

May 2016

# Consequences of Loss of an Abundant Pollinator: An Experimental Study

Allysa Hallett

*University of Wisconsin-Milwaukee*

Follow this and additional works at: <https://dc.uwm.edu/etd>



Part of the [Biology Commons](#), [Ecology and Evolutionary Biology Commons](#), and the [Plant Sciences Commons](#)

---

## Recommended Citation

Hallett, Allysa, "Consequences of Loss of an Abundant Pollinator: An Experimental Study" (2016). *Theses and Dissertations*. 1147.  
<https://dc.uwm.edu/etd/1147>

This Thesis is brought to you for free and open access by UWM Digital Commons. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of UWM Digital Commons. For more information, please contact [open-access@uwm.edu](mailto:open-access@uwm.edu).

CONSEQUENCES OF LOSS OF AN ABUNDANT POLLINATOR FOR  
POLLINATION SUCCESS: AN EXPERIMENTAL STUDY

by

Allysa C. Hallett

A Thesis Submitted in  
Partial Fulfillment of the  
Requirements for the Degree of

Master of Science  
in Biological Sciences

at

The University of Wisconsin-Milwaukee

May 2016

# CONSEQUENCES OF LOSS OF AN ABUNDANT POLLINATOR FOR POLLINATION SUCCESS: AN EXPERIMENTAL STUDY

by

Allysa Hallett

The University of Wisconsin-Milwaukee, 2016  
Under the Supervision of Professor Jeffrey D. Karron

Pollinator populations are declining worldwide, and this may lower the quantity and quality of pollination services. Since pollinators often compete for floral resources, loss of an abundant pollinator species may release others from competition and potentially alter floral visitation rates. We explored how the removal of a frequent pollinator, bumble bees, influenced pollination success of whorled milkweed (*Asclepias verticillata*). In three small and three large populations we quantified pollinator visitation rates and pollination success for control plots and for plots where bumble bees were experimentally excluded. We found that exclusion of bumble bees did not reduce *A. verticillata* pollination success. Visitation by *Polistes* wasps increased markedly (293%) following bumble bee exclusion, especially in large populations (400%). Because *Polistes* wasps were just as efficient as bumble bees at pollen transport, increased wasp visitation offset lost bumble bee pollination services. This study provides a vivid example of the challenges associated with forecasting how pollinator declines may influence pollination success. When pollinator loss is followed by a shift in the composition of visiting pollinator species, implications for pollination success will depend on the net change in the quantity and quality of pollination services.

## TABLE OF CONTENTS

	PAGE
Abstract .....	ii
List of Figures .....	iv
List of Tables .....	v
SECTION	
I. Introduction .....	1
II. Methods .....	3
Study species .....	3
Study populations .....	4
Experimental manipulation: bumble bee exclusion .....	4
Pollinaria receipt and pollinia removal .....	5
Pollinia transport .....	6
III. Results .....	9
Pollinator visitation rates .....	9
Pollinaria receipt and pollinia removal .....	9
Pollinia transport .....	10
IV. Discussion .....	10
V. Conclusions .....	13
V. Figures .....	14
VI. Tables .....	19
VII. References .....	23

## LIST OF FIGURES

	PAGE
Figure 1 .....	14
Figure 2 .....	15
Figure 3 .....	16
Figure 4 .....	17
Figure 5 .....	18

## LIST OF TABLES

	PAGE
Table 1 .....	19
Table 2 .....	20
Table 3 .....	21
Table 4 .....	22

## INTRODUCTION

Nearly 90% of flowering plant species depend on animal pollinators for pollen transport (Ollerton et al. 2011), and plant reproductive success may therefore be sensitive to loss of pollination services (Potts et al. 2010, Burkle et al. 2013, González-Varo et al. 2013). Evidence is accumulating that pollinator populations are declining worldwide (Potts et al. 2010), as indicated by decreased population sizes and/or reduced ranges, especially for bumble bees (Goulson et al. 2008, Grixti et al. 2009, Cameron et al. 2011, Kerr et al. 2015) and butterflies (Parmesan et al. 1999, Breed et al. 2012). In turn, these declines may influence the amount and source of pollen deposited on stigmas of flowering plant species (Aizen and Harder 2007). Pollinator species often differ substantially in their contributions to plant reproductive success due to differences in the number of visits per flower and the amount of pollen deposited per visit (Motten et al. 1981, Sahli and Conner 2007). Therefore, the severity of the effects of pollinator loss for a focal plant species will depend on the effectiveness of the remaining pollinators, and the subsequent net change in pollination services.

Pollinators often compete for floral resources (Fort 2014), so the loss of an abundant pollinator may release competing pollinator species from competition. This may increase visitation rates by less frequent visitors, or may lead to recruitment of additional pollinator species to a focal plant species or population (Makino and Sakai 2005, Nagamitsu et al. 2010, Brosi and Briggs 2013, Song and Feldman 2014). The release of pollinators from competition may thus strengthen existing plant-pollinator interactions or allow new plant-pollinator interactions to form (Mommott et al. 2007, Kaiser-Bunbury et al. 2010).

How the decline or loss of a pollinator influences plant reproductive success depends on both the lost pollinator's visitation frequency prior to decline and its pollen transfer efficiency

(i.e. the proportion of pollen transferred from an insect's body to a receptive stigma; Inouye et al. 1994, Theiss et al. 2007). If a declining pollinator species was historically a frequent visitor and efficient pollinator (Vázquez et al. 2005, Sahli and Conner 2007), plant reproductive success may decrease unless the pollinator decline is offset by increased visitation from other pollinator species. Increased visitation by other pollinators may sustain or even increase pollination success following pollinator decline (Madjidian et al. 2008), depending on the magnitude of pollinator recruitment and the pollen transfer efficiency of the remaining pollinator species.

The effects of pollinator loss may also vary as a function of plant population size. Small populations may have fewer pollinator species (Lamont et al. 1993, Rathcke and Jules 1993) and lower visitation rates (Mustajärvi et al. 2001). In addition, following pollinator loss, pollinators may preferentially recruit to large populations (Mustajärvi et al. 2001), which provide greater floral rewards than small populations. Consequently, loss of a common pollinator species may have a disproportionate effect on pollination success of small and large populations (Bernhardt et al. 2008).

Here we explore how a change in pollinator species composition interacts with plant population size to influence pollination success. Bumble bees, wasps, and honey bees are all effective pollinators of whorled milkweed, *Asclepias verticillata* (Theiss et al. 2007). By experimentally removing bumble bees, a frequent visitor to *A. verticillata* at our study site, we induce a shift in the assemblage of pollinators visiting this species. Our manipulation allows us to address whether bumble bee exclusion: 1) influences the visitation rate of competing pollinator species; 2) influences whorled milkweed pollination success; and 3) differentially influences visitation rates and pollination success in small and large whorled milkweed populations.



## METHODS

### Study species

*Asclepias verticillata* is a self-incompatible perennial herb that is pollinated by a diversity of nectar-foraging hymenoptera (Macior 1965, Willson et al. 1979, Theiss et al. 2007). Plants typically produce a single stem with 2-5 umbels, each with 8-15 small white flowers (Fig. 1a). Each flower has 5 reflexed petals and 5 nectar-containing tubular floral hoods (Fig. 1b). Pollen grains are packaged en masse in paired saccate pollinia (Fig. 1c). Each pollinarium (two pollinia joined via translator arms to a corpusculum) contains 60-75 pollen grains (Wyatt et al. 2000, Fig. 1c). Pollinaria are presented in between the floral hoods, for a total of 5 pollinaria (10 pollinia) per flower. Flowers have two ovaries, each with 30-60 ovules (Wyatt and Broyles 1994, Wyatt et al. 2000). Stigmatic chambers are also located in between the floral hoods, for a total of 5 stigmatic chambers per flower. As a pollinator forages for nectar, its legs settle in between the floral hoods, inadvertently picking up pollinia (Fig. 2). As the pollinator continues foraging, some of the removed pollinia are inserted into stigmatic chambers of flowers on other plants in the population (Macior 1965, Theiss et al. 2007).

In the present study 27% of pollinator visits to *A. verticillata* plants in control plots were by the bumble bee *Bombus griseocollis*, 10% were by *Bombus impatiens*, 31% were by wasps, and 32% were by honey bees. Of the three most common wasp genera, 62% of the visits were by *Polistes* (*P. fuscatus* and *P. dominula*), 28% were by *Vespula* spp., and 10% were by *Sphex* (*S. pensylvanicus* and *S. ichneumonous*). In field plots we were able to reliably distinguish visitors to genus.

## **Study populations**

We studied six naturally occurring *A. verticillata* populations at the University of Wisconsin-Milwaukee Field Station (Saukville, Wisconsin, USA). All six populations occur in old agricultural fields with sandy soils. The three ‘small’ populations have 200-300 plants, and the three ‘large’ populations have 3,000 to 4,000 plants. All six populations were separated from neighboring populations by at least 50 meters. *A. verticillata* blooms from early August to early September in our study area. We performed our field research during the first three weeks of August, 2014.

## **Experimental manipulation: bumble bee exclusion**

To explore how changes in pollinator species composition influence pollination success, we experimentally excluded bumble bees from plots at each study population. We compared pollinia removals and pollinia insertions in both control plots, and in plots with bumble bees excluded. We also monitored pollinator visitation to determine whether visitation rates by other pollinators changed in response to bumble bee exclusion.

In all six populations we established 4 plots, each with an area of 0.91 m<sup>2</sup>. No species other than *A. verticillata* were flowering in these plots. In each plot we controlled plant density by removing individual plants so that density was limited to 11-13 plants/ m<sup>2</sup>. In two replicate plots we experimentally excluded bumble bees by gently tapping approaching bees with a 1.2m white fiberglass rod. This technique chased the bee out of the plot, but did not harm the bee, disrupt other visiting pollinators, or prevent visitation to plants outside the plot. The remaining

two replicate plots in each population were ‘controls’ and did not receive the bumble bee exclusion treatment. In each exclusion plot, an observer prevented bumble bee visitation during the peak period of pollinator visitation: 9:30 to 15:30 local daylight savings time. Bumble bee exclusion and control treatments were performed in three populations at a time over a 4- or 5-day study period. The entire exclusion study lasted 9 days: 4 days for the first set of three populations and 5 days for the second set. For each study period, we chose three populations based on flowering phenology. We used two large populations and one small population during the first study period, and one large and two small during the second study period. All plots were caged outside the window of pollinator exclusion to ensure that no visits occurred. Prior to caging we removed flowers that were already open to ensure that flowers collected at the end of the experiment were visited only during the exclusion window.

During bumble bee exclusion and control treatments we monitored the number of pollinator arrivals to each plot during two 20-minute observation periods each day, for a total of 160 or 200 minutes of observation per plot over a 4- or 5-day period. Total observation time across populations was 80 hours.

### **Pollinaria removal and pollinia receipt**

At the end of the last day of bumble bee exclusion we sampled *A. verticillata* flowers to quantify pollinaria removal and pollinia receipt. Most flowers were open for 2-5 days during the pollinator exclusion window, and we preferentially collected older flowers to ensure adequate exposure to pollinator visitation. In each of the 4 plots in all 6 populations we collected 8 flowers from each of 2 umbels on each of 10 plants (16 flowers/plant). Flowers were frozen until

dissection. Using a dissecting microscope, we counted the number of pollinaria removed and the number of pollinia inserted for each flower. Pollinators remove pollinia in pairs (each pollinia pair is called a pollinaria), but frequently insert only a single pollinium into a stigmatic chamber. Multiple insertions rarely occur within a single stigmatic chamber, which we counted as a single insertion because a single pollinium is sufficient for full seed production. Insertions describe pollination success since, once inserted, pollen grains germinate and grow down the style toward the ovaries.

### **Pollinia transport**

To better understand the role of bumble bees, wasps, and honey bees in pollinia transport, we collected pollinator specimens near our study populations on two days within a week of completion of the exclusion experiment. Specimens were haphazardly collected on and near flowers at midday. We sampled at least 19 individuals in each pollinator group, and counted the number of corpuscula and pollinia attached to the mouthparts and legs of each individual using a dissecting microscope. Since no other *Asclepias* species flower concurrently in the area, all corpuscula and pollinia present on collected pollinators were from *A. verticillata*. Because corpuscula remain attached to insects after pollinia deposition, we used corpuscula load to estimate pollinia transfer in addition to pollinia load (Kephart and Theiss 2003, Theiss et al. 2007). Corpuscula load, which includes corpuscula with and without attached pollinia, provides a measure of the number of pollinia removed by an individual pollinator. In contrast, corpuscula lacking connected pollinia (Fig. 2a,b) approximate the number of pollinia that have been inserted into *Asclepias* flowers. A ratio of these two variables represents the approximate proportion of

pollen transfer (Inouye et al. 1994, Theiss et al. 2007). Therefore, we could estimate pollinia transfer efficiency as:

$$[ (2 \times \text{corpuscula load}) - \text{pollinia load} / (2 \times \text{corpuscula load}) ].$$

Corpuscula load includes corpuscula without attached pollinia, corpuscula with one pollinium, and corpuscula with two pollinia. Multiplying corpuscula load by two approximates the number of pollinia removed by an insect. Since pollinia load is a measure of individual pollinia, subtracting pollinia load from the number of pollinaria removed accounts for whether one or two pollinia remain attached to a given corpusculum. This measure assumes that pollinia are only inserted into flowers and are not lost during transport.

## **Data analyses**

### *Pollinator visitation rates*

Statistical analyses were conducted with JMP v. 12.0.1 (SAS Institute, Inc., Cary, NC). We used ANOVAs to explore how exclusion of bumble bees and population size class influenced pollinator visitation to *Asclepias verticillata*. We first tested whether visitation by the three main pollinator groups (*Bombus*, wasps, and *Apis*) was affected by bumble bee exclusion and population size class. We then compared visitation among the different wasp visitors (*Polistes*, *Vespula*, and *Sphex*) to interpret the overall response of wasps to bumble bee exclusion. Lastly, we tested whether overall visitation (total visitation summed across pollinator groups) was influenced by bumble bee exclusion. For each model, we included bumble bee exclusion (control

or exclusion) and population size class (small or large) as fixed main effects. We also included a bumble bee exclusion by population size class interaction in the model, as well as population and plot terms. Population was nested within population size class and plot was nested within bumble bee exclusion, population, and population size class. The bumble bee exclusion  $\times$  population size class interaction term helps determine whether the effect of bumble bee exclusion varied with population size. There were 24 samples in each analysis, representing the mean number of visits to each of the 24 plots (4 plots in each of 6 populations) for each pollinator group. For all visitation models plot effects were non-significant ( $p > 0.2$ ) so plot was pooled with error.

#### *Pollinaria removal and pollinia receipt*

We used ANOVAs to test for an effect of bumble bee exclusion on pollinaria removal and pollinia receipt. Bumble bee exclusion and population size class were our fixed main effects. We included a bumble bee exclusion by population size class interaction in the model, as well as population and plot terms. Population was nested within population size class, and plot was nested within bumble bee exclusion, population, and population size class. There were 230 samples in each analysis, representing the number of pollinaria removed and pollinia inserted to 16 flowers of each of 230 plants (10 plants from each of 23 plots). Of the 24 original plots, one plot (10 plants) was excluded from the analysis because wasps infiltrated the exclusion cage during the experimental window. Plot effects were non-significant ( $p > 0.5$ ) for both removal and receipt models, so plot was pooled with error.

#### *Pollinia transport*

We used one-way ANOVAs to test for differences in pollinia load, corpuscula load, and transport efficiency between pollinator groups (*Bombus*, *Polistes*, and *Apis*). We then performed a *post-hoc* Tukey's HSD test to determine which pollinator groups differed significantly from one another. We sampled a total of 80 individual pollinators: *Bombus* [n = 24], *Polistes* [n = 37], and *Apis* [n = 19].

## RESULTS

### Pollinator visitation rates

In control plots, the three main pollinator groups (*Bombus*, wasps, and *Apis*) each represented approximately one third of the total floral visitation to *A. verticillata* (Fig. 3A). Following bumble bee exclusion, the rate of wasp visitation increased significantly (293% overall, regardless of population size; Table 1), with a 92% increase in small populations and a 313% increase in large populations (Fig. 3A). *Polistes* accounted for 70% of the increase in wasp visitation (Fig. 3B). Visitation by *Polistes* increased in both small and large populations (Table 2), but the increase was especially pronounced (400% increase) in large populations (Fig. 3B). *Apis* visitation decreased by 26% in small populations and increased by 118% in large populations following bumble bee exclusion (Table 1, Fig. 3A). Overall visitation showed a strong interaction between bumble bee exclusion and population size class, with an overall increase in visitation upon exclusion (fixed effect model;  $p = 0.005$ ) and an even stronger response in larger populations (interaction  $p = 0.0002$ , Fig. 3a).

### **Pollinaria removal and pollinia receipt**

Pollination success did not vary in response to bumble bee exclusion treatment or population size class ( $p > 0.4$  for all effects in the removal model except for population size class, for which  $p = 0.07$ ; Table 3, Fig. 4). Although marginally significant, the population size class effect is too small to be biologically meaningful even if it were significant (a 2.5% difference between size classes; means and standard errors are  $2.04 \pm 0.01$  and  $1.99 \pm 0.02$  for large and small populations, respectively; Fig. 4). The number of pollinaria removed and pollinia inserted were also unaffected by population size class, and there was no interaction between bumble bee exclusion and population size class (Table 3).

### **Pollinia transport**

Pollinia load, corpuscula load, and pollinia transport efficiency differed significantly among pollinator groups (Table 4, Fig. 5). *Polistes* and *Apis* individuals carried more *A. verticillata* pollinia and corpuscula than *Bombus* (Fig. 5A,B). However, despite carrying fewer pollinia and corpuscula, *Bombus* pollinia transport efficiency was nearly equal to that of *Polistes* (Fig. 5C). *Apis* transport efficiency was significantly lower than that of both *Bombus* and *Polistes* (Fig. 5C).

## **DISCUSSION**

Exclusion of a frequent and efficient pollinator did not reduce *A. verticillata* pollination success. Wasp visitation increased nearly three-fold following bumble bee exclusion, suggesting that



wasps were released from competition. Increased wasp visitation compensated for lost visitation by bumble bees.

Several recent papers have suggested that pollinator declines are likely to lower pollination success (Biesmeijer et al. 2006, Lundgren et al. 2013, Thomann et al. 2013). In one of the few studies to explore this hypothesis experimentally, Brosi and Briggs (2013) removed the most locally abundant bumble bee species from populations of *Delphinium barbeyi*. The authors found that *Delphinium* reproductive success declined following manipulation, even though plants still received pollination services from several other pollinator species. Our results, in contrast, provide evidence that pollination services offered by competing pollinator species can offset loss of an abundant pollinator. Increased visitation by wasps preserved pollination success and prevented decline in pollination function despite bumble bee loss. This finding underscores that pollinator losses may not always reduce pollination success, and that a direct link between pollinator decline and plant reproduction should not be assumed.

Whether a change in the composition of visiting pollinator species affects pollination success may depend on the effectiveness of the lost and remaining pollinator species. While we found no difference in pollinia transport efficiency between bumble bees and *Polistes* wasps, increased visitation by *Polistes* wasps compensated for the loss of bumble bee visits. Because both taxa are highly efficient at removal and insertion of *A. verticillata* pollinia (Theiss et al. 2007), the compensation in visitation rate played a crucial role in maintaining *A. verticillata* pollination success. Changes in visitation rate may be particularly important indicators of the consequences of pollinator declines for plant reproductive success (Vázquez et al. 2005, Madjidian et al. 2008), especially in cases where the net change in pollinator efficiency is minimal.

The magnitude of post-decline pollinator recruitment may depend on the size of flowering plant populations. We found that more *Polistes* wasps were recruited to large *A. verticillata* populations than to small populations. Bumble bee exclusion likely reduced nectar consumption by bumble bees. Increased nectar availability in plots with reduced bumble bee visitation may, therefore, have promoted increased foraging by other pollinator species (Thomson 1988). Furthermore, because large plant populations may sustain more pollinator individuals, exclusion plots in large populations may have attracted proportionally more *Polistes* wasps from the surrounding population than exclusion plots in small populations. This suggests that pollinator recruitment to small plant populations may be limited by the number of pollinator individuals or species, which may restrict the ability of remaining pollinators to offset the effects of pollinator loss. Though we found that plant population size did not independently influence pollinator visitation, pollinator composition, or pollination success, the size of a plant population may influence patterns of subsequent pollinator recruitment.

The present study explored how pollinator loss in local patches influenced visitation by competing pollinator species. However, patterns of pollinator visitation and pollination success may differ with declines across larger landscapes. Following small-scale pollinator loss the remaining pollinators may readily compensate for the local decline of a common pollinator. In contrast, a landscape-wide decline might exhaust the capacity of other pollinators in the region to increase recruitment. Therefore, it is possible that reduction in pollination services following broad, landscape-wide declines of bumble bees would not have been offset by recruitment of *Polistes* wasps.

Our findings, in conjunction with those of Brosi and Briggs (2013), suggest that the effects of pollinator decline on pollination success can vary among plant species and ecological

contexts. This emphasizes the need for additional experimental studies of the effects of pollinator loss on pollination success. Such studies may be especially important for assessing how changes in pollination services influence populations of rare or endangered plant species, and can also be used to explore the implications of pollinator decline at the community level, especially in fragmented habitats.

## **Conclusions**

We temporarily excluded bumble bees from experimental plots in small and large populations of *A. verticillata*. Our results demonstrate that pollinator loss need not always lead to a decline in pollination success. However, the effects of pollinator decline on pollination success may vary widely among species, and may even vary among populations. Our work suggests that pollinator declines may shift the composition of visiting pollinator species, and that the consequences of decline or loss may hinge on the net change in quantity and quality of pollination services.

## FIGURES

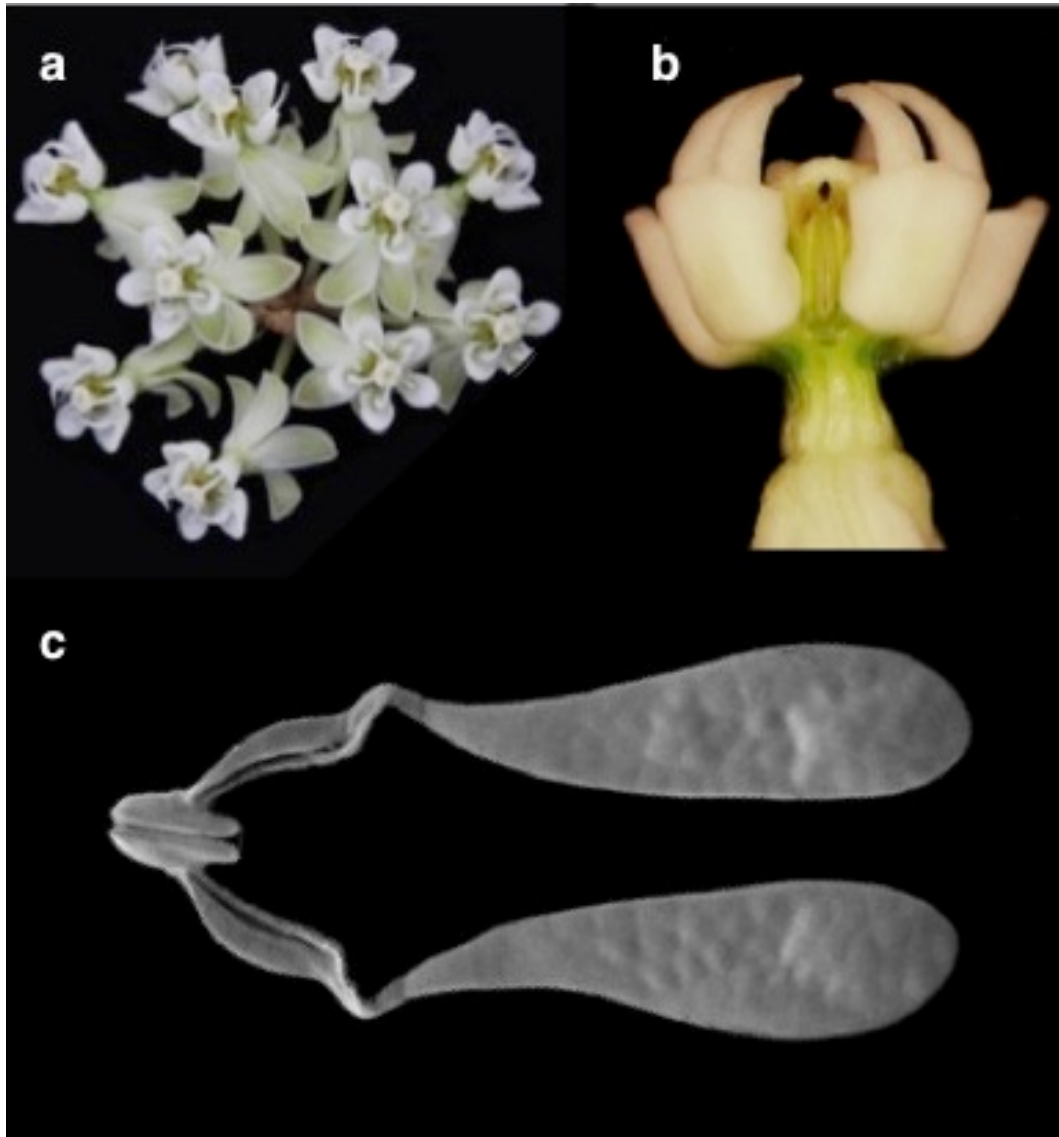


Figure 1. Floral morphology and pollinarium structure for *Asclepias verticillata*. a) umbel with 11 flowers; b) view of a corona showing the corpuscula of a pollinarium between adjacent tubular hoods; c) scanning electron micrograph of an *A. verticillata* pollinarium taken at 80x magnification. The corpusculum is the oval structure between the two pollinia of each pollinarium.

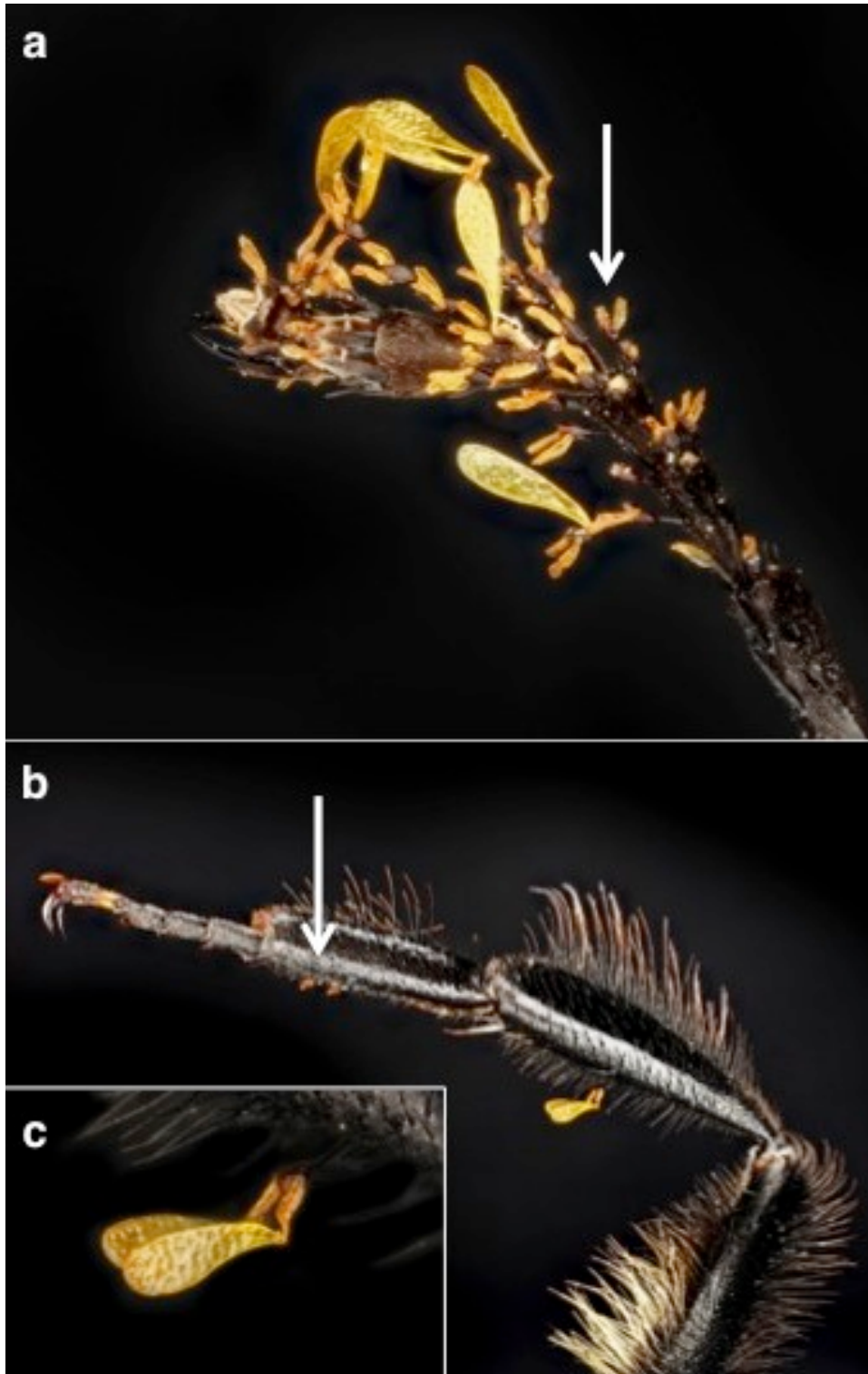


Figure 2. Pollinia and corpuscula loads on a) a *Polistes* tarsus and b,c) a *Bombus* claw, tarsus, and tibia. Pollinia may travel alone (a) or in pairs (b, c) and are always attached to a corpusculum when on insect legs. The white arrows (a,b) point to corpuscula with remaining translator arms.

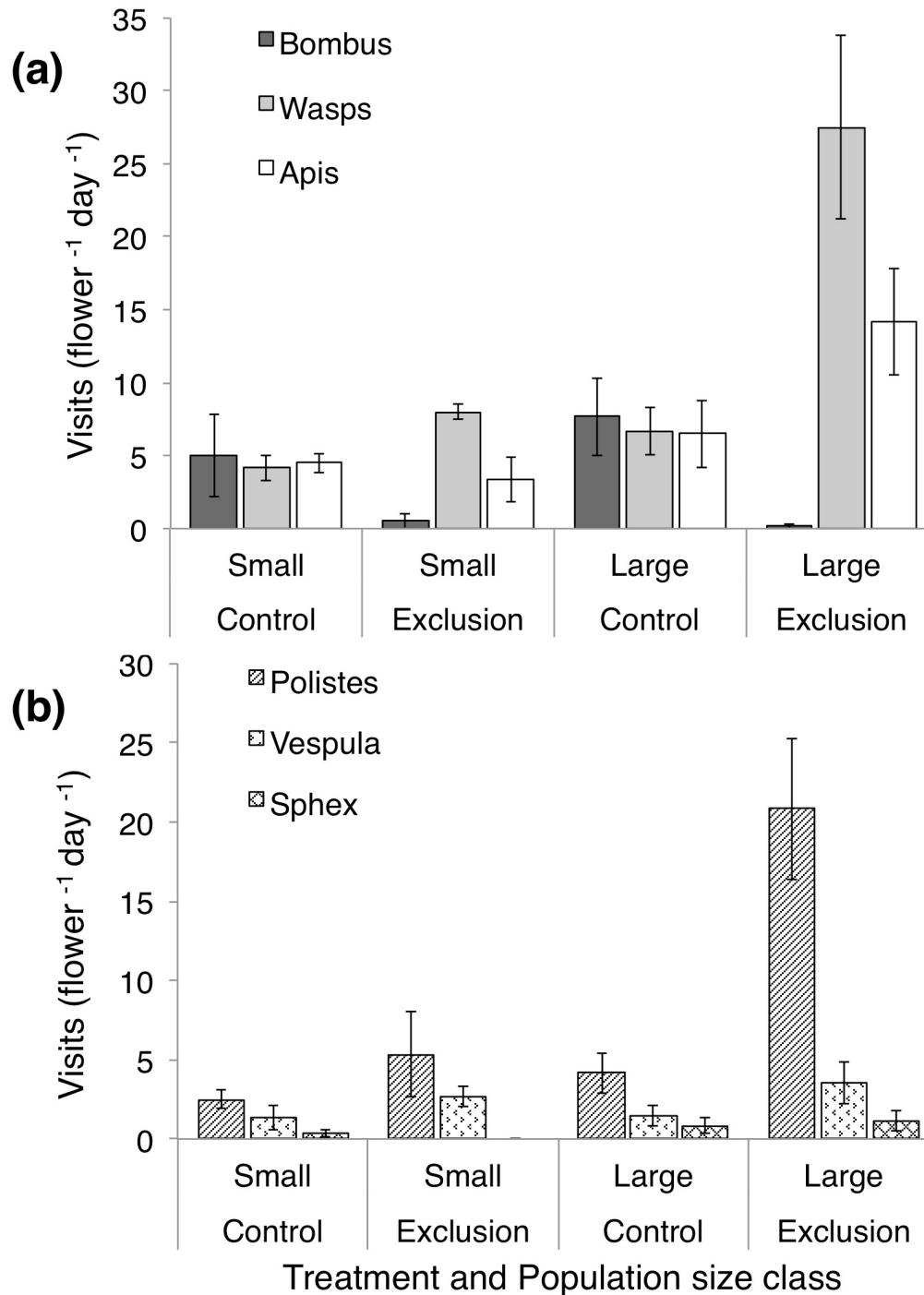


Figure 3. Rate of pollinator visitation to *Asclepias verticillata* flowers in control and bumble bee exclusion plots, and in either small or large populations of *A. verticillata*. Panel A) shows visitation by *Bombus*, wasps, and *Apis*. Panel B) shows visitation by the most common wasp genera composing the wasp category in the first panel, *Polistes*, *Vespula*, and *Spheg*. Bars display means  $\pm$  SE of plot-level data ( $n = 6$  plots/bar, measured over 4 or 5 days depending on population).

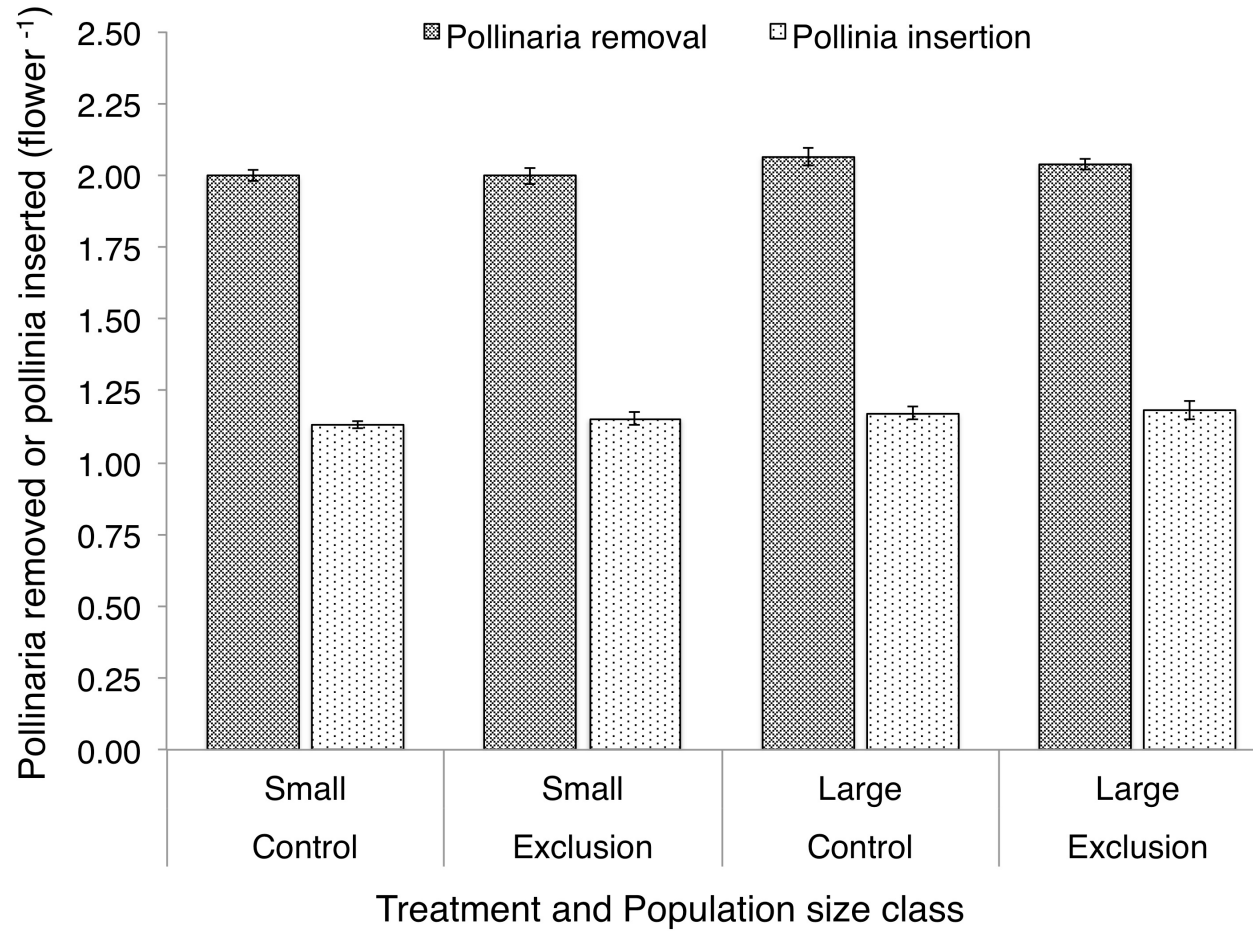


Figure 4. Number of *Asclepias verticillata* pollinaria removed (dark grey) and pollinia inserted (light grey) per flower in control and exclusion plots, and in either small or large populations. Each pollinaria represents two pollinia. Bars display means  $\pm$  SE of plant-level data ( $n = 60$  plants/bar).

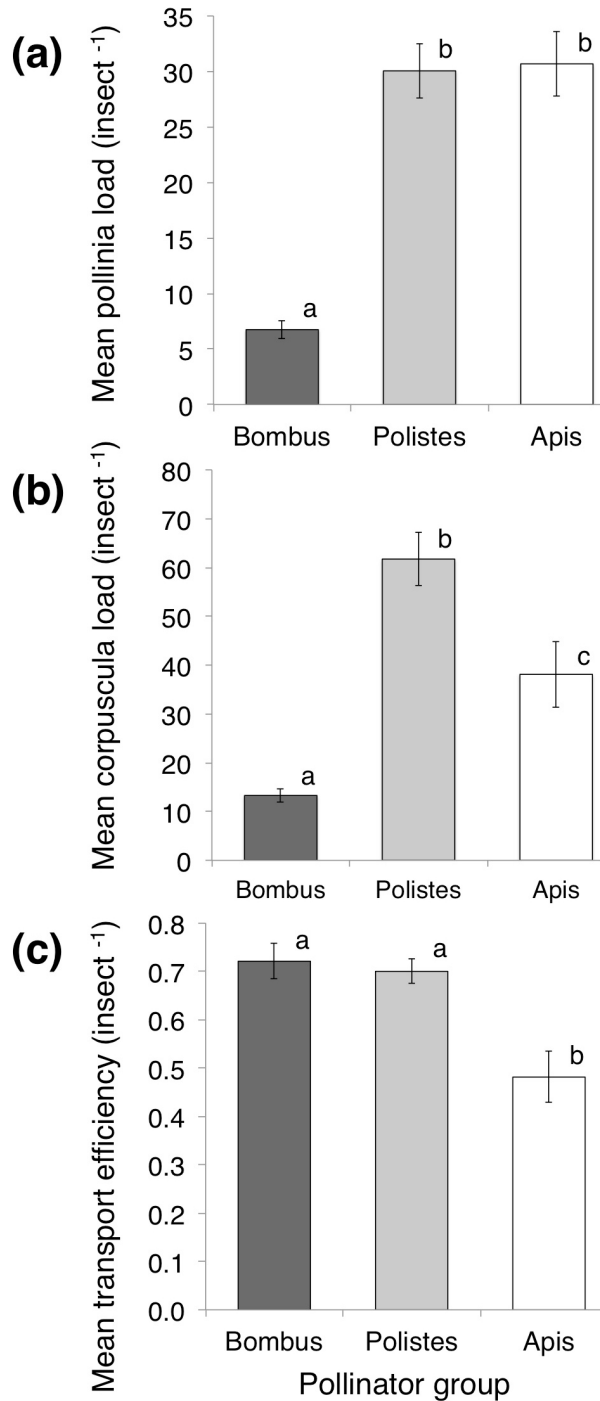


Figure 5. A) Numbers of *Asclepias verticillata* pollinia per insect across pollinator groups. B) Numbers of *A. verticillata* corpuscula per insect across pollinator groups. C) Efficiency of transport of *A. verticillata* pollinia across pollinator groups. Pollinia transport efficiency is a measure of the proportion of *A. verticillata* pollinia removed by a pollinator that are subsequently inserted into other *A. verticillata* flowers. Bars display means  $\pm$  SE for each pollinator group (*Bombus* [n = 24], *Polistes* [n = 37], and *Apis* [n = 19]). Letters denote significant differences between pollinator groups (*post-hoc* Tukey's HSD test).



## TABLES

Table 1. Effect of bumble bee exclusion and population size class on pollinator visitation to *Asclepias verticillata*. We used ANOVAs to compare visitation rates by three pollinator groups (*Bombus*, wasps, and *Apis*). We calculated mean visitation rate to each of 24 plots (2 replicate plots of each treatment per population for 6 populations).  $R^2 = 0.59$  (*Bombus*), 0.80 (wasps), and 0.68 (*Apis*). Significant values are in bold.

Response	Source	df	MS	F	<i>p</i>
<i>Bombus</i>	Bumble bee exclusion / control	1	12.1126	<b>12.24</b>	<b>0.003</b>
	Population size class	1	0.2926	0.12	0.7
	Bumble bee exclusion $\times$ population size class	1	0.5859	0.59	0.5
	Population [population size class]	4	2.4542	2.48	0.09
	Error	16	0.9892		
Wasps	Bumble bee exclusion / control	1	48.0251	<b>20.31</b>	<b>0.0004</b>
	Population size class	1	36.1376	3.45	0.14
	Bumble bee exclusion $\times$ population size class	1	21.7551	<b>9.20</b>	<b>0.008</b>
	Population [population size class]	4	10.4630	<b>14.60</b>	<b>0.01</b>
	Error	16	2.3646		
<i>Apis</i>	Bumble bee exclusion / control	1	3.6038	3.30	0.08
	Population size class	1	12.7604	3.48	0.14
	Bumble bee exclusion $\times$ population size class	1	5.9004	<b>5.40</b>	<b>0.03</b>
	Population [population size class]	4	3.6705	<b>3.36</b>	<b>0.04</b>
	Error	16	1.0931		

Table 2. Effect of bumble bee exclusion and population size class on wasp visitation to *Asclepias verticillata*. We used ANOVAs to compare visitation by wasps in the genera *Polistes*, *Vespula*, and *Sphex*. We calculated mean visitation rate to each of 24 plots (2 replicate plots of each treatment per population for 6 populations).  $R^2 = 0.81$  (*Polistes*), 0.44 (*Vespula*), and 0.71 (*Sphex*). Significant values are in bold.

Response	Source	df	MS	F	p
<i>Polistes</i>	Bumble bee exclusion / control	1	30.6004	<b>23.79</b>	<b>0.0002</b>
	Population size class	1	21.8504	3.81	0.12
	Bumble bee exclusion $\times$ population size class	1	13.9538	<b>10.85</b>	<b>0.005</b>
	Population [population size class]	4	5.7283	<b>4.45</b>	<b>0.013</b>
	Error	16	1.2865		
<i>Vespula</i>	Bumble bee exclusion / control	1	0.8251	<b>3.50</b>	<b>0.080</b>
	Population size class	1	0.1001	0.20	0.7
	Bumble bee exclusion $\times$ population size class	1	0.0651	0.28	0.6
	Population [population size class]	4	0.4920	2.09	0.13
	Error	16	0.2354		
<i>Sphex</i>	Bumble bee exclusion / control	1	0.0001	0.01	0.94
	Population size class	1	0.1751	1.27	0.3
	Bumble bee exclusion $\times$ population size class	1	0.0301	1.55	0.3
	Population [population size class]	4	0.1376	<b>7.12</b>	<b>0.002</b>
	Error	16	0.0193		

Table 3. Effect of bumble bee exclusion and population size class on removal and receipt (insertion) of *Asclepias* pollinia. We measured mean pollinaria removal and pollinia receipt for 10 plants (16 flowers per plant) from each of 23 plots (2 replicate plots of each treatment per population for 6 populations, one plot excluded).  $R^2 = 0.03$  for both pollinaria removal and pollinia receipt.

Source	df	MS	F	p
Pollinaria removal				
Bumble bee exclusion / control	1	0.0039	0.10	0.7
Population size class	1	0.1307	6.64	0.07
Bumble bee exclusion $\times$ population size class	1	0.0174	0.47	0.5
Population [population size class]	4	0.0201	0.54	0.7
Error	222	0.0373		
Pollinia receipt				
Bumble bee exclusion / control	1	0.0145	0.50	0.5
Population size class	1	0.0436	1.26	0.3
Bumble bee exclusion $\times$ population size class	1	0.0102	0.35	0.6
Population [population size class]	4	0.0345	1.19	0.3
Error	222	0.0291		

Table 4. One-way ANOVAs testing for differences amongst pollinator groups in numbers of *Asclepias* pollinia, numbers of *Asclepias* corpuscula, and pollinia transport efficiency by individual insects. Pollinia transport efficiency is a measure of the proportion of *A. verticillata* pollinia removed by a pollinator that are subsequently inserted into other *A. verticillata* flowers. We sampled a total of 80 individual pollinators (*Bombus* [n = 24], *Polistes* [n = 37], and *Apis* [n = 19]).  $R^2 = 0.45$  (pollinia load), 0.45 (corpuscula load), and 0.24 (pollinia transport efficiency). Significant values are in bold.

Source	df	MS	F	p
Pollinia load				
Pollinator group	2	4648.4500	<b>32.03</b>	<b>&lt; 0.0001</b>
Error	77	145.1500		
Corpuscula load				
Pollinator group	2	17228.7000	<b>31.55</b>	<b>&lt; 0.0001</b>
Error	77	546.1000		
Pollinia transport efficiency				
Pollinator group	2	0.4123	<b>12.48</b>	<b>&lt; 0.0001</b>
Error	77	0.0330		

## LITERATURE CITED

- Aizen MA, Harder LD (2007) Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* 88:271–281.
- Bernhardt CE, Mitchell RJ, Michaels HJ (2008) Effects of population size and density on pollinator visitation, pollinator behavior, and pollen tube abundance in *Lupinus perennis*. *Int J Plant Sci* 169:944–953.
- Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffer AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin W (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313:351–4.
- Breed GA, Stichter S, Crone EE (2013) Climate-driven changes in northeastern US butterfly communities. *Nat Clim Chang* 2:1–4.
- Brosi BJ, Briggs HM (2013) Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proc Natl Acad Sci USA* 110:13044–8.
- Burkle L, Marlin J, Knight T (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 80:1611–1615.
- Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL (2011) Patterns of widespread decline in North American bumble bees. *Proc Natl Acad Sci USA* 108:662–7.
- Fort H (2014) Quantitative predictions of pollinators' abundances from qualitative data on their interactions with plants and evidences of emergent neutrality of emergent neutrality. *Oikos* 123:1469–1478.
- González-Varo JP, Biesmeijer JC, Bommarco R, Potts SG, Schweiger O, Smith HG, Steffan-Dewenter I, Szentgyörgyi H, Woyciechowski M, Vilà M (2013) Combined effects of global

- change pressures on animal-mediated pollination. *Trends Ecol Evol* 28:524–30.
- Goulson D, Lye GC, Darvill B (2008) Decline and conservation of bumble bees. *Annu Rev Entomol* 53:191–210.
- Gixti JC, Wong LT, Cameron SA, Favret C (2009) Decline of bumble bees (*Bombus*) in the North American Midwest. *Biol Conserv* 142:75–84.
- Inouye DW, Gill DE, Dudash MR, Fenster CB (1994) A model and lexicon for pollen fate. *Am J Bot* 81:1517–1530.
- Kaiser-Bunbury CN, Muff S, Memmott J, Müller CB, Caflisch A (2010) The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. *Ecol Lett* 13:442–52.
- Kephart S, Theiss K (2003) Pollinator-mediated isolation in sympatric milkweeds (*Asclepias*): do floral morphology and insect behavior influence species boundaries? *New Phytol* 161:265–277.
- Kerr JT, Pindar A, Galpern P, Packer L, Potts SG, Roberts SM, Rasmont P, Schweiger O, Colla SR, Richardson LL, Wagner DL, Gall LF, Sikes DS, Pantoja A (2015) Climate change impacts on bumblebees converge across continents. *Science* 349 :177–180.
- Lamont BB, Klinkhamer PGL, Witkowski ETF (1993) Population fragmentation may reduce fertility to zero in *Banksia goodii*: a demonstration of the Allee Effect. *Oecologia* 94:446–450.
- Lundgren R, Lázaro A, Totland Ø (2013) Experimental pollinator decline affects plant reproduction. *J Pollinat Ecol* 11:46–56.
- Macior LW (1965) Insect adaptation and behavior in *Asclepias* pollination. *Bull Torrey Bot Club* 92:114–126.

- Madjidian JA, Morales CL, Smith HG (2008) Displacement of a native by an alien bumblebee: lower pollinator efficiency overcome by overwhelmingly higher visitation frequency. *Oecologia* 156:835–845.
- Makino TT, Sakai S (2005) Does interaction between bumblebees (*Bombus ignitus*) reduce their foraging area?: bee-removal experiments in a net cage. *Behav Ecol Sociobiol* 57:617–622.
- Mayfield M, Waser N, Price M (2001) Exploring the “most effective pollinator principle” with complex flowers: bumblebees and *Ipomopsis aggregata*. *Ann Bot* 88:591–596.
- Memmott J, Craze PG, Waser NM, Price MV (2007) Global warming and the disruption of plant-pollinator interactions. *Ecol Lett* 10:710–7.
- Motten AF, Campbell DR, Alexander DE, Miller HL (1981) Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica*. *Ecology* 62:1278–1287.
- Mustajärvi K, Siikamäki P, Rytkönen S, Lammi A (2001) Consequences of plant population size and density for plant-pollinator interactions and plant performance. *J Ecol* 89:80–87.
- Nagamitsu T, Yamagishi H, Kenta T, Inari, N, Kato, E (2010) Competitive effects of the exotic *Bombus terrestris* on native bumble bees revealed by a field removal experiment. *Popul Ecol* 52:123–136.
- Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? *Oikos* 120:321–326.
- Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thoma, CD, Descimon H, Huntley B, Kaila L, Kullberg J, Tammaru T, Tennent WJ, Thomas JA, Warren M (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399:579–583.

- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25:345–53.
- Rathcke B, Jules E (1993) Habitat fragmentation and plant-pollinator interactions. *Curr Sci* 65:273–277.
- Sahli HF, Conner JK (2007) Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *Am J Bot* 94:203–209.
- Song Z, Feldman MW (2014) Adaptive foraging behaviour of individual pollinators and the coexistence of co-flowering plants. *Proc R Soc B* 281:20132437.
- Theiss K, Kephart S, Ivey CT (2007) Pollinator effectiveness on co-occurring milkweeds (*Asclepias*; Apocynaceae, Asclepiadoideae). 94:505–516.
- Thomann M, Imbert E, Devaux C, Cheptou P (2013) Flowering plants under global pollinator decline. *Trends Plant Sci* 18:353–359.
- Thomson JD (1988) Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. *Evol Ecol* 2:65–76.
- Vázquez DP, Morris WF, Jordano P (2005) Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol Lett* 8:1088–1094.
- Willson MF, Bertin RI, Price PW (1979) Nectar production and flower visitors of *Asclepias verticillata*. *Am Midl Nat* 102:23–35.
- Wyatt R, Broyles SB (1994) Ecology and evolution of reproduction in milkweeds. *Annu Rev Ecol Syst* 25:423–441.
- Wyatt R, Broyles SB, Lipow SR (2000) Pollen-ovule ratios in milkweeds (Asclepiadaceae): an exception that probes the rule. *Syst Bot* 25:171–180.