cactus of Mexico. Biotropica 36: 492-504.

Menges, E. S. 1991. Seed Germination Percentage Increases with Population Size in a Fragmented Prairie Species. Conservation Biology 5 (2):158-164.

Menges, E.S. 1995. Factors limiting fecundity and germination in small populations of *Silen regia* (Caryophyllaceae), a rare hummingbird prairie forb. American Midland Naturalist 133: 242-55.

Moody-Weis, J.M. and J.S. Heywood. 2001. Pollination Limitation to Reproductive Success in the Missouri Evening Primrose, *Oenothera macrocarpa* (Onagraceae). American Journal of Botany 88 (9):1615-1622.

Murdock, N.A. 1992. Endangered and threatened wildlife and plants: *Echinacea laevigata* (Smooth Coneflower) determined to be endangered. Federal Register 57:46340-46344

Murdock, N.A. 1995. Recovery Plan for Smooth Coneflower (*Echinacea laevigata*). Prepared for the U.S. Fish and Wildlife Service.

Nielsen, R. 2006. Why sex? Science 311: 960-961.

Olesen, J.M. and S.K. Jain 1994. Fragmented plant populations and their lost interactions. Conservation Genetics (eds. V. Loeschke, J. Tomiuk, & S.K. Jain), pp. 417-426.

Paland, S. and M. Lynch. 2006. Transition to asexuality result in excess animo acid substitutions. Science 311: 990-992.

Pavlik, B.M., N. Ferguson, M. Nelson. 1993. Assessing limitations on the growth of endangered plant populations: 2. Seed production and seed bank dynamics of *Erysimum capitatum ssp. angustatum* and *Oenthera deltoids ssp. howellii*. Biological Conservation 65: 267-78.

Peters, M. 2005. Genetic analysis of the federally endangered *Echinacea laevigata* using amplified fragment length polymorphisms (AFLP)—Inferences in population genetic structure and mating system. Masters thesis. North Carolina State University.

Philippi, T. B. Collins, S. Guisti, and P.M. Dixon. 2001. A multistage approach to population monitoring for rare plant populations. Natural Areas Journal 21: 111-116.

Rathcke, B.J. and E.S. Jules. 1993. Habitat fragmentation and plant-pollinator interactions. Current Science 65 (3).

SAS INSTITUTE INC. 2000. SAS User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Schafale, M. P. and A. Weakley. 1990. Classification of the Natural Communities of North Carolina. 3rd Approximation. North Carolina Natural Heritage Program. Divisionof Parks and Recreation Department of Environment, Health and Natural Resources.

Sih, A. and M. Baltus. 1987. Patch size, Pollinator behavior, and Pollinator Limitation in Catnip. Ecology 68 (6): 1679-1690.

Silander, J.A. and R.B. Primack. 1978. Pollination Intensity and seed set in Evening Primrose (*Oenothera fruticosa*). American Midland Naturalist 100 (1): 213-216.

Sipes, S.D., and V.J. Tepedino. 1995. Reproductive Biology of the Rare Orchid, *Spiranthes Diluvialis*: Breeding System, Pollination, and Implications for Conservation. Conservation Biology 9 (4): 929-938.

Slapcinsky, J. 1994. The Vegetation and soils associated with Diabase in Granville and Durham Counties, North Carolina. Masters thesis. North Carolina State University.

Smith, C.W. 1986. The occurrence, distribution, and properties of dispersive soil and saprolite formed over diabase and contact metamorphic rock in a Piedmont landscape in North Carolina. Masters Thesis. North Carolina State University.

Spira, T.P. 2001. Plant-pollinator interactions: a threatened mutualism with implications for the ecology and management of rare plants. Natural Areas Journal 21:78-88.

USDA, NRCS. 2006. The PLANTS Database, 6 March 2006 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Wagenius, S. 2000. Performance of a prairie mating system in fragmented habitat: self incompatiblilty and limited pollen dispersal in *Echinacea angustifolia*. PhD Dissertation. University of Minnesota.

Wagenius, S. 2004. Style persistence, pollen limitation, and seed set in the common prairie plant *Echinacea angustifolia* (Asteraceae). Journal of Plant Science 165 (4): 595-603.

Wagenius, S. 2006. Scale dependence of reproductive failure in fragmented *Echinacea* populations. Ecology-In press.

Walck, J.L., T.E. Hemmerly, & S.N. Hidayti 2002. The Endangered Tennessee Purple Coneflower, *Echinacea tennesseensis* (Asteraceae): Its Ecology and Conservation. Native Plants Journal 3(1).

Yates, CJ. And PG. Ladd. 2005. Relative Importance of Reproduction Biology and Establishment Ecology for Persistence of a Rare Shrub in a Fragmented Landscape. Conservation Biology 19 (1): 239-249.

Table 1: Populations of Smooth Coneflower used for this study.

Population	Year included in study	Location	No. flowering rosettes	Habitat
Picture Creek Diabase Barrens	2004, 2005	36.06965°N 78.73682°W	50,000	60 meter wide powerline right-of-way, bordered by pine-oak-hickory forest
Snow Hill Road	2004	36.07539°N 78.86295°W	23	Roadside, adjacent to an oak forest
Knap of Reeds Creek	2004	36.13067°N 78.79366°W	31	Narrow, abandoned, and overgrown powerline right-of-way, adjacent to Knap of Reeds Creek
Briardale Road	2005	36.08833°N 78.88911°W	15	Roadside on the edge of an oak forest
Lakeside Drive	2005	36.09081°N 78.89060°W	17	Roadside on the edge of an oak forest
Freudenberg	2005	36.06965°N 78.86100°W	48	small forest clearing adjacent to an abandoned railroad easement

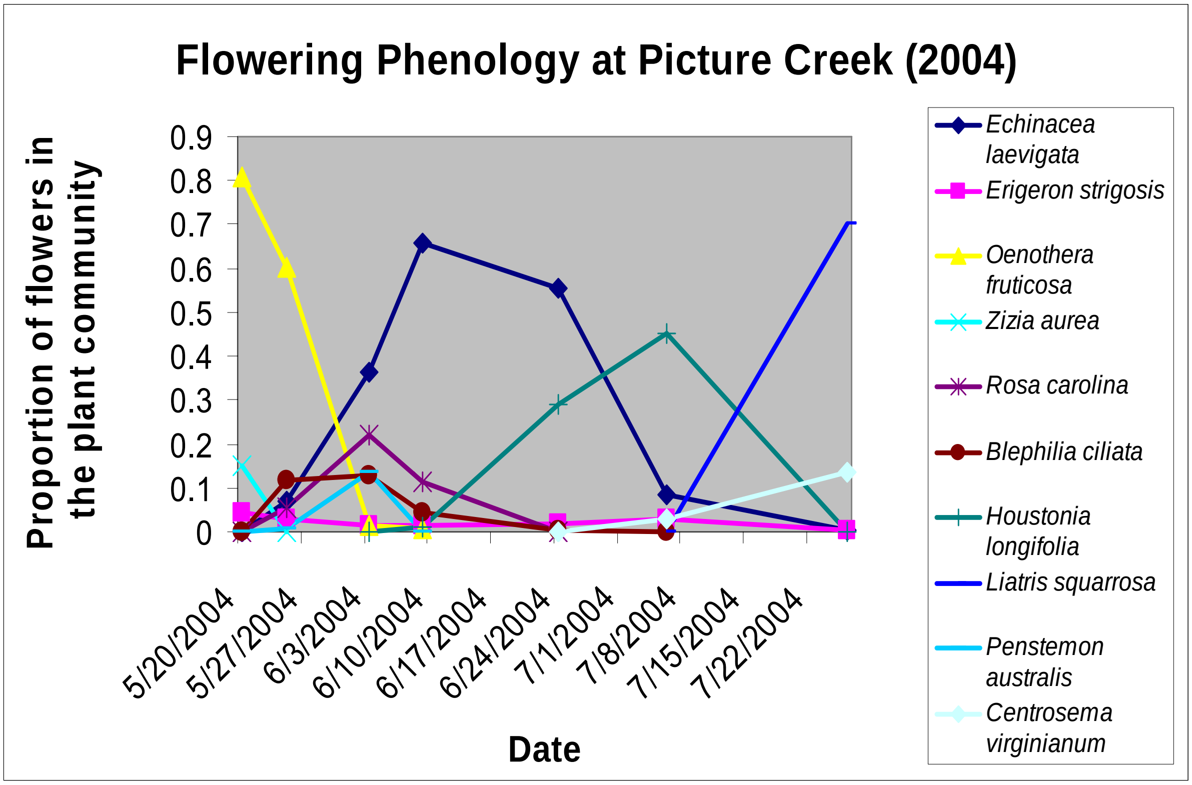

Figure 1: Flowering Phenology at Picture Creek

Table 2: The most effective pollinators of smooth coneflower.

Pollinator group	n[a]	Location of pollen grains on body		Avg. # coneflower pollen in pollen load sample		Avg. % coneflower pollen in pollen load sample		Average visits/head/15 minute n=77[b]		Sum of group ranks	Rank of group sum
			Rank		Rank		Rank		Rank		
Bombus spp.[c]	13	3.8	7	51	7	72	5	40	8	27	**8**
Xylocopa virginica (L.)	3	4	8	110	8	86	6	0.6	4	26	**7**
Megachile spp.[d]	13	3.4	5	36	5	72	5	5	6	21	**6**
Lygaeus kalmii Stal	3	3.5	6	14	4	92	7	0.5	3	20	**5**
Apis mellifera	1	1	3	2	1	95	8	0.7	5	17	**4**
Halictidae[e]	1	3	4	42	6	64	4	0.3	1	15	**3**
Hesperiidae[f]	5	0.6	2	3	2	50	2	6	7	13	**2**
Nymphalidae[g]	3	0.03	1	4	3	60	3	0.3	2	9	**1**

[a] Number of insects evaluated for the first three criteria
[b] Represents 77 total observation periods at Picture Creek
[c] Includes *B. bimaculatus* Cresson, *B. griseocollis* Degeer, *B. impatiens* Cresson, *B. pennsylvanicus* Degeer and *Psithyrus citrinus* Smith.
[d] Includes *M. brevis* Say, *M.mendica* Cresson, *M. sculpturalis* Smith, *M. texana* Cresson, and *M. xylocopoides* Smith.
[e] Includes *Augochlorella gratiosa* Smith and *Halictus ligatus* Say
[f] Includes *Atrytone logan* Edwards, *Atrytonopsis hianna* Scudder, *Epargyreus clarus* Cramer, *Polites* sp. probably *themistocles* Latreille and *Thorybes bathyllus* J.E. Smith
[g] Includes *Euptoieta claudia* Cramer, *Speyeria cybele* F., and *Vanessa virgineinsis* Drury

Table 3: Average head visitation rates and visitor species richness in coneflower populations.

Population	# 15 min. intervals (n)	Avg. no. visits/ head/ 15 min.	Avg. visitor species richness
2004			
Picture Creek	41	1.8 a [1]	4.1 a
Knap of Reeds	7	0.5 b	1.0 b
Snow Hill Road	5	0.3 b	0.7 b
2005			
Picture Creek	36	4.9 d	4.0 c
Briardale Road	12	1.5 e	2.3 d
Lakeside Drive	6	2.6 e	2.3 d
Freudenberg	6	9.2 c	1.8 d

[1]Lsd groupings were from analysis of square-root transformed data.
α-value for lsd <0.05

Means within a column within individual years that do not have a letter in common are significantly different using the lsd procedure.

Table 4: Average seed production from cross- and open- pollinated treatments within each population.

Year	Population	Pollination treatment	# heads initiated for each treatment	# heads for each treatment that survived	Average % viable seeds per head	P-value[1]
2004	Picture Creek	Open	24	24	42 a[2]	0.31
		Open + Cross	10	10	47	
	Snow Hill Rd.	Open	6	6	52 a	0.36
		Open + Cross	4	2	62	
	Knap of Reeds	Open	15	15	50 a	-
		Open + Cross	0	0	-	
2005	Picture Creek	Open	10	10	56 c	0.002
		Open + Cross	10	8	35	
	Briardale Rd.	Open	15	15	22 e	0.4
		Open + Cross	13	8	27	
	Lakeside Dr.	Open	8	8	38 d	0.37
		Open + Cross	6	4	27	
	Freudenberg	Open	10	10	42 d	0.29
		Open + Cross	6	6	49	

[1] P-values indicate the difference between seed production from open- and cross-pollination treatments within each population.
[2] Lower case letters indicate significant differences based on lsd groupings in seed production among open-pollinated treatments from each population within individual years

Table 5: Average percent viable seeds per head for different numbers of bee visits per day (Picture Creek 2005)[1]

Number visits per day	**Average % viable seeds per head**
0	0
0.5	14 c[2]
1	13 c
3	36 b
5	19 c
Open-pollinated	56 a

[1] n=12 flowering heads for each treatment, α=.05
[2] Lower case letters indicate significant differences based on lsd groupings in seed production among visitation treatments.

Table 6: Average seed germination, seedling survivorship, and seedling fitness in the field and greenhouse

	Population and treatment	**Seed Germination (%)**	**Seedling Survivorship (%)**	**Number of leaves per seedling**
Field	Picture Creek open-pollination	40.5 a[1]	87.9 a[1]	5.07 a[1]
	Snow Hill Road open-pollination	54.5 a	92.1 a	4.05 b
	Population and treatment	**Seed Germination (%)**	**Seedling Survivorship (%)**	**Seedling dry weight (g)**
Greenhouse	Picture Creek open-pollination	65.5 a[1]	97.4 a[1]	21.99 a
	Snow Hill Road open-pollination	55.5 a	98.8 a	14.35 b

[1] Lsd groupings from analysis of arc sine transformed data

Means within a column within separate experimental environments that do not have a letter in common are significantly different using the lsd procedure