POLLEN LIMITATION OF NATIVE SPECIES ON CHICAGO GREEN ROOFS

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BY KELLY KSIAZEK

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Abstract

With increasing urbanization and creation of novel habitat types, green roofs can provide usable habitat for many species. To date, most research on green roofs has focused on minimizing the environmental impacts of buildings but little is known about the ecological services they provide. Previous research has shown that although green roofs are visited by numerous bees, fewer species visit flowers on roofs than those on the ground. This lack of potential pollinators on green roofs could result in pollen limitation, poor seed production, reduced seed germination, and reproductive failure of many plant species. This study aims to determine whether pollination services on green roofs are sufficient for these novel ecosystems to function as sustainable habitats. Ten native Illinois prairie plant species and their pollinator communities were studied on green roofs and ground-level locations in the Chicago area. Pan traps were used to assess pollinator communities and supplemental pollination treatments were used to evaluate pollen limitation. Seed quality was assessed using seeds weights and a germination test of viability. All species showed significantly reduced seed set when pollinators were excluded but no significant difference between supplemental and open pollination treatments. Green roofs and ground locations differed significantly in the percent maximum seed set: surprisingly green roofs had a higher overall mean percent maximum seed set. Seed quality was either higher or not significantly different on green roofs compared to ground level sites for the majority of species. Our results support previous studies, showing lower numbers and diversity of bees on green roofs compared to the ground level. Together, these data suggest that although green roofs may contain a smaller and less diverse community of pollinators, the insects that are present provide sufficient pollinator services for many native plants.

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Chapter 1: Introduction

As human civilization has spread across the globe, cities have increased both in size and in number, with approximately 50% of the current global population living in cities (Erickson 2006) and an expected to increase to 6.4 billion by 2050, almost double the 3.3 billion people living in cities in 2007 (United Nations 2008). In the United States where urban areas have seen rapid growth, the population is currently over 311 million (United States Census Bureau 2011) and the land area occupied by cities is expected to double between 2000 and 2050 (Nowak and Walton 2005). As the patterns of increasing population and urban expansion continue, the pressures exerted on natural ecosystems are likely to increase as a result of habitat loss, increased pollution, introduction and spread of invasive species, fragmentation, and decreased persistence of historical disturbance regimes. These pressures, among others, can negatively affect plant communities and the wildlife that they support (Aguilar, Ashworth et al. 2006).

Because plants are sessile organisms and cannot relocate, they are particularly affected by dramatic changes in their environment. These changes can alter the species composition of an area and result in changes in species richness. Plant diversity can decrease as a result of smaller patch size and extinction of native species (Prober and Thiele 1995). On the other hand, species diversity can increase if fragmentation is accompanied by introduction of exotic species or horticultural varieties. Dominance of invasive species can lead to biodiversity loss and can easily occur in urban environments (Walker, Grimm et al. 2009), even in instances where urban fragments have been designed specifically to conserve and preserve native species (Drayton and Primack 1996).

In addition to the global loss of many native plant species (Pitman and Jorgensen 2002; Thomas, Telfer et al. 2004) and consequent homogenization of ecosystems (Olden and Poff 2003), there is clear evidence that diversity and richness of pollinators are also declining (Potts, Biesmeijer et al. 2010). Urban environments have been shown to be particularly susceptible to pollinator decline, as fragmentation and increased infrastructure affect pollinator movement, diversity, and density (McIntyre and Hostetler 2001; Tommasi, Miro et al. 2004; Winfree, Aguilar et al. 2009). Urban patches are less accessible to pollinators like bees and hummingbirds causing pollinators alter their foraging behavior to maximize their floral rewards (Goverde, Schweizer et al. 2002; Andrieu, Dornier et al. 2009; Hadley and Betts 2009). Smaller fragments in or near urban areas are often dominated by *Apis mellifera* (the introduced European honeybee) and are characterized by decreases in native (Aizen and Feinsinger 1994; Gonzalez-Varo, Arroyo et al. 2009), oligolectic, or pollen collecting specialists (Cane, Minckley et al. 2006; Steffan-Dewenter, Klein et al. 2006) and above-ground nesting bee species (Steffan-Dewenter, Klein et al. 2006). However, because they forage in edge habitats, of which there are more in habitat fragments, butterfly species benefit from the increased number of patches (Tscharntke et al. 2002, Ockinger et al. 2009, Bender et al. 1998), suggesting that some pollinators may be able to make use of the patchy matrix of urban green space.

The effects of urban fragmentation on mutualistic plant-pollinator relationships are often seen in the wide spread occurrence of pollen limitation, generally taking place when plant reproductive success is not attained due to a deficiency in either pollen quality or quantity (Aguilar, Ashworth et al. 2006; Aizen and Harder 2007). Many studies have found that pollen limitation is common in angiosperms (Bierzychudek 1981; Larson and Barrett 2000; Knight, Steets et al. 2005), particularly herbaceous species and those with self-incompatible breeding systems (Larson and Barrett 2000; Aguilar, Ashworth et al. 2006; Nayak and Davidar 2010). Globally, over half of all animal-pollinated species are expected to be pollen limited (Burd 1994) though there is significant variation in the amount of pollen limitation experienced in various angiosperm families (Larson and Barrett 2000).

If the trend of pollinator decline in highly disturbed urban environments continues (Winfree, Aguilar et al. 2009), pollen limitation could become increasingly common. However, the complete array of community-wide effects of pollen limitation and the long-term consequences are not completely understood as many biotic interactions may contribute (Ashman et al. 2004, Steffan-Dewenter et al. 2006). As the community is affected, complex cascades of extinction throughout the community could result and would be very difficult to accurately predict (Bronstein, Gouyon et al. 1990; Rathcke and Jules 1993; McIntyre and Hostetler 2001; Ashman, Knight et al. 2004).

In the wake of urban expansion and the resulting fragmentation of habitats, some urban environments are being designed within the framework of reconciliation ecology (Rosenzweig 2003). This approach modifies the human-dominated landscape to harbor a greater diversity of species, thereby expanding species ranges beyond natural areas or nature preserves (Rosenzweig 2001). The concept of reconciliation ecology is particularly applicable to green roofs, an increasingly common novel environment across Europe and North America (Francis and Lorimer 2011). The number of green roofs has increased dramatically since the turn of the century with the help of policies and technology standards employed by many cities (Carter and Fowler 2008). For example, while self-reported projects only represent a fraction of the green roofs throughout the world, the number of reported green roofs increased from 93 in 2000 to nearly 1,200 in 2011 (Greenroofs.com 2011). The advantages of these novel ecosystems are the subject of an increasing amount of research. Environmental benefits include reduction in stormwater runoff, decrease in the urban heat-island effect due to an increase in transpiration, pollution abatement, and as much as 58% reduction in energy use by buildings for heating and cooling (Getter and Rowe 2006; Oberndorfer, Lundholm et al. 2007; Carter and Butler 2008; Dunnett, Nagase et al. 2008; Spala, Bagiorgas et al. 2008; Rowe 2010).

Typically, the environmental benefits of green roofs are achieved by planting a mixture of hardy succulent species that are able to retain water and withstand the increased temperatures and wind experienced on green roofs (Monterusso, Rowe et al. 2005; Dunnett, Nagase et al. 2008; Lundholm, MacIvor et al. 2010). Species from the genus *Sedum* are commonly recommended for green roof plantings due to their drought tolerance and water-saving metabolism (Crassulacean acid metabolism or CAM) (Snodgrass and Snodgrass 2006; Dunnett and Kingsbury 2008). Although these succulents exhibit superior establishment and survivorship (Monterusso, Rowe et al. 2005; Carter and Butler 2008), native species have been shown to contribute positively to stormwater retention (Dunnett, Nagase et al. 2008) and overall ecosystem function (Lundholm, MacIvor et al. 2010). In addition to these environmental benefits, native species have the potential to support a greater diversity of organismal groups including native birds and pollinators.

Despite the progress that has been made to increase our understanding of how green roofs function, very little research has focused on the specific ecosystem processes that occur on green roofs and how they compare to natural areas. Ecosystem functions, such as the ability of green roofs to support communities of native pollinators, have yet to be investigated in detail. The few published studies that have focused on community functions on green roofs have found that incorporation of native flora or designing with specific conservation goals in mind increases the available habitat for plants as well as native pollinators, herbivores, and birds (Baumann 2006; Brenneisen 2006; Grant 2006; Kadas 2006; Colla, Willis et al. 2009; Tonietto 2009; Fernandez-

Canero and Gonzalez-Redondo 2010; Tonietto, Fant et al. 2011). Only a handful of published studies compare the abundance and diversity of pollinators on green roofs to nearby or similar habitats at the ground-level. Those that have found marginal to no significant differences between overall pollinator communities on green roofs versus those on the ground (Colla, Willis et al. 2009; MacIvor and Lundholm 2011(a)) although green roofs in the Chicago area have slightly decreased pollinator abundance and species richness (Tonietto, Fant et al. 2011). If the pollinator communities on green roofs have fewer individuals and are less diverse, this could lead to a reduction in pollinator visitation and increased pollen limitation, resulting in reduced seed set and reproductive success of green roof plants and therefore failure to meet basic ecosystem function requirements in the long-term. The relationship between pollinator abundance and plant reproductive success has not previously been investigated.

This study directly addresses the potential results of plant-pollinator interactions on green roofs in the Chicago area. We tested the following hypotheses: (1) plants on green roofs are pollen limited because they are not visited by an adequate number of effective pollinators, and (2) seed set is lower in green roof plants compared to the same species in nearby gardens or natural areas because green roofs represent novel habitats with fewer pollinators than more traditional ground-level habitats. To test these hypotheses, pollinator effectiveness on green roofs was measured through pollen limitation and seed set studies.

Chapter 1: Materials and Methods

Site Locations and Characteristics

Chicago, Illinois, USA and its surrounding suburbs were chosen for the location of this study, as there are currently over 500 green roofs completed or in the process of being built in this area (Kamin 2010). Experiments were conducted at four green roof locations and four ground level locations in the Chicago region (Table 1); the green roofs varied in size, distance from ground-level, time since establishment, and number of plant species. Three of the ground and green roof locations selected were paired, with ground sites adjacent to the buildings which contained the green roofs. All ground locations were landscaped with a mixture of prairie plant species native to Illinois and additional horticultural species. Three of the four green roofs had more than 30 species, most of which were species native to northern Illinois prairies. The ground locations each contained a minimum of six of the species that were also located on the green roofs.

Species Selection

Nine species were selected for analysis in the pollen limitation study: *Allium cernuum* (nodding onion), *Amorpha canescens* (lead plant), *Aquilegia canadensis* (red columbine), *Baptisia alba* (wild white indigo), *Baptisia australis* (blue wild indigo), *Dalea purpurea* (purple prairie clover), *Monarda fistulosa* (wild bergamot/bee balm), *Penstemon digitalis* (foxglove penstemon), and *Zizia aurea* (golden Alexander). Species were chosen based on their presence at a minimum of three study sites, status as an Illinois native species, possession of animal-pollinated flowers, and production of seeds large enough to be contained within the mesh of pollinator exclusion bags. Species were only used if there were more than ten individuals present at each site so as not to disrupt future persistence of the population by over-collection of fruits and seeds or manipulation. Many of the selected species were previously reported to be pollinated by bees, butterflies, or both (Davis and Hendrix 2008).

Autogamy

Six individuals per species were haphazardly chosen at each location and a pollinator exclusion bag (Delnet® Pollinator Bags, Delstar Technologies, Inc.) was placed over a single bud or inflorescence in bud. All pollinator exclusion bags were cut and sewed to appropriate size based on flower/inflorescence length and secured with wire. Exclusion bags were left on the plant for the duration of the flowering period to exclude pollinators and to determine the rate of spontaneous autogamy (self fertilization) in each species.

Pollen Limitation

Plants can shunt resources, directing them to developing fruits of flowers that have received supplemental pollen (Stephenson 1981; Haig and Westoby 1988; Kearns and Inouye 1993). To control for this effect, plants were assigned to one of the two conditions. Twenty-four individuals of each species were haphazardly chosen for study at each site. For each species, half of the individuals (12) were selected for use in the open-pollination and the remaining 12 were used for the supplemental pollination treatment. Colored wires were attached to the portion of the plant being used in the treatment in order to differentiate between the open and hand-pollinated individuals. Flowers with supplemental pollen and open pollination were studied simultaneously to account for differences in weather and pollinator regimes (Kearns and Inouye, 1993).

Traditional pollen limitation experiments include supplemental pollen being applied to the stigmas of flowers and comparisons of fruit or seed set with flowers pollinated naturally (Bierzychudek 1981). For this treatment, pollen was collected into 1.5-ml Eppendorf tubes from at least six unmarked individuals of the same species at the same time and combined to make species-specific pollen mixtures. Pollen was either used on the same day as collection or stored in an air-tight box filled with silica and used within 48 hours to ensure pollen viability. To initiate the release of pollen from *P. digitalis*, entire anthers were removed, placed in open 1.5-ml Eppendorf tubes and placed in air-tight boxes with silica overnight.

When the stigmas of the marked flowers were visible, sticky, and appeared to be viable, the pollen mixture was applied using a small sterile brush of appropriate size and texture, depending on the species. All brushes were washed, soaked in ethanol for 30 minutes, rinsed, and allowed to dry overnight to ensure that interspecific pollen did not inadvertently clog the stigmas of the supplemented individuals. Flowers of each species were hand-pollinated once during the week and time of day that was appropriate for each species when stigmas appeared most receptive. No pollen was applied to the flowers in the open-pollinated condition. All flowers were then allowed to be pollinated naturally for the remainder of their flowering period (Table 2).

After the stigmas were no longer receptive and pistils were withered, pollinator bags (described previously) were placed over developing fruits to protect them from herbivores and were kept in place until seeds were mature. Once fruits were fully formed, they were taken to the Reproductive Biology Lab at the Chicago Botanic Garden, where seeds were extracted from fruits and counted using a dissecting microscope or seed counter (Seedburo™ 801 Count-A-Pak ®, Seedburo Equipment Company). When entire inflorescences were collected, flower number was counted or estimated using measurements of the inflorescence length before seeds were extracted, depending on the structure of each individual species. Seed set measurements for each species are listed in Table 2.

Seeds were dried and stored in paper envelopes at 15°C for seven months. Seed weight was then used to ascertain seed quality by determining the mean seed weight for each population. Ten to 20 seeds from each individual were weighed and the total mass was divided by the number of measured seeds. When individuals had fewer than ten seeds, they were excluded from the analysis. Mean seed weight per population was calculated using the mean weight of one seed per individual.

Insect Collection and Processing

Insect pan traps were used at all sites to assess the insect pollinator community. Pan traps were constructed from Solo® plastic (PETE) 3.25-oz dishes approximately 165 mm in diameter and 60-mm deep painted with white, blue, and yellow fluorescent spray paint as suggested by Kearns and Inouye (1993) and demonstrated in other studies of green roof pollinators (Colla, Willis et al. 2009; Tonietto, Fant et al. 2011). The three fluorescent colors were used to mimic the range of wavelengths represented by the petal colors of our selected species. Each color attracts different insects (Kearns and Inouye 1993), so those captured in the combination of colors should represent the entire community. Five pan traps of each color were haphazardly placed throughout each site and were filled with water that had a few drops of Dawn® dish detergent to decrease surface tension. Due to the presence of dense prairie vegetation at the ground-level sites, pan traps were mounted on wooden poles at a height of approximately 130 cm when vegetation was more than 1 m tall. Traps were only used on days with no precipitation and less than 60% cloud cover and either set up before 0800 hr and removed after 1600 hr or left for a period of 24 hours. Insects were collected once a month for three months to account for temporal differences in availability of floral resources. Trapped insects were rinsed with water and stored in Whirl-Pak® bags with a 70% ethanol mixture within 24 hours of being collected

(Davis and Hendrix 2008). All insects were identified to order using Bland and Jaques (1978). Bees from the genera *Apis*, *Bombus, Megachile,* and *Xylocopa* were identified to genus while all others were classified by body length, based on classification from previous studies (Cane, Minckley et al. 2006; Winfree, Aguilar et al. 2009).

Pollinator observations were made at least twice per month at each site when there was less than 20% cloud cover and the air temperature was over 20°C. Photographs were taken at each of the sites periodically to confirm the presence of certain pollinators that the traps may have missed, specifically larger bee genera such as *Xylocopa* and *Bombus* (Roulston, Smith et al. 2007).

Statistical Analyses

We used the statistical program R, version 2.10.1 (R Development Core Team 2009), to test for the relationship between closed and open pollination treatments for each species. Because linear models revealed significantly lower seed set in the closed condition for all species, data from the bagged treatment were then removed from the data set for the remainder of the analysis. We ran a standard least square model using JMP statistical software (version 5.1.2) to evaluate the effect of treatment and site on seed set. Backward elimination of linear models was performed in R and used to determine significant effects of site type (roof or ground) and treatment (open or supplemented) on pollen limitation. Pollen Limitation Values (PLVs) were defined as PLV = ((SP-OP)/SP) x 100, per Zorn-Arnold and Howe (2007), by comparing the mean number of naturally pollinated seeds ("open" condition; OP) with the number of seeds resulting from supplemental pollination ("supplemented" condition; SP) produced per flower. In the case of species with multiple flowers clustered in an inflorescence, the number of seeds per inflorescence or length of inflorescence was used rather than counting all individual flowers

(Table 2). As an additional measurement of pollen limitation across all species, the percent maximum seed set (PMSS) was calculated within each species by determining the percent difference between the individual with the highest seed set across all sites (H) and all other individuals (I): PMSS = (1-((H-I)/H)) x 100. Backward elimination of linear models was performed in R and used to determine significant effects of site type and treatment on PMSS.

Seed weight was used to assess seed quality and to evaluate potential tradeoffs between seed number and seed size. To compare mean seed weight across all species, the percent of the maximum weight (PMW) for each species was calculated: PMW = (1-((H-I)/H)) x 100. Again, R was used to determine significant effects of site type and treatment on PMW by performing backward elimination of linear models.

As the number of bees captured was small, pan traps of all colors and dates were pooled and the total counts per site from the three collection periods and analyzed by site. We used a generalized linear model in R to evaluate the relationship between the number of bees from different size categories by site type. A chi-square analysis was performed to determine significant variations from expected values in bee communities between green roofs and ground-level sites. Pollinator observations were used to verify presence or absence of pollinators and were not analyzed further.

To view the pollen limitation data in light of the pollinator collections, the relationship between the mean PMSS of all species combined was plotted against the total number of bees caught at each location. The bee data from the green roof at the Chicago Center for Green Technology was excluded due to the lack of PMSS data.

Chapter 1: Results

Autogamy

The linear regression to test the effect of treatment on seed set revealed that the closed condition had significantly fewer seeds ($p<0.01$) than the open treatments in all cases (Table 2).

Pollen Limitation

Analysis of the effects of site, treatment and their interaction on seed set for each species revealed significant effects ($p<0.05$) of site except in *Amorpha canescens* ($p>0.05$). There was no significant effect of treatment for all species, except for *Allium cernuum* ($p=0.001$) or site and treatment interaction, except for *Aquilegia canadensis* ($p=0.004$) and *Penstemon digitalis* ($p=0.025$). *Allium cernuum* had higher mean seed set at both green roofs (1.50 ± 0.11 seeds/flower) and ground level sites (1.10 ± 0.10 seeds/flower) in the supplemented treatment compared to the open pollination treatment (1.04 ± 0.07 seeds/flower at green roofs; 0.98 ±0.06 seeds/flower at ground level). Supplemental pollination only decreased seed set for *A. canadensis* at the Chicago Botanic Garden green roof (102.83 ± 10.63 mean seeds/flower in open treatment, $N=12$; 48.17 ± 24.66 mean seeds/flower in supplemented treatment, $N=6$) and increased seed set for *P. digitalis* at Waters School Community Garden (44.38 ± 14.31 mean seeds/flower in open treatment, $N= 8$; 110.0 ± 0 mean seeds/flower in supplemented treatment, $N=3$).

According to PLVs, the majority of species (6 of 9) exhibited a small degree of pollen limitation at either green roof or ground sites; *Allium cernuum* and *Baptisia australis* were the only species found to be pollen limited at both site types (Table 2). Overall, mean PLVs were low with many species not showing evidence of pollen limitation (Table 2) and no significant difference was found between mean ground PLV and mean roof PLV when data from all species was combined by site type (mean ground PLV = 9.04 ± 4.07; mean roof PLV = 13.65 ± 9.17: Figure 1)

We used percent of the maximum seed set (PMSS) to pool data from all species by site type to test the effects of site type on seed set. There was no significant effect of treatment or treatment by site type interaction on mean PMSS, so these terms were removed from the model. Site type was significant (ANOVA, $p<0.001$, $F_{19,797}$; Figure 3) with plants on the ground having a significantly lower mean PMSS (28.70% ±1.087, N=449) than those on the green roofs (35.86% ±1.642, N=350). Treatment and its interaction with site type and species did not have significant effects on PMSS, but site type, species and their interaction were significant (ANOVA, $p<0.001$ in all cases, $F_{17, 781}(0.05) = 11$). The majority of species (6 of the 9 tested) had a higher PMSS on the roof when compared to the ground, although most of these relationships were not statistically significant (Table 3). Of those species that did have a significantly strong relationship, *Baptisia alba* ($p<0.001$) and *Baptisia australis* ($p<0.001$) had a lower PMSS on the roofs than the ground, while *Dalea purpurea* ($p=0.015$) had a higher PMSS on the roofs than on the ground (Table 3).

Similar to the PMSS measurements, we used percent of the maximum seed weight (PMW) to pool the data from all species and test the effects of treatment and site type on seed weight (Table 3). Again, no effects of treatment or its interaction with site type was found, so these terms were removed from the model. Site type did have a significant effect on seed weight (ANOVA, $p<0.001$, $F_{21,770}$) with plants on the green roofs having a higher overall mean PMW (65.84 ± 2.38, *N*=353) than plants on the ground (54.74 ± 1.61, *N*=419).

Insect Collections

No significant effect of bowl color or the interaction of bowl color with site type was found on the total number of bees captured. The overall abundance of bees at green roofs (*N*=133) was less than the abundance at ground level sites (*N*=281). The composition of insects between the site types differed, with ground level sites having more *Bombus*, large dark, large metallic green, small dark, small metallic green, and tiny dark bees than green roofs (Table 4). Green roofs had more *Apis*, *Megachile*, and *Xylocopa* than ground sites. Large dark bees and *Xylocopa* species deviated from predicted values when number of bees captured in each body type category was separated by site type (contingency table, chi-square test; Table 4).

The ground communities were dominated by small dark bees (41.28%), with large numbers of large dark, tiny dark, and *Bombus* species and the remaining categories (*Apis*, large metallic green, *Megachile*, and small metallic green) present in lower numbers (Table 4). The communities on green roofs were dominated by small and tiny dark bees (29.32% and 27.07% respectively) with large dark bees and *Apis* also common (Table 4) and *Megachile*, small green metallic, *Bombus*, and *Xylocopoda* present in low numbers (Table 4). When grouped by body length (small (<10mm), medium (10 – 15mm), and large (>15mm)), the number of large and medium bees captured deviated from predicted values for green roof and ground level sites (Chi-square test of the contingency table; Figure 3) with the green roofs having more medium and fewer large bees than the ground. There was a significant negative correlation between the PMSS and the number of bees captured (slope = -3.4669 ± 0.8415; Figure 4).

Chapter 1: Discussion

Although this study found that green roofs support fewer and lower diversity of bees than the surrounding urban green space, we found no evidence that green roofs are more pollen limited than nearby ground locations. In contrast to our original hypotheses, seed set of green roof plants was not significantly lower than the same species on the ground.

All the plant species we examined depended on pollinators for maximum reproductive output. This finding is supported by previous ground-level research for *Dalea* (Cane 2006), *Penstemon* (Dieringer and Cabrera R. 2002), *Zizia* (Lindsey 1982), *Baptisia* (Haddock and Chaplin 1982), *Amorpha* (Cruden 1977), *Monarda* (Cruden, Hermanutz et al. 1984), *Allium* (Bell 2007), and *Aquilegia* (Eckert and Schaefer 1998) species. This highlights the importance of having viable pollinator communities present to sustain native species on green roofs.

The observed overall lack of increased seed set with supplemental pollination indicates that reproductive output of plants at both site types is not affected by lack of pollen quality or quantity. Though pollen limitation can occur in species with self-compatible breeding systems (DeMauro 1993) such as the species in our study, pollen limitation may be more pronounced in species with self-incompatible breeding systems (Knight, Steets et al. 2005). As our sites range from the city center, perimeter, and suburbs, this suggests that the lack of pollen limitation may be common throughout the metropolitan region; a finding supported by other studies of fragmented landscapes (Steffan-Dewenter, Klein et al. 2006). Other research shows that habitat patch size does not necessarily affect seed set or fruit set of prairie plant species (Slage and Hendrix 2009); even small patches of habitat on top of buildings can provide necessary floral resources for pollinators. But caution must be taken when applying the observed trends too broadly, as pollen limitation of individuals does not necessarily translate to pollen limitation of populations (Ashman, Knight et al. 2004).

In addition to pollen, maternal resources contribute to the reproductive success of plants. Some studies report that when plenty of resources are available, pollen limitation may be a larger contributor to lack of seed production (Haig and Westoby 1988). The lack of evidence of pollen limitation in the study suggests that nutrient levels are likely to be a significant contributor to the observed reproductive output of our plant species. If nutrients were at a constant level between site types, we would have expected the higher PMSS on the roof to have been associated with a tradeoff in seed size, as found in previous research (Jakobsson and Eriksson 2000). However, this study demonstrated that plants on green roofs actually had both a higher PMSS and higher PMW than the same species on the ground, providing further support that nutrient levels may vary between site types. In the engineered habitats of green roofs, organic matter is a small component of the growth media mixture (Dunnett and Kingsbury 2008) and has been found to fluctuate over time (Kohler and Poll 2010). Since the standard commercially available growth media mixes strive for low-fertility with only 10%-20% of the mixture being organic matter (Dunnett and Kingsbury 2008), it is likely that the amount of nutrients available to native plants on green roofs is low though the precise composition has not been determined at our particular sites. On the ground, none of the sites were reported to have been given any fertilizer mixes, and nutrient availability is unknown.

Of the nine species studied, *A. cernuum* was the only species with significantly greater seed set when provided with supplemental pollen. However, this effect was observed at both ground and green roof sites, indicating that pollen limitation was not confined to green roofs and suggesting the absence of effective pollinators for this species throughout the region. Schuett and

Vamosi (2010) found that *A. cernuum* was usually pollinated by *Bombus* and *Andrena* species. *Bombus* have experienced population declines in the Chicago area (Grixti, Wong et al. 2009) and *Andrena* were not found in large numbers at any of the study sites, supporting the idea that effective pollinators of this species may be lacking in these urban sites. No significant differences in treatment were found in the remaining eight species on both the ground and green roofs. However, *B. australis* exhibited pollen limitation at both site types, providing evidence that supplemental pollination does improve seed set in some cases.

One surprising exception to the supplemental pollination treatment was the decreased seed set in the supplemented population of *A. canadensis* on the green roof at the Chicago Botanic Garden. This species is highly self-compatible and capable of spontaneous autogamy (Eckert and Schaefer 1998), so it can produce seeds in the absence of pollinators. Supplemental pollination would still be expected to increase seed set, hence the decline could be a result of the fragile stigmas being damaged during hand-pollination or strong storms destroying some of the selected plants and resulting in a small number individuals (*N*=6) in the supplemented treatment

Honeybees (*Apis mellifera*) have been found to be poor pollinators of native species (Westerkamp 1991; Nayak and Davidar 2010) so individuals at sites dominated by honeybees would be expected to experience an increase in magnitude of pollen limitation compared to individuals at sites with lower proportions of honeybees (Knight, Steets et al. 2005). Both Chicago City Hall and the Peggy Notebaert Nature Museum had managed honeybee hives present on the roofs for portions of the study period and, as such, we expected the bee communities to be dominated by this species. Although more *A. mellifera* were present at roofs than ground sites, they were not the most prevalent bee type. At both site types, small native bee genera were most dominant in the community. As body size is positively correlated to homing and foraging distance (Gathmann and Tscharntke 2002; Greenleaf, Williams et al. 2007), the high number of small genera might suggest these species are living on or nearby these green roofs.

An interesting trend observed was that many species had a higher mean PMSS on green roofs when compared to ground-level sites. The abundance of pollinators was expected to impact the seed set at each of the locations, particularly since our study species rely on pollinators, however, the trend was opposite to what was expected, with a strong negative relationship between mean PMSS across species and the number of bees captured at each site (Figure 4). This suggests that although there are few bees on green roofs relative to the ground level, those that are present are able to pollinate native species just as effectively, if not more so, than those at ground level sites. This higher PMSS was only significant for *D. purpurea* on green roofs, but it is likely that with repeated experiments or a more robust data set, the five other species would show a significantly higher PMSS on green roofs as well.

The significant negative association between number of bees and mean PMSS could be due to interplant competition for pollinators. The green roof sites were generally smaller in area than the ground sites but had a very high density of species, as they were designed to be aesthetically pleasing. Also, the green roofs had a high number of individuals of the same species grouped together, allowing for decreased foraging distance by pollinators and a greater density floral display. Other investigations have found pollen limitation to be more prevalent in sites or larger areas with high plant species diversity where pollinators frequently passed over individuals due to an abundance of floral resources (Gonzalez-Varo, Arroyo et al. 2009). The close proximity of species with similar morphology has also been found to decrease the amount of pollen an individual plant receives as pollinators collect and distribute pollen of many different species at the same time (Schuett and Vamosi 2010). In contrast, urban gardens that have a high density of plants of the same species tend to receive more conspecific pollen (Werrell, Langellotto et al. 2009). Accurately measuring species diversity and floral density at all sites may provide further insight into this perceived relationship though effects of varied floral density would be expected to be detected by a supplemental pollination study such as ours.

Many factors could account for the unexpected relationship between pollinator number and seed set, nonetheless, our data demonstrate that pollen limitation does not lead to low seed set on green roofs. Reproductive output is influenced by many factors other than pollination, including seed herbivory and availability of resources for the maturation of fertilized ovules (Knight, Steets et al. 2005). Pollinator exclusion bags protected the developing fruits from herbivory and hence seed herbivory was observed only on *B. alba* by the weevil *Apion rostrum*; a widespread seed herbivore of the genus (Petersen, Lindsey et al. 1998; Petersen, Petersen et al. 2006).

Overall, this study provides support that native plant species on green roofs do not experience pollen limitation in the Chicago region. As green roofs continue to be built across Europe and North America, this study demonstrates that they have the potential to provide habitat for native plant and animal species in urban environments. In fact, designing green roofs with native plants and pollinators in mind will not only provide a more stable population for both types of organisms, but possibly nearby economically important crops that rely on pollinators (Colla, Willis et al. 2009). Future urban areas could be designed to provide a steady and abundant food source for bees (Tommasi, Miro et al. 2004) and other insect guilds. In fact, during this study, many species of butterflies and birds were seen foraging on the green roofs. With urbanization increasing, native pollinators with limited ranges risk becoming extinct in fragmented environments unless appropriate habitat corridors are available (Townsend and Levey 2005). This study provides evidence of the important role green roofs can play as part of such corridors, not only for native insects but for the other animals that prey upon them. Some studies have suggested that native Midwest plants will not survive without an additional water supply (Monterusso, Rowe et al. 2005; Carter and Butler 2008). However, we feel that with careful selection of species from water limited ecosystems in the area, such as sand or gravel-hill prairies, green roofs could be designed with such concerns in mind to increase the likelihood that they are self-sustaining habitats.

Additional long-term studies would improve the validation of our current study, especially as pollen limitation has been found to fluctuate each growing season (Aizen and Harder 2007). Further research could contribute to the conservation of pollinators (McIntyre and Hostetler 2001) and insects (Hunter and Hunter 2008) as pollinator loss has been documented across the globe (Potts, Biesmeijer et al. 2010). Bees are found in urban parks, but their abundance depends on the quality of the surrounding vegetation (McFrederick and LeBuhn 2006; Tonietto 2009; Tonietto, Fant et al. 2011), as proper foraging and nesting habitats need to be available to support insect populations for the long-term (Kearns, Inouye et al. 1998). Urban gardens are known to contribute to overall biodiversity and it has been suggested that they should be studied from the perspective of the entire matrix in which they exist, rather than as individual habitats (Goddard, Dougill et al. 2009). Green roofs should be incorporated into these studies of the larger urban green spaces and habitat connectivity. As green roofs continue to provide an increasing amount of habitable space for native fauna, the ecological relationships that occur in these novel habitats must be understood, fostered and protected.

Native species on green roofs have the potential to play an important role in reconciliation ecology practices, in which management techniques allowing humans and native wild species to occupy the same geographic ranges are used (Rosenzweig 2001). At a time when urbanization is causing biotic homogenization (McKinney 2006), the preservation of local native species is critical. While green roofs are certainly not the only technique needed in urban areas for preservation of future biodiversity, they represent a new piece of the conservation puzzle that has the potential to contribute to the diversity of urban landscapes (Francis and Lorimer 2011).

Table 1. Site locations

Site Name	City	Type	Latitude	Longitude
Chicago Botanic Garden	Glencoe	Roof and Ground	42.14443	-87.78591
Chicago City Hall	Chicago	Roof	41.88385	-87.63240
Chicago Center for Green Technology	Chicago	Roof and Ground	41.88916	-87.70072
Peggy Notebaert Nature Museum	Chicago	Roof and Ground	41.92662	-87.63490
Waters School Community Garden	Chicago	Ground	41.96338	-87.69181

Table 2. Seed set measurements, flowering period, mean seed set measurements for the open-pollinated condition for each species. (-) indicates absence of the species from the site. Site locations: Chicago City Hall (CH), Chicago Botanic Garden (CBG), Peggy Notebaert Nature Museum (Note), Chicago Center for Green Technology (CCGT), and Waters School Community Garden (Waters). Significance of pollination indicates the difference between open and closed treatments.

			Mean Seed Set Value in Open Treatment							**Significance of Pollination**	
			Ground				**Roof**				
	Seed set measurement	Flowering Period	CBG	Note	CCGT	Waters	CH	CBG	Note	*p*	Adjusted R^2
Allium cernuum	seeds/flower	Aug.	1.15	0.81	0.95	-	0.99	1.30	0.82	<0.0001	0.65
Amorpha canescens	seeds/raceme length (mm)	June-July	1.42	-	-	-	1.41	-	1.32	<0.0001	0.87
Aquilegia canadensis	seeds/flower	May-June	45.3	97.00	-	78.50	93.33	102.83	38.20	<0.001	0.10
Baptisia alba	seeds/flower	June-July	12.95	0.00	1.03	-	-	-	0.20	<0.01	0.19
Baptisia australis	seeds/flower	May-June	3.81	-	-	7.74	0.18	-	-	<0.001	0.19
Dalea purpurea	seeds/inflor. length (mm)	July	1.95	-	-	-	3.63	2.52	2.98	<0.001	0.17
Monarda fistulosa	seeds/inflorescence	July-Aug.	374.00	-	285.83	518.58	649.64	333.08	-	<0.0001	0.57
Penstemon digitalis	seeds/flower	June-July	44.00	50.33	41.75	44.38	33.42	47.50	-	<0.0001	0.18
Zizia aurea	seeds/inflorescence	May-June	12.64	32.75	-	103.7	99.83	-	87.00	<0.01	0.11

Table 3. The mean percent of the maximum seed set (PMSS), pollen limitation value (PLV) and percent of the maximum seed weight (PMW) for each species at ground level and green roofs sites. Negative PLVs were converted to zeros, indicating lack of pollen limitation. PMSS difference is the mean roof PMSS - mean ground PMSS. A double asterisk (**) indicates significance at the $p<0.05$ level. See Methods for PMSS, PLV and PMW equations.

	Ground					**Roof**					
	PMSS *N*	Mean PMSS ± SE	PLV	PMW *N*	Mean PMW ± SE	PMSS *N*	Mean PMSS ± SE	PLV	PMW *N*	Mean PMW ± SE	PMSS Difference
Allium cernuum	70	28.14 ± 2.53	11.36	70	59.29 ± 1.62	63	33.58 ± 3.67	30.78	67	70.68 ± 1.62	+5.44
Amorpha canescens	22	45.51 ± 5.17	0.00	23	47.19 ± 1.70	47	47.84 ± 6.58	2.24	50	64.78 ± 1.95	+2.33
Aquilegia canadensis	55	31.67 ± 3.81	0.00	58	70.72 ± 1.48	44	39.12 ± 5.64	0.00	48	60.23 ± 2.04	+7.45
Baptisia alba	49	26.59 ± 3.94	0.00	34	57.98 ± 3.31	7	0.85 ± 9.30	0.00	3	41.12 ± 29.45	-25.74**
Baptisia australis	34	30.24 ± 4.42	30.93	33	79.21 ± 1.91	22	2.34 ± 6.85	81.96	9	86.03 ± 4.30	-27.90**
Dalea purpurea	22	23.66 ± 5.17	0.00	24	57.62 ± 2.53	64	44.64 ± 6.39	0.00	75	70.58 ± 1.38	+20.98**
Monarda fistulosa	70	33.54 ± 3.57	0.00	77	44.74 ± 1.31	46	46.47 ± 5.44	7.87	50	57.72 ± 2.06	+12.94
Penstemon digitalis	68	25.61 ± 3.60	13.32	51	41.32 ± 2.17	44	21.51 ± 5.50	0.00	33	46.07 ± 3.41	-4.10
Zizia aurea	59	20.92 ± 3.74	25.76	49	41.81 ± 1.45	13	35.94 ± 7.45	0.00	18	55.79 ± 4.93	+15.02

Table 4. Number and proportion of bees captured at ground level and on green roofs. Deviance of adjusted R^2 values from expected results are indicated by an asterisk (*). For body size categories: small <10mm, medium 10-15mm, large >15mm.

		Ground		**Roof**	
	Body Size Category	Number	Community Proportion	Number	Community Proportion
**Apis*	medium	15	5.3%	20	15.00%
**Bombus*	large	30	10.7%	1	0.80%
Large Dark	large	44	15.7%	26	19.60%
*Large Green Metallic	large	16	5.7%	0	0.00%
**Megachile*	medium	1	0.4%	8	6.00%
*Small dark	small	116	41.3%	39	29.30%
*Small Green Metallic	small	17	6.0%	2	1.50%
*Tiny Dark	small	42	15.0%	36	27.10%
Xylocopa	large	0	0.0%	1	0.80%

Figure 1. Mean pollen limitation value (PLV) for all species combined at ground level sites and green roofs sites. Mean PLV was not significantly different between site types ($p > 0.05$). See Methods for PLV equation.

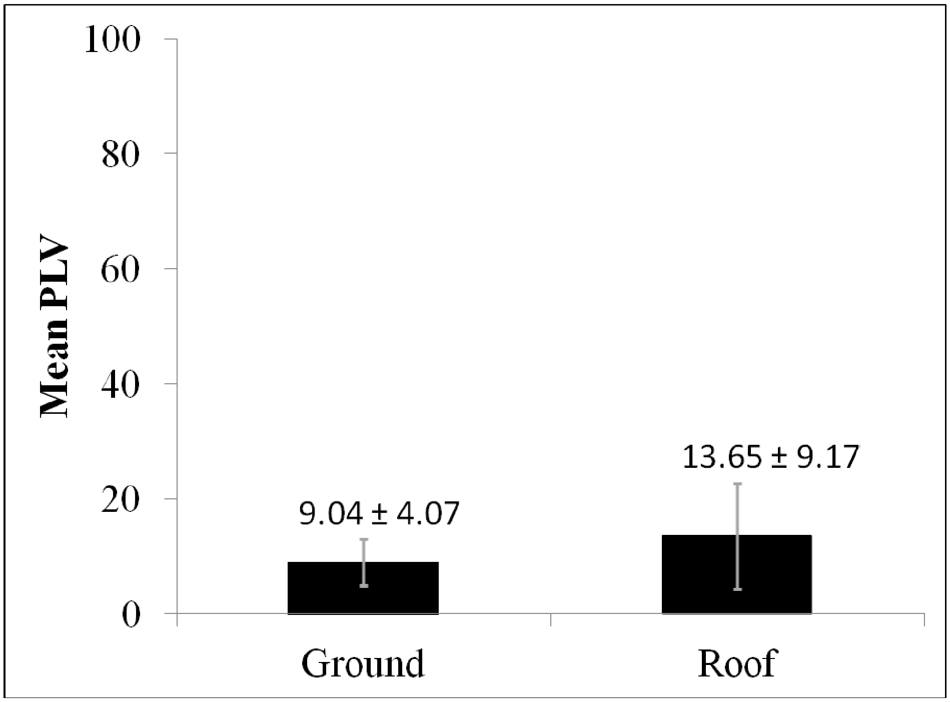


Figure 2. Mean percent of the maximum seed set (PMSS) for all species combined. No significant differences were found between the PMSS in the open and the supplemented (Supp.) treatments at either site type ($p > 0.05$).

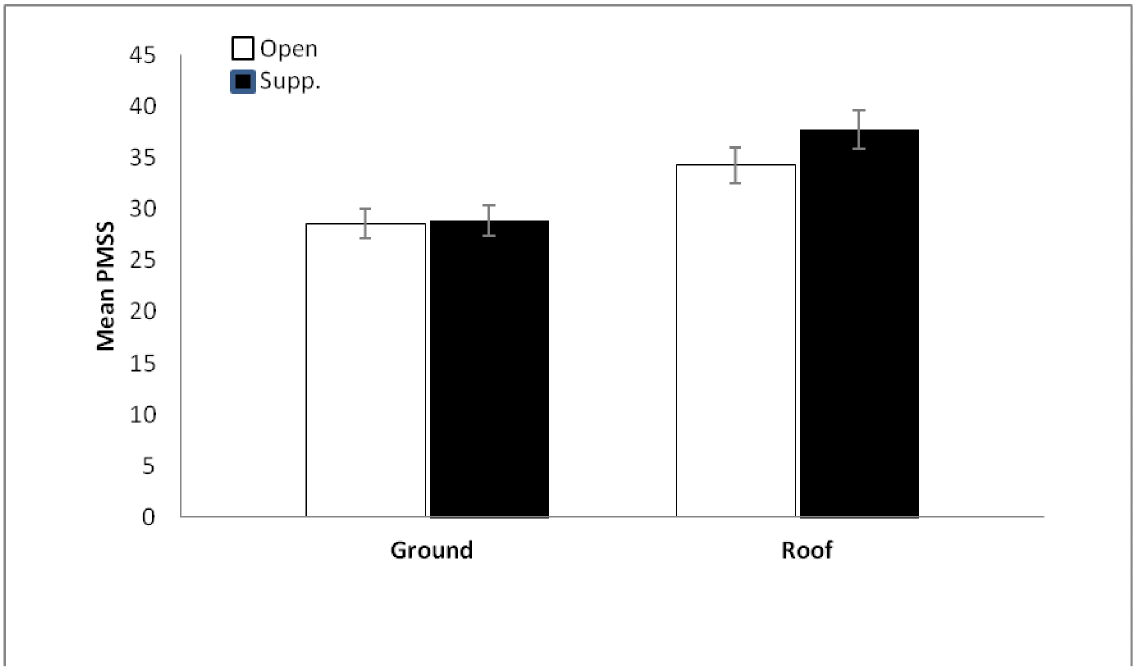


Figure 3. Total number of bees captured in pan traps at ground-level and green roof sites grouped by bee body size. Medium bees on green roofs were significantly more abundant (adjusted residual value >2.0) while large bees on green roofs were significantly less abundant than expected (adjusted residual value <2.0) and green roofs had fewer bees overall.

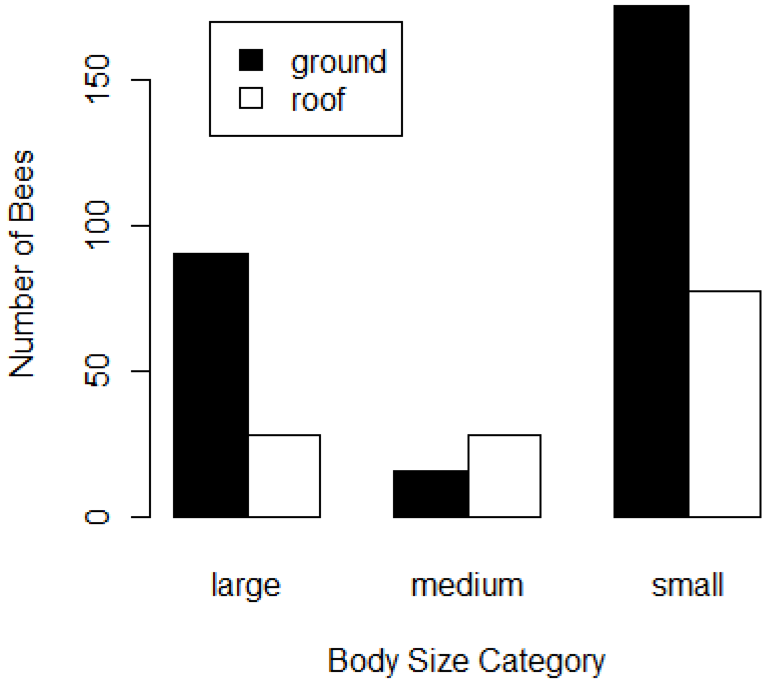
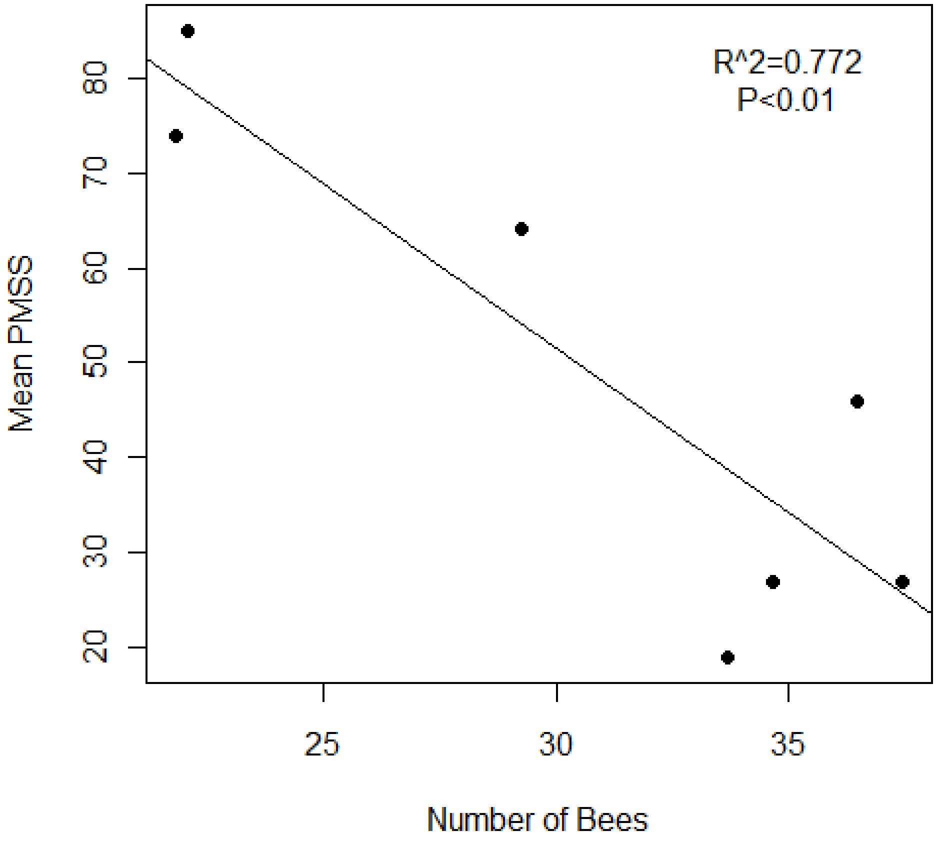


Figure 4. Relationship between mean percent of the maximum seed set (PMSS) for all species combined and total number of bees captured at each site.



Chapter 2: An assessment of pollination services on Chicago green roofs.

Conference proceedings prepared for CitiesAlive! 2011; Green Roofs for Healthy Cities' 9th Annual Green Roof & Wall Conference.

December 2, 2011.

Chapter 2: List of Figures

Figure 1

(a). Green roof sites, from left: Chicago Botanic Garden, Chicago City Hall, and Peggy Notebaert Nature Museum.

(b). Ground-level sites, from left: Chicago Botanic Garden, Chicago Center for Green Technology, Waters School Community Garden, and Peggy Notebaert Nature Museum.

Figure 2. Mean germination of seeds from ten native Illinois prairie species at ground-level sites and green roofs ± standard error. ("*" indicates a significant difference at the $p<0.05$ level and "**" indicates significance at the $p<0.001$ level.)

Figure 3. Proportion of seeds germinated by days since transfer from cold stratification for nine native Illinois prairie species at ground level sites (solid lines) and green roofs (dashed lines). *Baptisia alba* seeds from the green roofs had a total germination of 0% and therefore data for are not included in the figure.

Chapter 2: List of Tables

Chapter 2: Introduction

Organisms living in regions dominated by human populations have been, and will continue to be, affected by multiple anthropogenic factors. With approximately half the world's people living in cities and an expected increase to 6.4 billion city dwellers by 2050 (United Nations 2008), the pressures exerted on natural habitats are likely to be felt in all ecosystems across the globe. Changes in habitat structure and function commonly experienced in urban areas as a result of habitat loss and fragmentation can negatively impact the survival and reproduction of many plant communities (Aguilar et al. 2006) as well as wildlife species they support (Savard et al. 2000, Winfree et al. 2009). Plant extinction rates are high in urban environments and are influenced by the amount of vegetation remaining after natural habitat is replaced with buildings and agriculture (Hahs et al. 2009). To complicate matters, the natural pollinators of many angiosperm species, including bees, are also experiencing global declines in both species diversity and richness (Potts et al. 2010). In urban environments, where smaller habitat patches are common, the typical foraging behavior of pollinators is altered to maximize floral rewards in a less accessible environment (Andrieu et al. 2009, Goverde et al. 2002, Hadley and Betts 2009).

Many human-dominated landscapes are starting to be redesigned to foster cooperative living between people and native ecosystems to promote a greater diversity of species, a concept known as reconciliation ecology (Rosenzweig 2001). Green roofs, an increasingly common novel habitat in many urban environments, are good examples of reconciliation ecology (Francis and Lorimer 2011) as they provide the opportunity for a single piece of land to be used for buildings and habitat for plants and animals. It is well known within the industry that green roofs provide many shared environmental benefits over traditional roofing materials, including reduced energy needed for building insulation and cooling, reduced stormwater runoff, reduced urban heat-island effect, and pollution abatement (Carter and Butler 2008, Dunnett et al. 2008(a), Getter and Rowe 2007, Oberndorfer et al. 2007, Rowe 2011, Snodgrass and Snodgrass 2006, Spala et al. 2008, Susca et al. 2001).

Green roofs could also play a role in adding to, and supporting biodiversity in urban environments (Oberndorfer et al. 2007), although the potential for long-term persistence of many species on green roofs has yet to be tested. There are many benefits that diverse plantings on green roofs can provide, especially when local native species are used on their own or to supplement traditional plantings of *Sedum* or other succulent species. Green roofs planted to mimic local natural habitats can attract uncommon or threatened species of birds (Baumann 2006, Brenneisen 2006, Fernandez-Canero and Gonzalez-Redondo 2010) and insects (Kadas 2006, Tonietto 2011), which may use the roofs for nesting, mating, or foraging sites or may serve as components of larger migration corridors. Plantings of native species such as grasses and forbs are more effective at retaining stormwater than *Sedum* species (Dunnett et al. 2008(a), MacIvor and Lundholm 2011(a)) and contribute positively to overall ecosystem function (Lundholm and MacIvor 2010). Experimental green roofs are being developed to assist in the *ex situ* conservation of threatened plant species. For example, *Hymenoxys acaulis var. glabra*, the endangered lakeside daisy that has been extirpated from Illinois (DeMauro 1993) and *Asclepias lanuginosa*, a rare milkweed, are both native to locally dry habitats and are now being cultivated for seed production on the green roof at the Rice Center for Plant Conservation at the Chicago Botanic Garden. The green roof populations of these species and others like them have the potential to be used as one component in the future restoration and conservation of such species.

It is recognized that not all local native species are suitable to be used on green roofs. For example, many Illinois native prairie grasses and perennials have large root systems that can penetrate several meters into the soil (Craine et al. 2003, Sun et al. 1997). It would be impractical to use such species on extensive green roofs which have only a shallow profile of growing media (Snodgrass and Snodgrass 2006). Other grassland species can pose fire risks during dry periods at the end of the growing season as uncompacted dried biomass acts as a fuel source (Wright and Bailey 1982) and therefore are not recommended as suitable fire resistant vegetation by the American National Standards Institute/Singly Ply Roofing Industry (Approved American National Standard and Single Ply Roofing Industry 2010). In extreme northern and southern latitudes, winter can pose an additional problem, as many native perennial species will not provide roof insulation when their tissues have dried and withered. Currently many native plant species used on green roofs require additional irrigation to become established (Carter and Butler 2008, Monterusso et al. 2005) and their persistence over multiple generations has yet to be documented in North America. Through proper selection it is likely that less demanding natives could be identified from habitats with site characteristics similar to green roofs such as areas with low soil profiles closely associated with bedrock, rocky outcrops, sand or gravely soils or even urban habitats with impervious-surfaces (Lundholm 2006).

The use of native plants that require biotic pollination for successful fertilization on green roofs may run the risk of not setting enough seed to sustain populations over time. To date, few studies have looked at the diversity and abundance of pollinator communities attracted to green roofs, especially compared to nearby ground-level habitats. Two investigations in Eastern Canada that have made this comparison found fewer species of Hymenoptera (bees, wasps and ants) and specifically decreased species richness, abundance, and diversity on green roofs compared to ground-level sites (Colla and Willis 2009, MacIvor and Lundholm 2011(a)). Though the analyses used in these previous studies revealed no statistically significant differences between site types, the analyses used in Chicago-area studies have demonstrated that green roofs have slightly lower pollinator abundance and species richness compared to nearby prairies, parks and gardens (Ksiazek et al. 2011, Tonietto et al. 2011). In a previous study, we found that although green roofs support fewer and less diverse pollinator communities, there was no evidence that native plants on green roofs were pollen limited (Ksiazek et al. 2011). Based on our study, all species examined relied on pollinators for production of maximum seed set but number of seeds produced by green roof plants was not significantly lower than for plants of the same species on the ground. In fact, when data from all species were compared it was found that seed set was higher on green roofs than on the ground for six of the nine species studied. While these results are contrary to our expectations, it may be that using other characteristics including seed viability, germination and seedling survivorship, may be more accurate measures of reproductive success.

The current study builds on our previous investigation, addressing reproductive success of native plant species on green roofs in the Chicago region. Reproductive success is not limited to measures of seed set, rather, differences in seed quality can result from resources limitation or environmental stress experienced by the maternal plant during seed maturation. Ground-level habitats with deeper soil profiles and plants with considerably more extensive root systems would be expected to have higher proportions of available below-ground organic matter compared to extensive green roofs. Plants in resource-limited environments can still produce many seeds, but the resource trade off associated with higher seed production can include smaller seed or lower viability (de Jong and Klinkhamer 2005). We tested the hypothesis that seeds produced on green roofs would be smaller and less viable than those from ground-level sites. To test this hypothesis, seeds collected from ten native prairie species growing on green roofs and ground-level habitats were counted, weighed and germinated under controlled conditions.

Chapter 2: Materials and Methods

Species and Site Selection

Ten native Illinois species were selected for analysis in the germination study: *Allium cernuum* (nodding onion), *Amorpha canescens* (lead plant), *Asclepias tuberosa* (butterfly weed), *Aquilegia canadensis* (red columbine), *Baptisia alba* (wild white indigo), *Baptisia australis* (blue wild indigo), *Dalea purpurea* (purple prairie clover), *Monarda fistulosa* (wild bergamot/bee balm), *Penstemon digitalis* (foxglove penstemon), and *Zizia aurea* (golden Alexander). Species that bloomed across the growing season (April through September) were chosen based on their status as native to Northern Illinois, and reliance on biotic pollinators such as bees and butterflies (Davis and Hendrix 2008) for maximum seed production. All species, with the exception of *A. tuberosa*, were used in our previous pollen limitation study.

Three green roofs (Figure 1a) and four ground-level locations (Figure 1b) in greater Chicago were chosen for the location of this research. The green roof at the Chicago Center for Green Technology was not included in the study because it lacked our selected plant species. The remaining green roofs differed in age, size, height from the ground, and diversity of plant species but each had at least five of the ten selected species present (Table 1). Staff at each of the research sites reported that all species had been planted intentionally during the construction of the site. Plant populations on green roofs were generally believed to have originated from plugs, although the exact origin of the individuals used in our study could not be determined.

Seed Germination

Seeds were collected from one of three pollination treatments: no pollination, untreated, and supplemental pollination. For pollinator exclusion, mesh bags (Delnet® Pollinator Bags, Delstar Technologies, Inc.) were placed over a single flower bud or inflorescence before they opened to test for the rate of self fertilization (Ksiazek et al. 2011). Untreated individuals were tagged but otherwise undisturbed while individuals given supplemental pollen received additional pollen applied using a fine brush. After fruits developed, seeds were collected, counted, dried and weighed; seeds were kept separate to prevent unbiased sampling from maternal lines with greater seed sets. All dried seeds were stored in a freezer (<-20°C) for a minimum of 9 months before commencement of the germination experiment.

Agar plates (95mm x 15mm) were prepared using a 0.75% solution of Agar (Bacteriology Grade Agar; Fisher BioReagents® 9002-18-0). Seeds requiring scarification (Table 2) were agitated between two sheets of medium grain sandpaper to nick the seed coat. Ten seeds per individual plant were randomly assigned one quarter of a prepared agar plate. For each site type, seeds from 3 – 77 individuals (mean 39.7 ± 5.02) were plated per species, depending on the availability of seed. Seeds were sterilized by dipping them briefly in a 10% bleach solution and were double rinsed in sterilized water before being plated. All plates were sealed with Parafilm® to retain moisture and placed in dark, moist, cold stratification at 4°C for the time period suggested by the common literature for each species (Table 2). Plates were then moved to a single incubator (Intellus environmental controller, Model 136LLVL; Percival Scientific, Inc.) set to a common Chicago springtime temperature diurnal schedule of 23/13°C (12:12 h) with a relative humidity of 60%.

Germination, defined as the presence of a root radicle, was recorded every two to four days until all seeds had germinated or until there was no change in overall percent germination over a one-week period. Viability was defined as the mean proportion of germinated seeds per individual. Days to germination were determined as the time for all viable seeds to germinate. To eliminate over influence of outliers associated with late germination, time to 50% germination of all viable seed was calculated for each species by site-type combination, using the asymptotic point at which half of the viable seeds had germinated. Time to 50% germination of viable seed was rounded up to the nearest whole day. Percent viable seed was also tested against percent maximum seed set (PMSS) and percent of the maximum seed weight (PMW) from previous study to test for correlations by site type across all species (Ksiazek et al. 2011).

Data Analyses

All data were analyzed using R version 2.10.1. (R Development Core Team 2009). Seed was first analyzed by pollination treatment using Student's t test to determine whether the assigned pollination groups from the previous study affected viability. Binominal linear models were used to compare viability between site types for each species. Tests for correlation between PMW and PMSS, PMSS and percent viability, and PMW and viability were performed to determine the relationship between seed set, seed weight, and total germination on green roofs and ground-level sites.

Chapter 2: Results

Mean viability of seeds collected from the no pollination (bagged) treatment (0.620 ± 0.04) was significantly lower than that of seeds from the untreated (0.732 ± 0.02) and supplemental pollination (0.728 ± 0.02) treatments (ANOVA, $p<0.001$, $F_{5.5, 791}$). The majority of seeds (87.1%) were obtained from untreated or supplemental pollination treatments that did not have significantly different viability ($p=0.876$ on 684 DF) and, as a consequence, data was pooled for the remaining analyses.

Seed viability varied by species such that species was a significant predictor of total germination proportion (ANOVA, $p<0.001$, $F_{51,784}$). The majority of species (seven of ten) had higher seed viability on green roofs compared to ground level although binomial linear models revealed that these differences were not significant in most cases (Figure 2). The two exceptions were *Dalea purpurea*, which had a significantly higher mean germination (ANOVA, $p<0.001$, $F_{8,97}$) in green roof seeds (0.832 ± 0.02, $N=75$ plants) than seeds collected from the ground-level populations (0.682 ± 0.06, $N=24$ plants) and *Baptisia alba*, which had significantly higher (ANOVA, $p<0.05$, $F_{5, 35}$) mean germination on the ground (0.295 ± 0.04, $N=34$ plants) compared to the roof (no germination, $N=3$ plants; Figure 2).

When data from all species were pooled, seeds from green roofs had an overall higher mean germination proportion (0.786 ± 0.01) than those from ground-level sites (0.658 ± 0.02; $p<0.001$ on 792 DF). Germination proportion over time plots revealed little difference in germination rate by site type within each species (Figure 3). For all species combined, it took an average of seven days to reach 50% germination and 19 days to reach 100% germination of viable seed. These data were the same for both site types. Seeds from green roofs and ground-level sites had the exact same germination rate for three of the ten species (*A. canescens*, *A.*

tuberosa, and *M. fistulosa*). The largest within-species germination rate discrepancies between site types were for *B. australis* and *A. canadensis* with a three-day difference to 50% germination, and *A. cernuum* and *P. digitalis* with a four-day difference to 100% germination (Table 3), excluding data from *Baptisia alba*, as no *B. alba* seeds from green roofs germinated.

We found a significant positive correlation between percent of the maximum seed set (PMSS) and seed viability ($p<0.001$). Percent of the maximum seed weight (PMW) for each species was also significantly correlated to seed viability ($p<0.001$).

Chapter 2: Discussion

For native species to be economically-practical candidates for green roof plantings they need to be capable of reproducing and successfully sustaining their populations under extreme rooftop growing conditions so that re-planting and maintence costs do not become inhibitory. Production of an adequate quantity of seeds for long-term persistence is a necessary first step, but seeds must also be of sufficient quality to germinate, grow, and survive to reproductive maturity. This investigation supports recent findings (Ksiazek et al. 2011) that native plant species on green roofs have reproductive abililties similar to or better than those growing at ground-level. While the next step will be to verify successful seeds germination in green roofs settings, the current findings reveal that the native species we studied do not produce fewer or smaller seeds with inferior germination ability when grown on green roofs, as originally hypothesized.

Overall, seeds from green roofs had a higher mean percent germination, compared to seeds from ground sites. Only one species, *B. alba* had individuals with a significantly lower germination success in green roof plants but these data should be intrepreted cautiously as very few individuals on the roofs (*N*=3) produced enough seeds to be used in our analysis. The lack of seed production in *B. alba* could be a consequence of it requiring larger pollinators which are less abundant on green roofs (Colla and Willis 2009, Ksiazek et al. 2011, Tonietto et al. 2011), although providing supplemntal pollen did not increase seed set compared to individuals pollinated naturally (Ksiazek et al. 2011).

The rate of germination between plants on green roofs and ground level sites did not vary considerably, supporting a common pattern of germination for these native species. Long-term persistence of flowering species depends on the receipt of compatible conspecific pollen. Our data suggest that green roof natives will contine to produce viable seed, as similar germination patterns are likely to allow plant species to bloom at the appropriate time necessary to attract required pollinators during their appropriate foraging season.

Although data were colleted during a single field season, the results from this study and those of our previous study on pollen limitation provide evidence that native species can achieve reproductive success on green roofs similar to and sometimes surpassing levels observed on the ground. Both seed weight and seed set were correlated with germination success, whereby green roof populations having more, heavier seeds also had higher rates of viability than populations on the ground (Ksiazek et al. 2011). Traditionally, seed mass tends to correlate positively with seedling success, particularly during the early germination stage (de Jong and Klinkhamer 2005), a trend observed in our experiment. In contrast, greater seed set is not necessarily expected to correlate with increased viability because of the common tradeoff between seed number and seed weight (de Jong and Klinkhamer 2005) although under harsh environmental such as drought some plants will produce larger, heavier seeds to increase the likelihood of seedling survival (Moles and Westoby 2004). Though the populations in our study were all within a 20-mile corridor and did not vary greatly in the amount of precipitation received, the hotter, windier and drier conditions that are typically experienced on green roofs compared to adjacent ground level habitat (Carter and Butler 2008, Snodgrass and Snodgrass 2006, Susca et al. 2011, Takahiro et al. 2010). This additional stress on plants could affect seed production in green roof individuals. Increased exposure to heat on green roofs could also lead to longer growing seasons for these populations, allowing for an advantage of prolonged nutrient uptake compared to ground-level populations. Soil profiles as well as available resources in the soil may also vary between green roofs and ground-level sites; factors which were not measured in our investigations but would benefit from further research to assess maternal effects on seed number, size, viability, and subsequent growth.

Maternal effects from nutrient provisioning to seeds could explain the variation in seed mass and germination success in the green roof populations. Genetic effects could also account for differences in seed mass if seed sources used on green roofs in this study originated from maternal lines chosen for their robustness and ability to propagate easily, traits commonly selected for at many green roof installation companies (Snodgrass and Snodgrass 2006). Since the FLL Roof-Greening Guidelines require the use of nursey-derived seed for rooftop plantings rather than seed from wild populations (FLL 2002) and typical ground-level sites designed to mimic natural ecosystems are not restricted such regulations, there is a possibilty that green roof populations have increased reproductive output as a result of artifical genetic selection. As the maternal sources of seed is not know for either of our site types, this variable could not easily be discounted.

Developing a deeper understanding of the ecological processes occurring on green roofs is crucial at a time when these novel habitats are becoming increasingly prevalent and native habitats more fragmented throughout the world. Not only can native plants be included in species mixes that provide environmental benefits on green roofs (Lundholm and MacIvor 2010), but they also may contribute to biodiversity conservation efforts including supporting the birds and insects that use these habitats for nesting and foraging. While native plant conservation may not have been the original intention of green roof technology, the opportunity exists to design green roof habitats with conservation goals in mind which can make important contributions to biodiversity conservation, including specific plant and pollinator groups currently known to be threatened with extinction and loss of suitable habitat. The ability of a variety of species to reproduce on green roofs is encouraging and suggests that using native species may be a successful management tool. The presence of native plants in urban gardens have already been found to increase native pollinator diversity (Goddard et al. 2009, Burghardt et al. 2009) and green roofs, a new type of urban garden, could be incorporated into regional landscape-level studies of habitat connectivity (Brenneisen 2006, Oberndorfer et al. 2007).

Many questions about the ecosystem services provided by green roofs remain unanswered. While our data support the notion that native Illinois prairie species have the reproductive ability to successfully persist on green roofs, they have many other habitat requirements that need to be met for their long-term survival. We know that plant colonization (Archibold and Wagner 2007, Dunnett et al. 2008(b), Köhler 2006) and reproduction occur on green roofs although a common suite of traits needed by plants for successful colonization has yet to be established. We must monitor these habitats so they do not simply become seed sources for future ground-level colonization of invasive or weedy species, which tend to make use of resourece-limited habitats like green roofs. Most green roofs in North America are still in their early stages of ecological succession and how these habitats are designed and managed will determine the future trajectory of their species composition and their utility in biodiversity conservation efforts. Using a habitat-template approach to green roof design, where selected plant species originate from analogous habitat, can answer some questions about how green roofs mimic natural ecosystems (Lundholm 2006) and should be supported in a variety of geographic locales. While certainly not all habitats have native plant species suitable for growing on green roofs, many regions of the world do contain areas with high bedrock or rocky surfaces that serve as appropriate correlates for green roof habitats. By carefully selecting species from these analogous types of ecosystems within a region, green roofs can be designed with such concerns in mind to increase the likelihood that they are self-sustaining habitats which have the potential to play a very important role in future species conservation practices. Future research should address both the economic and ecological success of incorporating native species into the harsh environments of green roofs.

Table 1. Study site information. "*" indicates site not used for the current study.

Site Type	City	Latitude	Longitude	Green Roof Completion	Green Roof Height from Ground
Roof and Ground	Glencoe	42.14443	-87.78591	2009	2 stories
Roof	Chicago	41.88385	-87.63240	2001	13 stories
Roof* and Ground	Chicago	41.88916	-87.70072	2002	3 stories
Roof and Ground	Chicago	41.92662	-87.63490	2002	3-4 stories
Ground	Chicago	41.96338	-87.69181	N/A	N/A

Table 2. Seed germination selection, pretreatments and germination length.

Species	Number of Individuals (ground/roof)	Number of Sites (ground/roof)	Scarification Treatment Applied	Days in Cold Stratification	Days in Germination
Allium cernuum	70/67	3/3	no	56	17
Amorpha canescens	23/50	1/2	yes	16	21
Aquilegia canadensis	58/48	3/3	no	52	45
Asclepias tuberosa	15/8	2/1	no	31	14
Baptisia alba	34/3	3/1	yes	15	28
Baptisia australis	33/9	2/1	yes	15	17
Dalea purpurea	24/75	1/3	yes	0	25
Penstemon digitalis	77/50	3/2	no	31	20
Monarda fistulosa	51/33	4/2	no	0	29
Zizia aurea	49/18	3/2	no	57	20

Table 3. Germination of viable seed from native plant populations on the ground versus green roofs. *Baptisia alba* seeds from the green roofs did not germinate.

	Ground		Roof	
Species	**Days to 50%**	**Days to 100%**	**Days to 50%**	**Days to 100%**
A. cernuum	5	14	4	10
A. canescens	3	21	3	21
A. canadensis	17	37	14	37
A. tuberosa	5	7	5	7
B. alba	7	25	N/A	N/A
B. australis	7	17	10	17
D. purpurea	4	17	4	21
M. fistulosa	5	13	5	13
P. digitalis	11	25	10	29
Z. aurea	6	14	8	17

Figure 1

(a). Green roof sites, from left: Chicago Botanic Garden, Chicago City Hall, and Peggy Notebaert Nature Museum.

(b). Ground-level sites, from left: Chicago Botanic Garden, Chicago Center for Green Technology, Waters School Community Garden, and Peggy Notebaert Nature Museum.

Figure 2. Mean germination of seeds from ten native Illinois prairie species at ground-level sites and green roofs ± standard error. ("*" indicates a significant difference at the $p<0.05$ level and "**" indicates significance at the $p<0.001$ level.)

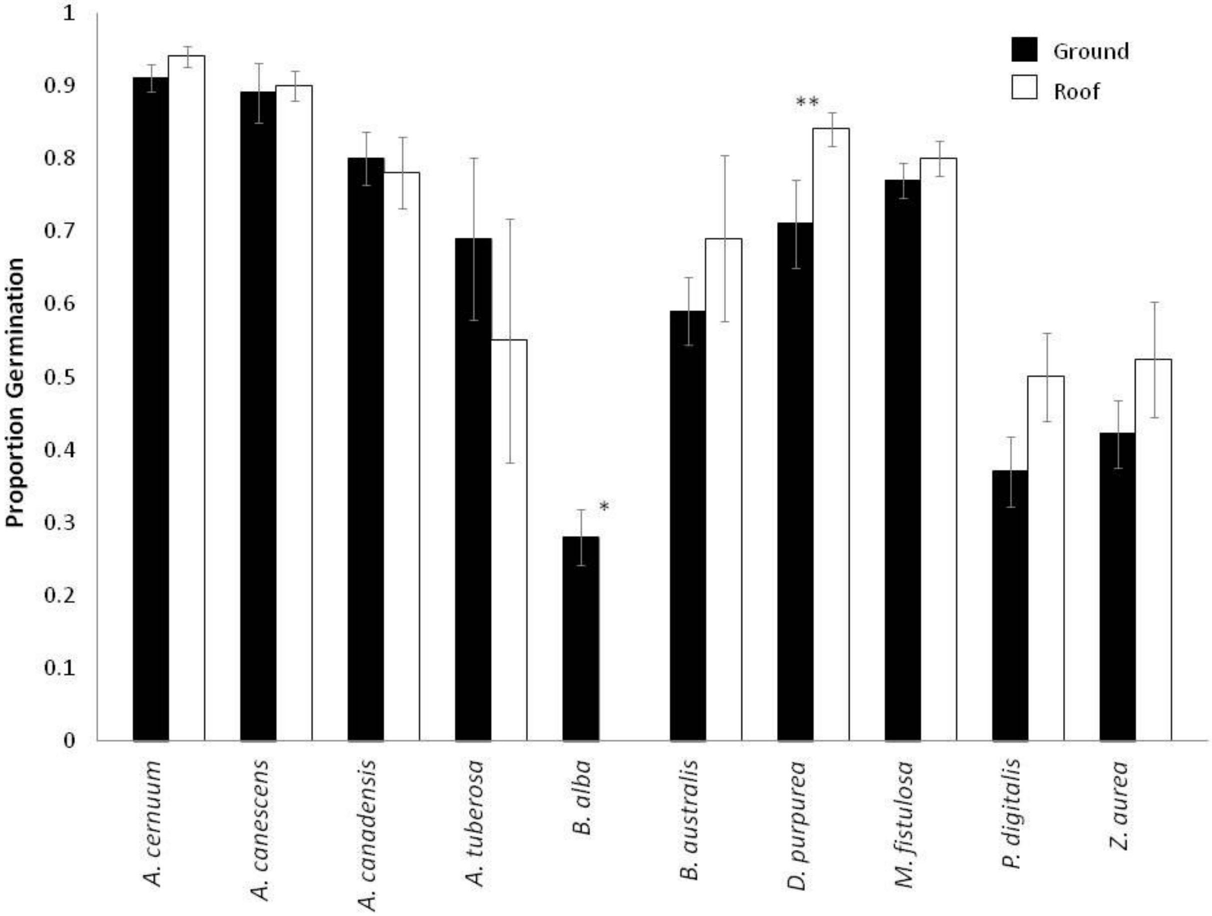
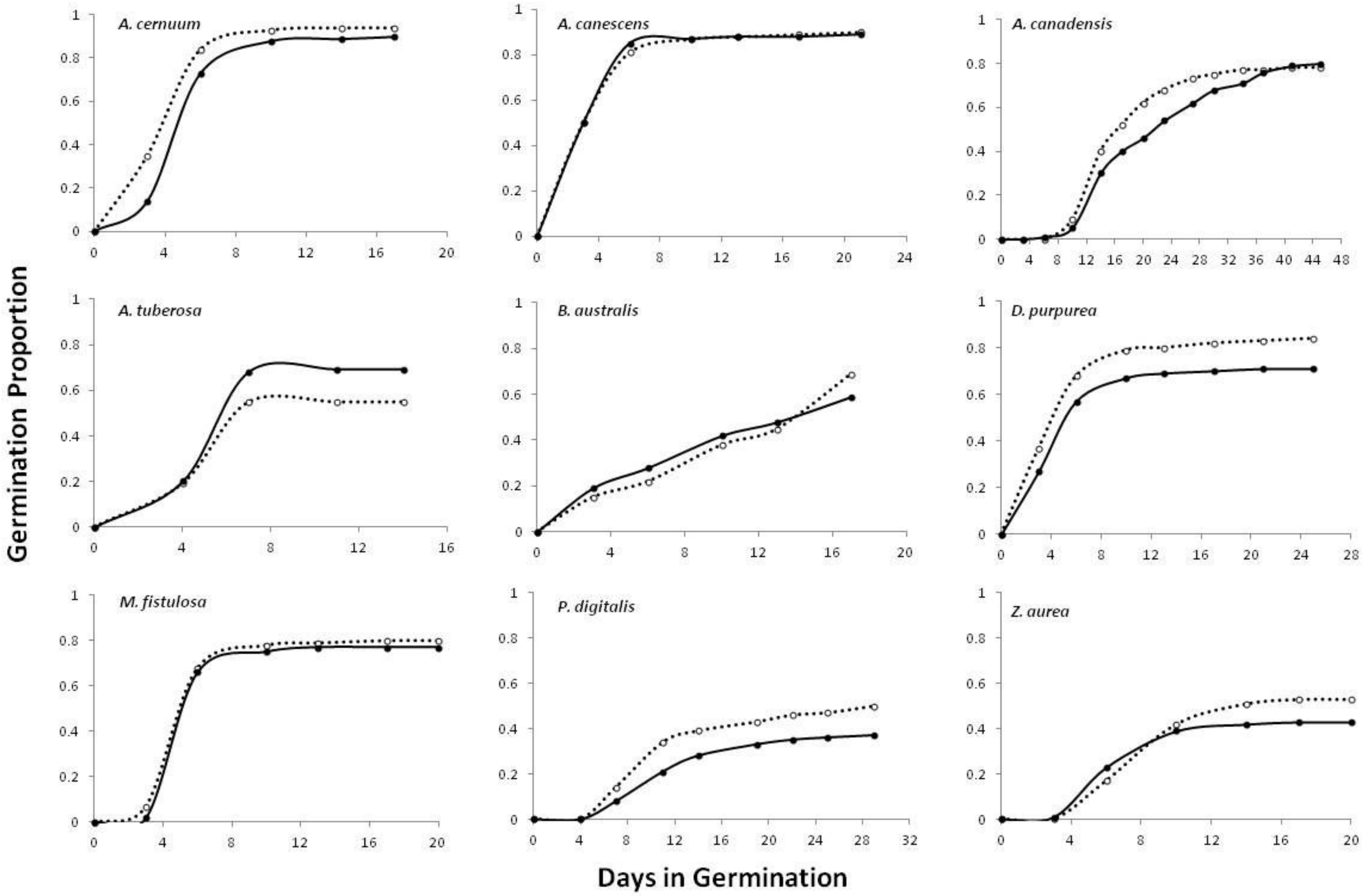


Figure 3. Proportion of seeds germinated by days since transfer from cold stratification for nine native Illinois prairie species at ground level sites (solid lines) and green roofs (dashed lines). *Baptisia alba* seeds from the green roofs had a total germination of 0% and therefore data for are not included in the figure.



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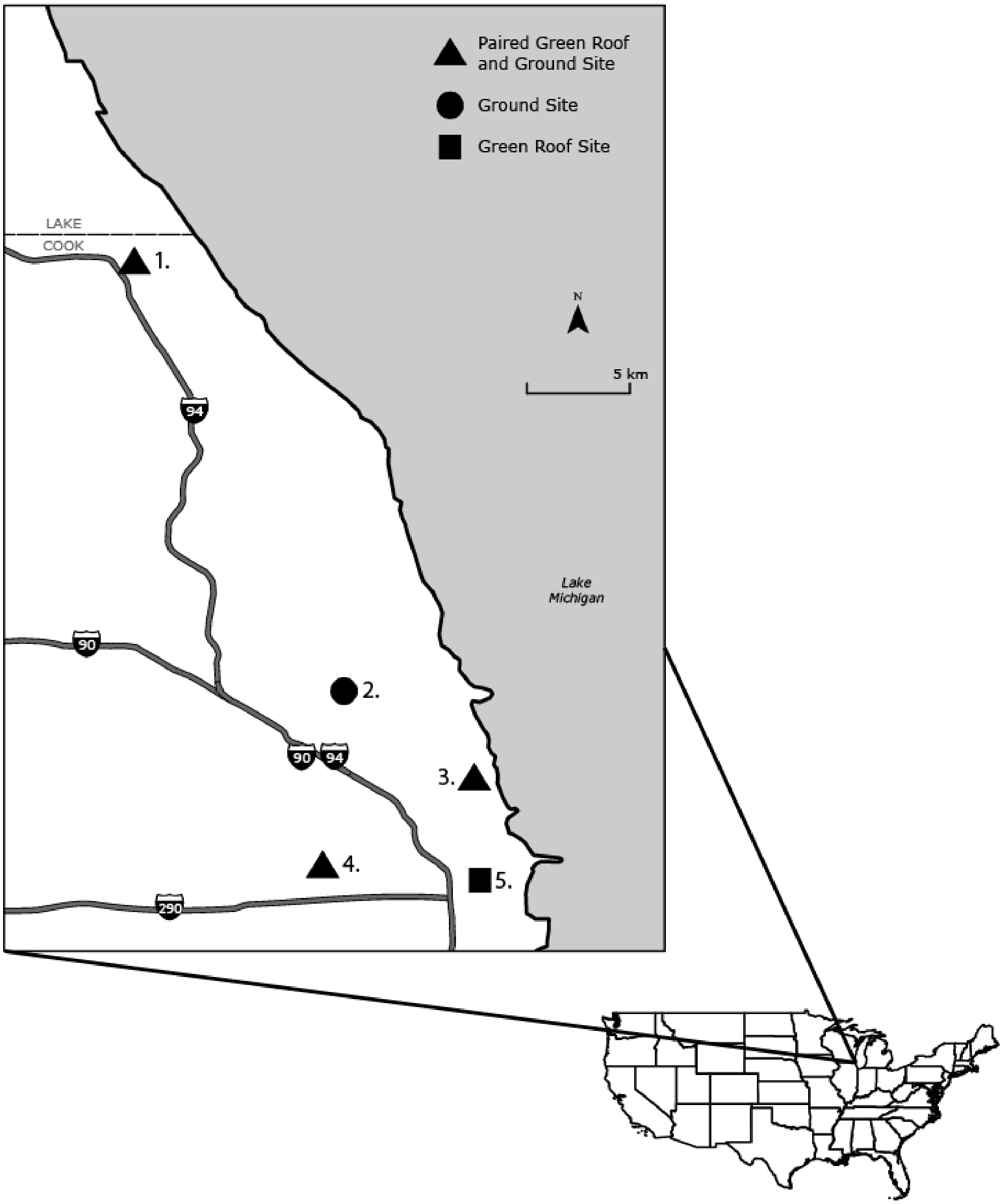
Appendix 1: Additional Site Information: Green Roofs

Site Name	**Plant Species Estimate**	**Approx. Size**
Chicago Botanic Garden	140+	1486 m^2
Chicago City Hall	202+	1886 m^2
Chicago Center for Green Technology	5+	246 m^2
Peggy Notebaert Nature Museum	30+	1644 m^2

Appendix 2: Additional Species Selection Information

Species	**Illinois Native**	**Number of Green Roof Locations**	**Number of Ground Locations**	**Typical Method of Reproduction**	**Observed Blooming Period for Individuals**	**Typical Pollinators**	**Other Notes**	**Chosen for this Study**
Allium cernuum	Yes	3	3	Seed	8/4 - 8/18 Ave. 12 days	Small bees, large green metallic (Halictid)		yes
Amorpha canescens	Yes	2	1	Seed	6/24 – 7/22 Ave. 25 days	Solitary Bees, Butterflies, *Bombus, Megachile*		Yes
Aquilegia canadensis	Yes	3	3	Seed	5/20 – 6/11 Ave. 13 days	*Bombus*, possibly hummingbirds, bees		Yes
Asclepias tuberosa	Yes	1	3	Seed		Long-tongued Bees, Butterflies	Requires deposition of polinia rather than pollen grains for fertilization – very difficult to perform by hand	No, still looked at flower/fruit ratio on G vs R
Baptisia alba macrophylla	Yes	1	4	Seed	6/16 – 6/25 Ave. 6 days	*Bombus* (inc. queens), Other Bees		Yes
Baptisia australis		1	2	Seed	5/20 – 6/17 Ave. 16 days	*Bombus* (workers)		Yes
Dalea purpurea	Yes	3	1	Seed	7/1 – 7/22 Ave 16 days	Butterflies, *Apis, Megachile*, green metallic bees		Yes
Monarda fistulosa	Yes	2	3	Seed	7/9 – 8/12 Ave 19 days	Small dark bees (*Doufouria*), Butterflies, *Bombus*		Yes
Penstemon digitalis	Yes	2	4	Seed	6/15 – 7/5 Ave 10 days	*Apis, Bombus, Megachile*, some large metallic bees		Yes
Sedum album	No	3	0	Seed and Vegetative Propagation		Various bees	Extremely tiny seeds, smaller than openings in exclusion bag	No
Sedum kamtschaticum	No	2	1	Seed and Vegetative Propagation		Various bees		No
Sedum reflexum	No	2	1	Seed and Vegetative Propagation		Various bees	Extremely tiny seeds, smaller than openings in exclusion bag	No
Sedum sexangular	No	2	1	Seed and Vegetative Propagation		Various bees	Extremely tiny seeds, smaller than openings in exclusion bag	No
Tradescantia ohiensis	Yes	2	3	Seed	Late spring-mid summer	*Bombus*, long-tongued bees	Bloom period very limited, 1 day/flower	No
Zizia aurea	Yes	3	3	Seed	5/26 – 6/17 Ave 15 days	Butterflies, large and small metallic bees, *Bombus*		Yes



Appendix 3. Spatial distribution of study sites in northeast Cook County, Illinois, USA. (1) Chicago Botanic Garden, (2) Waters School Community Garden, (3) Peggy Notebaert Nature Museum, (4) Chicago Center for Green Technology, and (5) Chicago City Hall.

Appendix 4: *Asclepias tuberosa* Seed Set Data

A. tuberosa, a native Illinois prairie species was present at the green roof on Chicago City Hall, the prairie at the Chicago Botanic Garden, and the prairie at the Peggy Notebaert Nature Museum. Because of the unique nature of pollination (the use of polinia rather than individual pollen grains), supplemental pollination treatments were not possible. However, I still estimated the fruit set at the three sites to see if there was any evidence of effective pollination occurring on the green roof. Overall, I found the mean fruit/flower ratio extremely low in all locations. However, the mean fruit/flower ratio on the roof (0.061 ± 0.017) was surprisingly higher than the mean fruit/flower ratio on the ground (0.021 ± 0.001) (Figure A4a). While only one green roof location was used for this comparison, there were butterflies present here frequently, particularly when *A. tuberosa* was in bloom (Figure A4b).

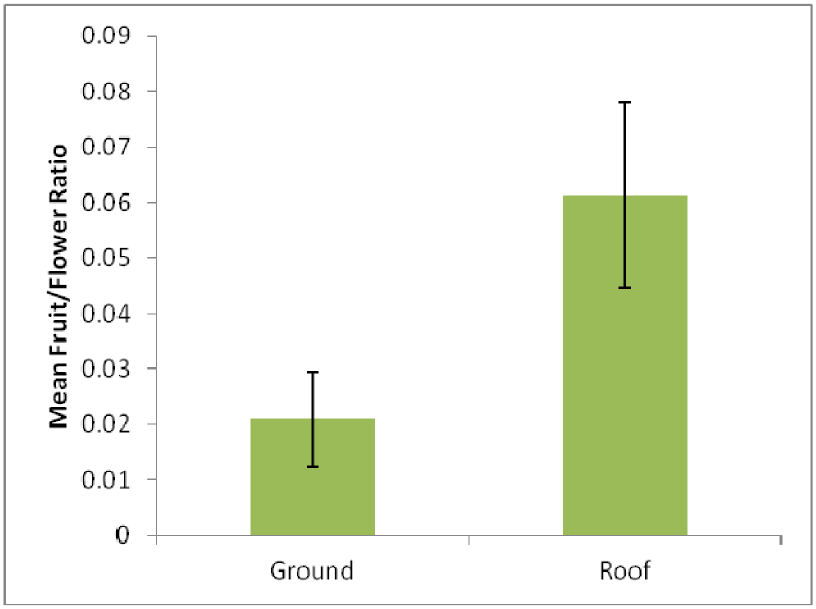


Figure A4a: Comparison of mean fruit/flower ratio in *A. tuberosa* in restored prairies versus a green roof.

Figure A4b: Butterflies were abundant on the green roof when *A. tuberosa* was in bloom.