MULTIYEAR STUDY OF POLLEN LIMITATION AND COST OF REPRODUCTION IN THE ITEROPAROUS *SILENE VIRGINICA*

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Abstract. We investigated whether pollen deposited onto stigmas limited female reproductive success in the hummingbird-pollinated, short-lived, iteroparous, *Silene virginica* (Caryophyllaceae). The study was conducted over a 4-yr span in a population occurring in a woodland area and over a 3-yr span in a second population occurring in a nearby open meadow. We contrasted average fruit set, seed set per fruit, and total annual seed production (in only the woodland site) between open-pollinated control plants and hand-pollinated experimental plants. We also followed surviving individuals in subsequent years and repeated the same treatments on plants when they flowered. All plants were monitored annually for survival and reproduction at both sites; growth was monitored at only the woodland site because of extensive herbivory in the meadow population.

Lack of pollen deposited onto stigmas significantly limited reproductive potential at the level of percentage fruit set throughout the study for both sites. In contrast, pollen deposition onto stigmas exhibited considerable site and year heterogeneity in its effect on seed production per fruit. In the cumulative test of pollen limitation, however, we detected no difference between total annual seed production between our open-pollinated control and hand-pollinated experimental plants in the woodland site during the 4-yr study. A weak negative trend was detected between fruit set per plant and average seed set per fruit among all plants in the woodland site, suggesting a limited role for an intraplant compensation mechanism. No significant trade-off was detected in probability of survival and flowering between the control and hand-pollinated experimental treatment groups at either site. In addition, no cost was detected in future growth and reproduction in the woodland population. Similar total seed production among individuals in the two treatment groups explains in part, why no difference was observed in future survival, growth, and reproduction between the control and hand-pollinated treatment groups.

Key words: cost of reproduction; multiyear study; perennial herb; pollen limitation; Silene virginica.

INTRODUCTION

Quantifying the extent to which pollen is limiting to female reproductive success is necessary for a more precise understanding of the selective forces responsible for the evolution of floral traits. For example, the degree of pollen limitation will determine the role of female mate choice in plants (e.g., Charlesworth et al. 1987), the opportunity for selection on floral traits to enhance seed production through increased pollination efficiency (e.g., Inouye et al. 1994), greater floral attractiveness (e.g., Dudash 1993) or changes in the mating system (e.g., Dudash and Ritland 1991, Fenster and Ritland 1994).

Lifetime effects of pollen limitation on female reproductive success in terms of the quantity of progeny produced among individuals can be readily assessed in annuals. However, studies of pollen limitation in iteroparous taxa conducted over a single year are less likely to predict the role of pollen limitation on lifetime reproduction for several reasons. Pollen limitation in

iteroparous taxa can vary among years (e.g., Campbell 1987, Vaughton 1991, Holm 1994) and can occur in conjunction with resource limitation (e.g., Zimmerman and Pyke 1988, Ehrlén 1992) either concurrently within a flowering season and/or subsequently exhibit a cost between flowering seasons. Thus, there may be an interaction between pollen limitation and future costs of reproduction (e.g., Janzen et al. 1980, Caswell 1986, Zimmerman and Pyke 1988). Therefore, pollen limitation of iteroparous taxa in a given year must be assessed within the context of how present reproduction affects future reproduction. One might expect a priori to observe greater future costs in plants subject to current reproductive enhancement via hand-pollinations than unmanipulated control plants. Thus the consistency of pollen limitation across years in iteroparous taxa and the cost of artificially enhanced reproduction in terms of future survival, growth, and reproduction relative to the benefits of artificially enhanced reproduction need to be assessed.

Pollen limitation is often examined at the level of seed and fruit set, but the definitive test is whether lifetime seed production differs between control and

hand-pollinated plants. Total seed production can be readily quantified in annual taxa, although it is rarely reported (but see Karoly 1992). In iteroparous taxa total seed production for one flowering season (e.g., Bierzychudek 1981, Holtsford 1985, Lawrence 1993, Lehtilä and Syrjänen 1995) and sometimes two flowering seasons (Flanagan and Moser 1985, Lubbers and Lechowicz 1989) has been assessed. In a recent review of the pollen limitation literature Burd (1994) found that 3% (8/258), 33% (86/258), and 80% (207/258) of the species investigated for pollen limitation were examined at the level of annual total seed production, seed set per fruit, and fruit set, respectively. Part of the reason for the limited number of studies investigating total seed set is the difficulty in quantifying the large numbers of flowers/fruits produced by single individuals, especially in iteroparous trees and shrubs. Nonetheless, it is important that we quantify the effect of pollen limitation on total seed production in order to fully understand its ecological and evolutionary significance.

Data are equivocal on the cost of reproduction in iteroparous plants exhibiting pollen limitation. Pollen limited seed set per fruit in the herbaceous *Viscaria vulgaris* (Jennersten 1991), seeds per plant in herbaceous *Geranium maculatum* (Ågren and Willson 1992), and fruit set and seeds per plant in the Australian shrub *Teleopa speciosissima* (Whelan and Goldingay 1989), for one season, while no cost was observed in response to the reproductive enhancement treatment the following year. Pollen limitation of fruit set in the shrub *Banksia spinulosa* differs between years with no evidence of cost between the control and hand-pollinated treatment groups (Vaughton 1991). Evidence of costs to reproduction in long-lived orchids is more consistent than the herbaceous/shrub literature and suggests that an increase in fruit production via supplemental hand-pollinations results in a reduction in growth and/or reproduction in subsequent year(s) (e.g., Ackerman and Montalvo 1990, Calvo and Horvitz 1990, Primack and Hall 1990). In *Primula veris,* pollen supplementation significantly enhanced: (1) seed production per plant compared to control plants; and (2) subsequent survival, growth, and reproduction compared to the control group the following year (Lehtilä and Syrjänen 1995).

To date, there are few multiyear, serial pollen limitation studies on iteroparous taxa and the potential subsequent fitness costs associated with artificially induced reproductive enhancement. Ultimately, we need to understand what factors contribute to variation among individuals in lifetime reproductive success and whether their relative importance changes as a function of life history strategy. Thus the primary goals of our study were to determine if iteroparous *Silene virginica* female reproductive effort was pollen limited in two sites during the 1991–1994 flowering seasons. Secondly we asked whether a difference in ''cost'' was incurred across years between the control and the hand-pollinated treatment groups. We anticipated that experimental plants, in which every flower produced over the course of the season was supplementally hand-pollinated, would have reduced survival, growth, and reproduction in subsequent years, relative to the open-pollinated control plants. We compared the effects of our two treatment groups at three levels: fruit set, average seed set per fruit, and annual total seed production per plant. Analysis of pollen limitation should be sensitive to examination at different levels within individuals, since it reflects the modular construction of reproductive parts in plants.

MATERIALS AND METHODS

Study organism

Silene virginica (Caryophyllaceae) is a short-lived perennial found in eastern North America. It flowers from late May to early July at our study sites near Mountain Lake Biological Station (Allegheny Mountains, Giles County, Virginia, elevation ≈ 1330 m). The bright red flowers are protandrous and highly outcrossing (C. B. Fenster and M. R. Dudash, *unpublished data*). The primary pollen vector of *Silene virginica* is the Ruby-throated Hummingbird, *Archilochus colubris,* with occasional visits by syrphid flies and small solitary bees (M. R. Dudash and C. B. Fenster, *unpublished data*). *Silene virginica* exhibits little evidence of clonal spread, and any additional leaf production occurs along the aboveground stem and is clearly interconnected upon inspection. A noctuid moth larva, *Hadena ectypa,* is a major herbivore on both flowers and developing fruits of *Silene virginica* at our study sites.

Study site

We investigated whether pollen limited female reproductive success of *Silene virginica* in a woodland site for 4 yr (1991–1994) and in an open meadow site for 3 yr (1991–1993). Monitoring of the meadow site was discontinued after 1993 owing to extensive herbivory by deer and woodchuck. Plants in the woodland site occurred in the shaded understory of an oak–hickory and formerly chestnut forest. Plants in the meadow site were growing without a canopy cover along a power line cut. Approximately 2.5 km separated the two study areas, and the meadow site is ≈50 m higher in elevation than the woodland site.

Pollen limitation experimental design

Each year in late May plants producing an inflorescence were randomly chosen along two permanent adjacent 10 m wide × 100 m long vertical transects in the woodland site and one 10 m wide × 100 m long vertical transect at the meadow site. At least 50 new plants per site were chosen each year in a stratified random fashion along the transect and randomly assigned to either of two treatment groups: (1) open-

pollinated (control), where each flower was labeled with a jeweler tag the day it became female; and (2) supplementally pollinated (experimental), where each flower was also labeled with a jeweler tag upon becoming female and hand-pollinated with pollen from donor plants at least 2.5 m away from the pollen recipient each day of the female phase (1–4 d). Plants were stratified by treatment such that an open-pollinated control always occurred near a supplementally hand-pollinated experimental. To confirm that newly chosen plants in our two treatment groups were indistinguishable from one another, we examined whether the two treatment groups in each site and year differed in total flower production, ovules per pistil, and plant, flower, and fruit herbivory levels. Percentage fruit set and average seed production per fruit among individuals in the two treatment groups were examined in the woodland and meadow site throughout 1991–1993. Because of extensive herbivory in the meadow site, total annual seed production is examined between the two pollination treatment groups only in the woodland site across the 4 yr of the study.

Any returning flowering plants that were included in the study in previous years were also followed and assigned the same treatments as in previous years. Percentage fruit set and average seed production per fruit were also examined in the woodland and meadow site, including both newly chosen plants in any given year and any returning individuals each year. Only 19 and 34 individuals flowered more than once and showed little herbivory in the woodland and meadow sites, respectively. A repeated-measures approach was not undertaken owing to the unpredictability of reappearance of individuals. In no instance did these returning plants change the outcome of the analyses for any variable measured. Thus additional analyses that differ in only the incorporation of both newly chosen plants and any returning (previously chosen) plants together for any given year are not shown since no new information was revealed.

Each fruit was collected 18 d after its last day of female phase, ≈4 d prior to natural fruit dehiscence. In the laboratory each fruit was scored for herbivory, fruit initiation, and total seed set per fruit in uneaten fruits under a dissecting microscope. Ovule number per pistil was the total number of seed, unfertilized ovules, and aborted seeds. Actual numbers of individuals in each treatment group by year are included on the figures. Sample sizes differ between data on fruit set and seed set per fruit because we can readily observe a fruit that formed but if upon closer inspection the seeds were eaten, we lost information on seed set per fruit for that data point. Finally, in the field we observed that not all flowers become functional females, e.g., some flowers have styles that often do not fully emerge from the corolla tube and are never receptive even if hand-pollinated. Since every flower was examined daily in both the open-pollinated and hand-pollinated group we could track which flowers never functioned as females in both groups, and they were omitted from the study.

Reproductive cost

Survival and reproductive status (flowering or nonflowering) of the two pollination treatment groups were monitored annually in both the meadow and woodland sites from 1991 to 1994. An additional cost assessment of both reproductive and nonreproductive performance was conducted in the woodland site, where total flower production, number of stems, total stem length, number of basal leaves, and the length and width of the longest leaf as well as a composite variable (length × width of the longest leaf) were measured each year. Relative growth was quantified as the ratio of growth of year ($t + 1$)/year t. Extensive herbivory in the meadow site each year prevented us from assessing differences in growth in this population.

STATISTICAL ANALYSES

Pollen Limitation

We performed t tests (PC SAS version 6.04, SAS 1987) to compare our control and experimental groups each year at each site and examine whether they differed in overall performance characters. The characters measured were total flower production (log transformed), average ovule production per pistil (square-root transformed), and percentage floral herbivory (arcsine square-root transformed), which might have influenced or biased the outcome of our contrasts between treatment groups.

ANOVA was performed (PROC GLM) to assess specifically whether pollen deposition onto stigmas limited percentage fruit set (arcsine square-root transformed), and mean seed set per fruit (square-root transformed) between the two treatment groups in a comparative study between the woodland and meadow site. For years 1991–1993, where both sites were part of the experiment, the dependent variables were analyzed with the same mixed-model ANOVA with year treated as a random effect and pollination treatment and site as fixed effects. We chose to make site a fixed effect owing to our a priori expectations that the enclosed understory habitat of the woodland site may differ in hummingbird visitation rates compared to the relatively exposed habitat of the open meadow site. However, we also performed all analyses with site as a random effect and in no case did the interpretation of the treatment effects differ. The SAS RANDOM statement with the TEST option was used to produce the error mean squares for the mixed-model ANOVA hypothesis tests. This approach requires that a linear combination of mean squares be constructed and F tests using a Satterthwaite (1946) approximation are generated. This approximation may generate fractional degrees of freedom in the denominator and/or negative F values (pooling the most nonsignificant mean square into the error

TABLE 1. Analysis of variance for the dependent variable ''arcsine square root of percentage fruit set per plant'' of the control and experimental treatment groups in *Silene virginica* during 1991–1993 in the woodland and meadow sites. Mean squares are based on type III sums of squares.

Source	df	MS	F	P
Year	2	1.88	1.41	0.417
Site	1	0.13	0.10	0.785
Pollination treatment	1	1.75	19.36	0.047
Year × Site	2	1.34	14.42	0.065
Year × Treatment	2	0.09	0.97	0.507
Site × Treatment	1	0.0002	0.002	0.967
Year × Site × Treatment	2	0.09	0.49	0.610
Error	264	0.19		

term usually produces a traditional positive term). The assumptions of ANOVA were met for all analyses. All figure treatment group means have been backtransformed for presentation along with 2 SE.

Data from the woodland site from all 4 yr (1991–1994) were incorporated into one additional analysis to examine whether percentage fruit set, average seed set per fruit per individual, and total annual seed production per individual (square-root transformed) varied significantly between the two treatment groups. Data were analyzed with a two-way mixed-model ANOVA with pollination treatment as a fixed effect and year as a random effect. An analysis of covariance was performed in conjunction with the total seed production per individual to determine if any other important variables were masking the effect of pollination treatment. The multiple covariates used in this analysis were total flower production and average ovule production per pistil by each plant in this portion of the study. Finally, to determine if an intraplant compensation mechanism might be occurring we performed Pearson product moment correlation analyses between percentage fruit set and average seed set per fruit among individuals for each year in the woodland site.

Reproductive cost

Survival and frequency of flowering in both the woodland and meadow sites of the control and hand-pollinated treatment groups were tested with chi-square analyses (Pyke and Thompson 1986), Fisher's exact test (Rosner 1990) where appropriate and a sequential Bonferroni test (Rice 1989). In the woodland site *t* tests were performed to determine if significant differences in relative growth occurred between the two treatment groups. Owing to low survivorship of plants across years, comparisons of relative growth between the open-pollinated control and hand-pollinated experimental groups were conducted by pooling plants across similar yearly intervals. Total flower production, number of stems, total stem length, number of basal leaves, and the length and width of the longest leaf as well as a composite variable (length × width of the longest leaf) were measured each year. Relative growth was quantified as the ratio of growth of year $(t + 1)$/year t.

RESULTS

Treatment group comparison

Total flower production, mean ovule number per pistil, and floral herbivory levels did not vary significantly among plants randomly assigned to the two pollination treatment groups at each site during each year of the study. None of the *t* tests demonstrated significant differences (maximum *t* value = 1.059, $P = 0.295$), and the rank order of the control and experimental pollination treatment groups oscillated from year to year and site to site. Annual average total flower production per individual during the study ranged from 2.7 to 5.1 and 3.7 to 5.5 in the woodland and meadow sites, respectively. Annual mean ovule number per pistil at the whole-plant level ranged during the study from 58 to 66 and 55 to 64 in the woodland and meadow sites, respectively. Overall annual yearly floral herbivory levels ranged from <1 % to 22% in the woodland site and from 11 to 32% in the meadow site throughout the study.

The recorded flower production and herbivory levels in the meadow do not take into account whole flower stem herbivory that frequently occurred prior to flowering at the meadow site. Therefore, overall size estimates of meadow individuals are greatly reduced compared to plants in the woodland site where essentially every flower was monitored that could be produced by each individual in the study.

Pollen limitation

Fruit production.—Supplemental hand-pollinations significantly increased the probability of fruit set during 1991–1993 (Table 1, Fig. 1). No significant main effects of year or site were detected. One marginally significant interaction occurred between year × site ($F_{2,2} = 14.42$, $P = 0.065$, Table 1). The inclusion of fruit set data from the woodland site during 1994 with years 1991–1993 again demonstrated an overall significant effect of pollination treatment ($F_{1, 3.88} = 22.87$, $P = 0.009$, Fig. 1a).

Seed production per fruit.—Supplemental hand-pollinations did not significantly increase average seed production per fruit once a fruit was initiated among
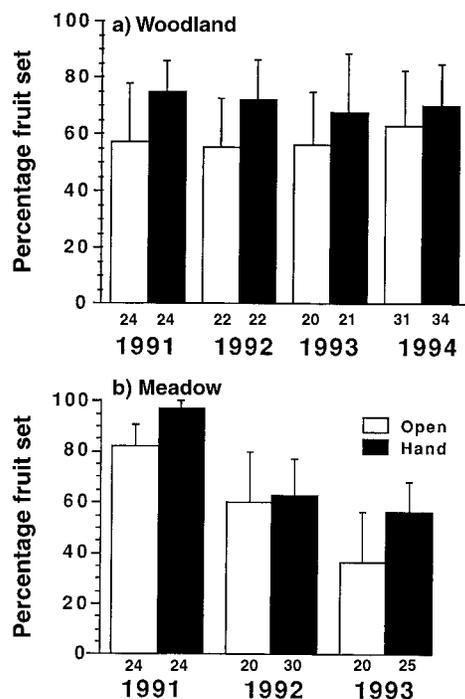
FIG. 1. Comparison of percentage fruit set of control and hand-pollinated treatment groups of *Silene virginica* across 4 and 3 yr in the (a) woodland and (b) meadow sites, respectively. Plants were randomly chosen each year, and sample sizes are below each bar. Error bars show 2 SE in one direction.
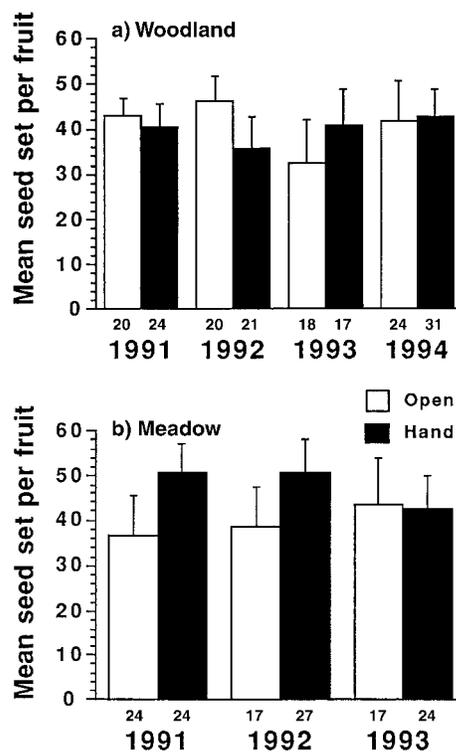

FIG. 2. Comparison of mean seed set per fruit of control and hand-pollinated treatment groups of *Silene virginica* across 4 and 3 yr in the (a) woodland and (b) meadow sites, respectively. Plants were randomly chosen each year, and sample sizes are below each bar. Error bars show 2 SE in one direction. Ovule number per pistil did not significantly vary between the control and treatment groups across years.

individuals at either site (Table 2, Fig. 2). No main effects of site or year were detected. A significant year × site × treatment interaction was detected ($F_{4, 241} =$ 4.60, $P = 0.026$), suggesting the presence of pollen limitation during 1991 and 1992 at the meadow site (Fig. 2b). No significant difference in average seed set per fruit between the treatment groups was observed at the woodland site alone from 1991 to 1994 (Fig. 2a, $F_{1, 3.04} = 0.06$, $P = 0.828$).

Correlation analyses.—We combined treatment groups across years in the woodland site to increase our sample sizes to examine the relationship between percentage fruit set and average seed set per fruit

among individuals (Table 3). Three of the four annual Pearson correlation coefficients were negative and one (1993) was very close to zero, allowing us to test whether the correlation coefficients were homogeneous (Sokal and Rohlf 1981; $\chi^2 = 3.083$, 3 df, $P = 0.379$). Since our yearly correlation coefficients were found homogeneous we asked whether there was an overall significant correlation since only year 1994 alone exhibited a significant relationship. A joint overall analysis that combined probabilities from the independent tests of significance provides marginal evidence of a weak negative relationship between percentage fruit set and average seed set per fruit among individuals across

TABLE 2. Analysis of variance for the dependent variable ''square root of mean seed set per fruit per plant'' of the control and experimental treatment groups in *Silene virginica* during 1991–1993 in the woodland and meadow sites. The year × site interaction was most nonsignificant and pulled into the error term to remove a negative F value. Mean squares are based on type III sums of squares.

Source	df	MS	F	P
Year	2	1.29	1.46	0.406
Site	1	4.63	1.01	0.371
Pollination treatment	1	4.00	4.50	0.166
Year × Treatment	2	0.86	0.19	0.831
Site × Treatment	1	8.37	1.83	0.247
Year × Site × Treatment	4	4.36	4.60	0.026
Error	241	1.64		

TABLE 3. The relationship between percentage fruit set and mean seed production per fruit among individuals of *Silene virginica* throughout 1991–1994 in the woodland site where both pollination treatment groups have been combined.

Year	Sample size	Correlation coefficient	P
1991	44	−0.09570	0.5366
1992	41	−0.18424	0.2488
1993	35	0.03882	0.8248
1994	55	−0.32602	0.0151

TABLE 4. Analysis of variance for the dependent variable ''square root of total seed production per plant'' of the control and experimental treatment groups in *Silene virginica* throughout 1991–1994 in the woodland site. Mean squares are based on type III sums of squares.

Source	df	MS	F	P
Year	3	63.24	3.78	0.152
Pollination treatment	1	8.79	0.53	0.519
Year × Treatment	3	16.74	1.91	0.129
Error	167	8.74		

the 4 yr in the woodland site independent of treatment group ($r = -0.166$, $P = 0.119$; Sokal and Rohlf 1981).

Total seed production per plant.—In the woodland site from 1991 to 1994 annual total seed production of flowering individuals was not limited by their access to pollen (Table 4). The analysis of multiple covariates (total flower production and ovule production per pistil) with total seed production per individual as the dependent variable did not alter the pollination treatment effects as shown in Table 4 (analysis of covariance not shown). Across all 4 yr the average annual seed production was ≈81 (ranging from 4 to 370 seeds) and 74 (ranging from 5 to 305) seeds per plant in the control and hand-pollinated treatment groups, respectively. Thus, on average, a plant is annually producing seed from slightly more than one mature fruit, while average individual flower production ranges from 2.7 to 5.1 flowers in the woodland site. Individuals that flowered during 2 yr of the study at the woodland site produced an average cumulative seed production of 181 seed (SD = 103.2, range 36–367) and 185 seed (SD = 96.9, range 97–289) for the 10 plants in the experimental treatment group and 3 plants in the control group, respectively. These cumulative estimates produce a yearly estimate of ≈ 90 seeds per plant. This is slightly greater than the overall estimates above. However, the three experimental plants that flowered during 3 out of the 4 yr averaged 160 seeds per plant (SD = 69.5, range 91–230), and on an annual basis their total seed production is considerably less than the overall and 2-yr annual seed production estimates per plant in the study.

Reproductive cost

Measured in terms of future survival and reproduction, we detected no cost associated with supplemental hand-pollination. There was an advantage, sometimes significant, in an unexpected direction, i.e., plants in the experimental group occasionally had a higher probability of surviving and reproducing than plants in the control group (Table 5: refer to meadow and woodland survival in both 1991–1992, 1991–1993, and 1991–1994 and meadow reproduction in 1991–1992). The control and hand-pollinated groups did not differ in their reproductive status (flowering vs. nonflowering), nor did they differ in total flower production, total stem length, number of stems, length and width of the longest leaf, and number of basal leaves at the woodland

site (Table 6). Three-year interval comparisons were not possible because of inadequate sample sizes associated with low survivorship.

DISCUSSION

Pollen limitation

Our study demonstrates the need to examine pollen limitation at more than one level. Pollen significantly limited the percentage of fruit set in the open-pollinated control group compared to the hand-pollinated experimental group at both sites for all years. However, we did not detect a main effect of pollen deposition on average seed set per fruit by individuals in the two treatment groups in either site but detected significant site × year × pollination treatment interactions. Furthermore, in the woodland site annual total seed production among individuals was not limited by access to pollen. We also detected a weak trend in the woodland site, suggesting that individuals maturing more fruit tend to mature fewer seeds per fruit. These data suggest caution when interpreting results investigating pollen limitation that do not reflect cumulative female reproductive effort in terms of total annual seed production at the whole-plant level. We conclude that the relatively long-lived and nectar-rich flowers (flowers may stay in the female phase up to 4 d and contain as much as 50 µL of nectar; M. R. Dudash and C. B. Fenster, *unpublished data*) appear to be adequately serviced in terms of pollen deposition by the Ruby-throated Hummingbird at the woodland site. However, both lowered fruit set and reduced seed set per fruit in some years at the meadow site suggest that total seed production among individuals may be limited by pollen deposition. Unfortunately, the extensive herbivory that we observed in the meadow precluded us from documenting the effect of pollen supplementation on total seed production at this site (Figs. 1b and 2b). In addition, it is possible that pollen limitation may have in some unstudied way affected the quality component of female reproductive success.

Our observation of yearly variation in pollen limitation at some levels is in agreement with studies conducted with other iteroparous species: fruit set of *Banksia spinulosa* (Vaughton 1991), seed set per inflorescence in *Spartina alternifolia* (Bertness and Shumway 1992), total seed production per plant in *Trillium grandiflorum* (Lubbers and Lechowicz 1989), seed set per fruit and total seed production per plant in *Aralia nudicaulis* (Flanagan and Moser 1985), seed set per fruit and fruit set in *Veronica cusickii* (Campbell 1987) and seed quality in *Betula* taxa (Holm 1994). Orchid species demonstrate more consistent pollen limitation in fruit set across years, e.g., *Tipularia discolor* (Snow and Whigham 1989), *Cyclopogon cranichoides* (Calvo 1990), *Cypripedium acaule* (Primack and Hall 1990), and *Epidendrum ciliare* (Ackerman and Montalvo 1990). Estimation of average seed set per fruit and total

TABLE 5. Percentage survival and reproduction of individuals in the experimental group where reproduction has been initially enhanced in 1991, 1992, and 1993 via supplemented hand-pollinations vs. unmanipulated controls over 1–3 yr in two natural populations of *Silene virginica*. Actual numbers of individuals that survived and reproduced over the 1–3 yr of the total are in parentheses. Cells with sample size too small to perform a chi-square or a Fisher's exact test comparison are denoted by ellipses.

	Meadow site			Woodland site		
Time period	Control	Experimental	χ^2	Control	Experimental	χ^2
One year						
Survival						
1991–1992	44% (11/25)	76% (19/25)	5.33	44% (11/25)	60% (15/25)	1.28
1992–1993	45% (15/33)	48% (15/31)	0.06	78% (21/27)	79% (19/24)	0.02
1993–1994	80% (20/25)	65% (17/26)	1.42	85% (23/27)	84% (21/25)	0.02
Average	55% (46/83)	62% (51/82)	0.79	70% (55/79)	74% (55/74)	0.42
Reproduction						
1991–1992	9% (1/11)	42% (8/19)	3.62	27% (3/11)	27% (4/15)	0.0
1992–1993	67% (10/15)	60% (9/15)	0.14	29% (6/21)	16% (3/19)	0.97
1993–1994	45% (9/20)	59% (10/17)	0.74	35% (8/23)	52% (11/21)	1.34
Average	43% (20/46)	53% (27/51)	0.88	31% (17/55)	33% (18/55)	0.04
Two year						
Survival						
1991–1993	4% (1/25)	48% (12/25)	12.58*	4% (1/25)	36% (9/25)	8.00*
1992–1994	39% (13/33)	35% (11/31)	0.10	63% (17/27)	54% (13/24)	0.39
Average	24% (14/58)	41% (23/56)	3.69	35% (18/52)	45% (22/49)	1.29
Reproduction						
1991–1993	0% (0/1)	58% (7/12)	...	0% (0/1)	56% (5/9)	...
1992–1994	46% (6/13)	55% (6/11)	0.17	18% (3/17)	38% (5/13)	1.56
Average	33% (6/14)	56% (13/23)	2.11	17% (3/18)	45% (10/22)	3.72
Three year						
Survival						
1991–1994	4% (1/25)	36% (9/25)	8.00*	0% (0/25)	24% (6/25)	...
Reproduction						
1991–1994	0% (0/1)	44% (4/9)	...	0% (0/0)	50% (3/6)	...

* $P < 0.05$ after a sequential Bonferroni test ($k = 24$).

seed production per plant is not feasible in orchids owing to the production of numerous dust-like seeds. Spatial variation among study areas in the presence of pollen limitation within a season for fruit set, seed set per fruit, and seed production per plant in *Lobelia car-dinalis* (Johnston 1991) and across seasons in fruit set for *Veronica cusickii* (Campbell 1987) is also consistent with our findings.

Overall, our results concur with Burd's (1994) recent survey of the literature pooling across all life history

TABLE 6. Yearly growth ratios for both flowering and nonflowering (vegetative) individuals of *Silene virginica* at the woodland site for 1- and 2-yr intervals following initial treatments. The 1- and 2-yr intervals are pooled across years, and the sample sizes for each comparison are in parentheses. None of the *t* test comparisons indicates a significant difference, with or without a sequential Bonferroni test ($k = 22$).

	Flowering			Nonflowering		
	Control	Experimental	t	Control	Experimental	t
One year						
Flower number	1.04 (17)	0.98 (17)	0.27			
Stem length	0.79 (16)	0.92 (16)	−0.80			
No. stems	0.84 (16)	1.10 (17)	−1.37			
Leaf length	1.08 (17)	0.85 (17)	1.06	0.92 (37)	0.81 (32)	1.65
Leaf width	0.88 (17)	0.93 (17)	−0.68	0.94 (37)	0.84 (32)	1.42
Length × Width	1.05 (17)	0.81 (17)	0.73	0.91 (37)	0.71 (32)	1.70
No. basal lvs.	0.70 (12)	0.83 (14)	−0.27	1.20 (22)	1.56 (19)	−0.66
Two year						
Flower number	0.65 (3)	1.06 (10)	−1.16			
Stem length	0.62 (3)	1.00 (9)	−1.37			
No. stems	0.83 (3)	1.11 (9)	−0.82			
Leaf length	1.02 (3)	0.93 (9)	0.45	0.70 (11)	0.78 (12)	−0.60
Leaf width	0.98 (3)	0.97 (9)	0.04	0.77 (11)	0.87 (12)	−0.53
Length × Width	0.97 (3)	0.93 (9)	0.18	0.62 (11)	0.78 (12)	−0.67
No. basal lvs.	0.11 (3)	0.40 (4)	−1.0	0.81 (10)	0.51 (7)	1.79

strategies, which demonstrated pollen limitation across taxa in fruit production, but less frequently observed effects in seed production per fruit. Burd's review covered 29, 86, and 207 species where percentage seed set, seed set per fruit, and percentage fruit set were examined, respectively. However, the magnitude in sample size differences among percentage seed set, seed set per fruit, and fruit set studies in the literature may be at least partly responsible for the less frequent detection of pollen limitation at the seed level rather than the fruit level. Finally, as reviewed above, few studies have examined cumulative annual and/or total seed production per plant in published studies of pollen limitation. The weakly documented intraplant compensation mechanism influencing fruit set and seed set per fruit within an individual may reflect either a packaging strategy to spread the risk of known herbivore attack by a commonly found noctuid moth larva on *Silene virginica* or perhaps an ongoing resource constraint.

Costs of reproduction

We detected no difference between unmanipulated open-pollinated control and hand-supplemented experimental groups in terms of future survival, growth, or subsequent reproductive bouts. This result is not surprising since we observed no difference in total annual seed production among individuals in our two treatment groups. Other researchers of long-lived perennials have quantified significant pollen limitation at one or more levels, while not detecting a cost to artificially enhanced reproduction as well. One-year follow up studies of detectable pollen limitation for seed set in a European herb *Viscaria vulgaris* (Jennersten 1991), for fruit set and seed set per plant in the Australian shrub *Teleopea speciosissima* (Whelan and Goldingay 1989), and for fruit set in a multiyear follow-up of a neotropical orchid species *Cyclopogon cranichoides* (Calvo 1990) demonstrated no demographic cost to artificially enhanced reproduction. Thus, the present study and those cited above demonstrate that current female reproductive success may often be pollen limited at some level but that higher levels of pollination would not necessarily lead to lower reproduction in the future.

Some comparisons of future survival from the first cohort in 1991 (Table 5) of *S. virginica* demonstrated a significant survival advantage of the hand-pollinated group compared to the open-pollinated control group. This suggests that in some instances supplemental hand-pollination had a positive influence on both future survival and probability of fruit set. Similar positive effects on future reproduction were observed in the long-lived European herbaceous perennial *Primula veris* (Lehtilä and Syrjänen 1995). Hypotheses put forward by Lehtilä and Syrjänen include the idea that the presence of plentiful resources precludes the detection of costs (Tuomi et al. 1983), and we cannot rule out this possibility since we were only able to detect a weak negative trend between fruit set and average seed set

per fruit among plants in the woodland site. They observed that plants with more developing fruits were able to accrue more resources overall, and enhance photosynthesis and leaf growth as well (Marshall 1990), but our growth data (Table 6) do not support this idea. Third, neither in this study nor in Lehtilä and Syrjänen (1995) was there a difference in flowering phenology between the control and treatment groups. Finally, perhaps the plants randomly placed in our pollen supplementation group in 1991 were more vigorous than the control group in some unmeasured way.

In contrast, a number of studies on perennial orchid species (reviewed by Calvo and Horvitz 1990) have demonstrated a demographic cost in reduction of growth and/or reproduction in subsequent year(s) following reproductive enhancement treatments. A 3-yr follow-up study on *Tolumnia variegata* demonstrated a relationship between the level of pollination and potential future growth and survival (Calvo 1993). Four years of data collected in two populations of the *Cypripedium acaule* (Primack and Hall 1990) demonstrated consistent pollen limitation on fruit set but inconsistent results for the detection of reproductive costs. In the year following experimental manipulation, one site exhibited an immediate decrease in plant size and probability of flowering. The other site, however, did not demonstrate these effects until 2 yr following the experimental manipulation, which significantly increased fruit set.

What factors may account for differences in the long-term consequences of pollen limitation on future survival, growth, and reproduction in perennial taxa? There are two approaches one can take to examine costs and the issue of scale. First, does a cost exist and if so, to what extent are the immediate subsequent survival and fecundity affected? Secondly, is the lifetime fitness of a plant pollen limited? Experimental manipulation of pollen enhancement addresses the short-term goal, predicting an immediate response. In perennial taxa, determination of pollen limitation on lifetime fecundity may not be attainable because of their longevity and numerous reproductive bouts. The detection of both short- and long-term costs to reproduction may also be linked to the degree of inflexibility in growth form. However, in annual and short-lived perennial taxa one can assess populations for numbers of years for the presence of pollen limitation. Abiotic and biotic environmental factors may influence these results for both annual and perennial taxa as well. Thus, variation in life history strategy among iteroparous species may influence the detection of reproductive costs on both the short- and long-term scales.

Our populations of *S. virginica* exhibited extensive mortality: only 6/50 and 10/50 plants survived for 3 yr in the woodland and meadow sites, respectively, after being chosen for the study (Table 5). Mortality measures 1 yr after each pollination treatment cohort averaged 70 and 74% for the control and treatment

groups, respectively, in the woodland site (Table 5), values similar to those found for the herb *Calathea ovandensis* (74.4% [low reproductive effort] vs. 72.9% [high reproductive effort]; Horvitz and Schemske 1988). Short-lived iteroparous species with high yearly mortality may not as readily have the opportunity to exhibit short-term trade-offs between present and future reproduction, but lifetime fecundity may be influenced because individuals have few opportunities to reproduce. In contrast, orchid species are often very long lived and can maintain either aboveground or subterranean presence for indefinite periods of time, and often exhibit a short-term cost following reproductive enhancement (e.g., Gill 1989, Calvo 1990, 1993, Primack and Hall 1990). However, the life history strategy observed here with *S. virginica* and with other short-lived perennials may minimize the long-term effects of pollen limitation influencing lifetime fecundity.

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