

## THE POLLINATION ECOLOGY OF *ORCHIS SPECTABILIS* L. (ORCHIDACEAE)<sup>1</sup>

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**ABSTRACT.** Three woodland sites were selected for collecting data on the frequency of pollination of *Orchis spectabilis* by bumblebees in northeast Ohio. A total of 82 hours was spent at 2 of these sites, which were chosen for observing the foraging of *Bombus* queens on spring ephemerals. The effect of *Aesculus glabra*, *Dicentra canadensis*, *Geranium maculatum*, *Iris pseudacorus*, and *Pedicularis canadensis* in attracting bumblebees in relation to *O. spectabilis* pollination was also studied. Of the 123 bumblebees captured, 74% foraged on flowers and 26% hunted for nesting sites.

Caging experiments on *O. spectabilis* indicated 4% self-compatibility and 100% outcrossing fertility and suggested a required animal pollen vector. In the two populations studied, 17–21% of the plants flowered while fruit production ranged 0–11%. Seed production measured by two methods averaged 3485 seeds/fruit and 6656 seeds/fruit, respectively. Both methods indicated more than adequate seed set to replace the existing populations even if only one fruit/season was produced.

*O. spectabilis* is a woodland plant species relying on chance visits by either woodland- or meadow-dwelling *Bombus* queens for pollination. The limited occurrence of this plant species in northeast Ohio has not been attributed to the low pollinator frequency it receives.

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### INTRODUCTION

*Bombus* queens emerge from hibernation in spring and effectively pollinate a variety of plant species blooming at this time (Macior 1968, 1978a). Orchid flowers are morphologically adapted for insect pollination (Pijl and Dodson 1966), and bumblebees have been documented as pollinators of some species (Ackerman 1975, Wright 1975). *Orchis spectabilis* is a native orchid endemic to the eastern United States. It is a member of the vernal flora growing in rich, moist humus of beech-maple woodlands (Auclair 1972). Gibson (1897) and Shuttleworth et al. (1970) have speculated that *O. spectabilis* is pollinated by bumblebees, but no field observations were made. The only recorded observations of *O. spectabilis* visitors were made by

Robertson (1924), who recorded *Bombus separatus* (= *B. griseocollis*) and *B. americanorum* (= *B. pennsylvanicus*). No mention of pollinial removal was made. Other observations on *O. spectabilis* were made by van Ingen (1887), Newell (1892), Ulke (1938), and Seymour (1970) regarding slits in the spur, pollination mechanism, and color variants. *Orchis* can be found growing throughout Ohio (Braun 1967) but seems to occur in small, disjunct populations. Larger, more continuous populations occur in protected areas of Wisconsin (Macior, pers. comm.).

Populations of *Aesculus glabra*, *Dicentra canadensis*, *Geranium maculatum*, *Iris pseudacorus*, *Orchis spectabilis*, and *Pedicularis canadensis* were selected for this study. All these plant species bloom in the spring and attract bumblebee queens as pollinators. A seasonal study of flower and bumblebee phenologies was designed to investigate the pollination frequency of *O. spectabilis*.

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The object of the study was to see whether *O. spectabilis* was receiving a low pollinator frequency relative to other spring flowers in the area. If so, could this be related to its limited occurrence in northeast Ohio? Nomenclature follows Gleason and Cronquist (1963) for plants and Mitchell (1962) for insects.

### STUDY SITES

The 3 largest populations of *Orchis* in the area were chosen as sites for a comparative study. Site I, located near Peninsula (Summit County), contained populations of *Dicentra*, *Aesculus*, *Pedicularis*, and *Iris*. Site II, located near Hiram (Portage County), contained mixed populations of *Geranium* and *Orchis*. Site III, located near Bath (Summit County), contained a population of *Orchis*. Site I had a population of *Orchis* too small (15 plants) for quantitative study.

Sites I and III were located within 0.5 km of farmland and open meadow, respectively. Site II bordered railroad tracks. The overstory vegetation of all sites was characteristic of a beech-maple association. Other plant genera included *Claytonia*, *Dentaria*, *Sanguinaria*, *Impatiens*, *Trillium*, *Hepatica*, *Cimicifuga*, *Viburnum*, *Botrychium*, and *Osmunda*.

### METHODS AND MATERIALS

In the spring of 1979 and 1980, I spent a total of 82 h observing insect visitors of the 6 plant species under study at sites I and II. Of these 82 h, 34.5 h were spent at site II. Observations in 1979 began 3 May and ended 9 June; in 1980 they began 28 April and ended 6 June. As many bumblebees were captured as possible and their behavior recorded. Site III provided data on the reproductive biology of *Orchis* without capturing pollinators.

Corolla reflectance for flowers of all plant species studied was determined in the laboratory using fresh flowers. A Bausch and Lomb Spectronic 20 reflecting spectrophotometer was used to measure the percent reflectance of 10 selected wavelengths. A Maxwell color diagram was plotted and the composite color determined. Bumblebees possess the ability to detect longwave ultraviolet light (Mazokhin-Porshnyakov 1962). Flowers of all species studied were examined for ultraviolet reflectance, but only those which did reflect ultraviolet light (360 nm) were photographed. Photographs

were taken using artificial ultraviolet light and a 35 mm camera equipped with a fused quartz lens and a Chance-Pilkington OX1 ultraviolet filter. I used the Kodak Grey Scale (fig. 1) to compare the degree of reflectance. The percent reflectance equivalents are as follows: 0.00 (40.5%), 0.10 (31.5%), 0.20 (19.2%), 0.30 (12.0%), 0.50 (7.0%), 0.70 (5.0%), 1.00 (3.0%), 1.30 (2.5%), 1.60 (2.0%), 1.90 (1.5%) (Macior 1978b).

Measurements were made of the tongues (prementum+glossa) of all bumblebees captured. Heads of all queens captured were soaked in 10% potassium hydroxide overnight then rinsed with water. Tongues dissected from them were mounted in glycerin jelly and measured microscopically using a calibrated ocular micrometer.

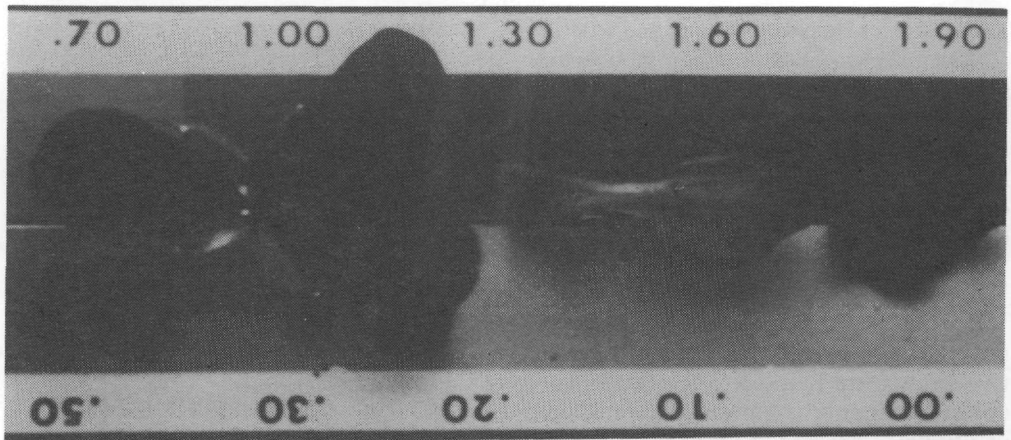
The length of the corolla tube from the point of petal fusion to the tip of the spur or tube was measured for 6 flowers per plant species.

Corbicular pollen of all bumblebees captured was dispersed in 70% ethanol and mounted in glycerin jelly tinted with methyl green. Pollen of the 6 plant species was identified microscopically, and pollen loads were classified as pure from the plant foraged on, foreign (alien to that plant species), or mixed.

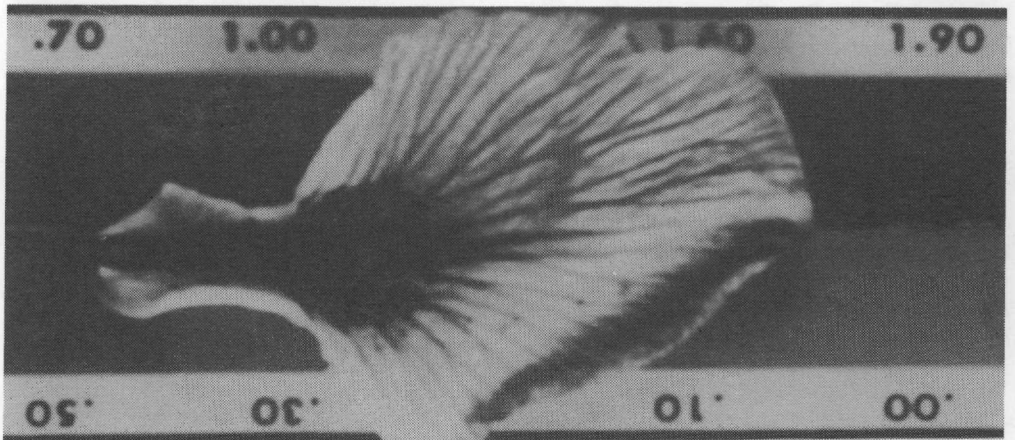
Caging of *Orchis spectabilis* was conducted at site II using galvanized mesh window screen while flowers were still in bud. A total of 14 plants with 46 flowers was caged; 25 flowers were artificially pollinated autogamously, one geitonogamously, and one xenogamously. The rest were left untouched. Extraction of pollinia was accomplished using a pencil tip. Cages were removed at the cessation of flowering.

The pollination mechanism of *Orchis* was tested 21 times using a pencil. The tip was inserted into the nectar spur and consequently came in contact with the rostellum breaking it open. The pollinia would become attached to the pencil via viscous pads originally located in the rostellum and could then be extracted from their enveloping sheaths. This is exactly what happens when a bumblebee thrusts its head into the nectar spur to suck nectar. A time period of 3 min was allowed for the caudicles to bend the pollen sacs anteriorly and horizontally. This would put the pollen sacs in a correct position for contact with the stigmatic surface if the pencil were reinserted into the flower and thereby effect pollination.

For comparative purposes, seed production per fruit capsule was measured by 2 methods. For both methods, the capsules were split and placed in a vial containing a 2:1 mixture of distilled water and 95% ethanol and allowed to stand overnight. In 1979 a 1-ml blow-out pipet was used to draw out 1 ml of solution and place one drop on a slide. In 1980 a 1-ml plastic syringe was used to draw out 0.5 ml and disperse on to quadrat ruled filter paper. The seeds were counted and the total seeds produced/capsule was estimated by accounting for dilution. Only one count/capsule was made for the



A



B

FIGURE 1. (A) Ultraviolet (360 nm) reflectance of the flower of *Geranium maculatum* (left), *Orchis spectabilis* hood and nectar spur (middle), and labellum (right). Kodak Grey Scale in background. (B) Ultraviolet (360 nm) reflectance of the fall of *Iris pseudacorus*. Kodak Grey Scale in background.

method used in 1979 and 3 were made for the method used in 1980. Seed production was determined for capsules only at site II.

Phenological records for plant and bumblebee species studied were maintained. Peak blooming for each plant species studied was only recorded in 1980 by visual determination of the greatest number of flowers in bloom during an observation week. Week assignment corresponded to consecutive 7-day periods of observation.

## RESULTS

**FLOWER AND BUMBLEBEE PHENOLOGIES.** The order of anthesis of the plants studied for both years was as follows: *Dicentra canadensis*, *Aesculus glabra*, *Geranium maculatum*, *Pedicularis canadensis*, *Orchis spectabilis*, and *Iris pseudacorus*. Peak blooming in 1980 for *Dicentra* was wk 2,

for *Aeculus* wk 3, for *Geranium* wk 5, for *Pedicularis* wk 4, for *Orchis* wk 3 and 4, and for *Iris* wk 6. All *Orchis* flowers remained in bloom through week 5.

Only *Bombus* queens were captured during the study; workers were not present. Queens emerged in the following order based on the earliest date captured: *B. bimaculatus*, *B. affinis*, *B. vagans*, *B. impatiens*, *B. perplexus*, *B. sandersoni*, *B. fervidus*, and *B. pennsylvanicus*. Bumblebee species site composition is shown in table 1. *Bombus vagans* composed 33% of the queens captured at site I in 1979 and 52% in 1980. At site II *B. vagans* comprised 60% in 1979 and 70% in 1980. The most frequent species at both sites was *B. vagans*. At site I *B. vagans* had a peak frequency of 3.00 bees/hr/wk during wk 6 in 1979 and 2.40 bees/hr/wk during wk 5 in 1980. At site II *B. vagans* had a peak frequency of 1.67 bees/hr/wk during wk 3 in 1979 and 1.00 bee/hr/wk during wk 5 in 1980. Clearly, *B. vagans* was the dominant bee species present in the area.

*Bombus fervidus*, *B. pennsylvanicus*, and *B. sandersoni* were the least represented and were not observed hunting nesting sites. *Bombus bimaculatus* was not observed in this behavior either but Macior (1978b) reports it to be woodland-dwelling. Bees in this study hunting nesting sites are considered to be woodland-dwelling; those not observed in this behavior are considered meadow- or field-dwelling. Bumblebees will forage in both habitats no matter which is preferred for nesting. Overall, the species composition and dominant species are relatively the same so that comparisons between sites are meaningful.

**POLLINATOR ACTIVITY.** Of the 123 bumblebees captured (table 1), 74% were foraging on flowers (table 2). *Bombus vagans* was the major pollinator or visitor to *Geranium*, *Orchis*, and *Iris*. Although not dominant visitors to any plant studied, *Bombus fervidus* and *B. pennsylvanicus* totalled 11% and 9%, respectively, of *Iris* pollinators.

TABLE 1  
*Bombus* queen site composition.

<i>Bombus</i> species	Total <i>Bombus</i> 1979		Queens 1980		Total	%
	Site		Site			
	I	II	I	II		
<i>affinis</i>	3	3	4	0	10	8.1
<i>bimaculatus</i>	3	0	1	1	5	4.1
<i>fervidus</i>	3	0	1	0	4	3.3
<i>impatiens</i>	14	12	5	1	32	26.0
<i>pennsylvanicus</i>	3	0	0	0	3	2.4
<i>perplexus</i>	3	0	1	1	5	4.1
<i>sandersoni</i>	1	2	0	0	3	2.4
<i>vagans</i>	15	26	13	7	61	49.6
Total	45	43	25	10	123	100.0

TABLE 2  
Total *Bombus* queens on plant species for sites I and II and total collecting time (hr).

Plant species	Time	<i>Bombus</i> species						Total
		<i>af*</i>	<i>bi</i>	<i>fe</i>	<i>im</i>	<i>pa</i>	<i>pe</i>	
<i>Aeculus glabra</i>	17.50	1			8	4		13
<i>Dicentra canadensis</i>	21.25	4	2					1 7
<i>Orchis spectabilis</i>	34.50							8 8
<i>Geranium maculatum</i>	34.50	1		1			2	18 22
<i>Iris pseudacorus</i>	10.50			4	3	3	1	26 37
<i>Pedicularis canadensis</i>	8.50	1	2					3
Total		6	5	4	12	3	4	3 53 90

\* *af* = *affinis*, *bi* = *bimaculatus*, *fe* = *fervidus*, *im* = *impatiens*, *pa* = *pennsylvanicus*, *pe* = *perplexus*, *sa* = *sandersoni*, *va* = *vagans*

The highest frequency of *B. vagans* roughly corresponds to peak flowering of *Geranium* and *Orchis* at site II and of *Iris* at site I in 1980. Because of varying weather conditions from year to year, optimal pollinator frequency will not correspond precisely to peak flowering of a plant species (Schemske et al. 1978).

*Orchis* had 8 *Bombus vagans* queens visit its flowers; 4 of which simply landed on

the labellum and then flew off. Only 4 queens thrust their heads into the nectar spur, and only one removed pollinia. In the other 3 instances, the pollinia had been previously removed.

**FLORAL ATTRACTION.** From an analysis of the visible spectral reflectance of corollas of the 6 plant species, 2 major divisions in visible color were noticed. Plant species having a peak in the violet-blue range (415-475 nm) included *Dicentra*, *Geranium*, and *Orchis*. Plant species lacking a peak in the violet-blue range and possessing one in the orange-red range (565-655 nm) included *Aesculus*, *Pedicularis*, and *Iris*. Ultraviolet reflectance occurs in the corolla of *Dicentra* (Macior 1978b), the hood and nectar spur of *Orchis* (fig. 1A), and the fall of *Iris* (fig. 1B).

Corolla tube and spur lengths were compared to *Bombus* queen tongue lengths (tables 3 and 4). Mean values for *Dicentra* and *Orchis* show a close relationship with *B. bimaculatus* and *B. vagans*. Both *Bombus* species were pollinators of *Dicentra* but *B. bimaculatus* emerges and dominates early during the spring. *Bombus vagans* emerges about the same time but does not dominate in frequency until later in the spring when *Orchis*, *Geranium*, and *Iris* are blooming. *Bombus bimaculatus* was poorly represented at site II. Both *Geranium* and *Iris* attracted the greater percentage of *B. vagans* at their respective site.

Only 7% of the *Bombus* queens captured carried pollen. With the exception of *Iris*, bees on all other plants carried mixed pollen loads. Those on *Iris* carried foreign pollen only. *Bombus vagans* was the most abundant pollen gatherer constituting 56% of the total.

The inflorescence height of *Geranium*, *Orchis*, and *Iris* was measured for 10 plants each. *Orchis* inflorescence height averaged 11.8 cm and ranged 10.5-13.5 cm. *Geranium* and *Iris* averaged 53.8 cm and 74.7 cm and ranged 46.4-62.9 cm and 49.5-108.0 cm, respectively.

**ORCHIS SPECTABILIS REPRODUCTIVE BIOLOGY.** The flower (fig. 2) has erect, con-

TABLE 3  
Corolla tube\* or spur lengths (mm).

Plant species	Mean	Range	SD	N
<i>Geranium maculatum</i> **	—	—	—	—
<i>Aesculus glabra</i>	7	7	0.00	6
<i>Iris pseudacorus</i>	10	10	0.00	6
<i>Pedicularis canadensis</i>	11	10-12	0.75	6
<i>Dicentra canadensis</i>	18	16-19	0.98	6
<i>Orchis spectabilis</i>	18	16-19	0.55	6

\* composed of fused or contiguous petals

\*\* no tube or spur

SD = standard deviation

N = sample size

TABLE 4  
*Bombus* queen tongue lengths (mm).

<i>Bombus</i> species	Mean	Range	SD	N
<i>sandersoni</i>	9.40	9.21-9.78	0.33	3
<i>perplexus</i>	10.35	9.00-12.69	1.67	4
<i>affinis</i>	10.54	8.33-12.16	1.24	9
<i>impatiens</i>	10.59	8.33-12.80	1.43	24
<i>vagans</i>	10.88	9.19-14.24	1.37	54
<i>bimaculatus</i>	11.28	9.33-12.69	1.45	5
<i>pennsylvanicus</i>	13.32	11.43-14.35	1.64	3
<i>fervidus</i>	13.94	13.67-14.45	1.36	4

SD = standard deviation

N = sample size

cave, ovate-lanceolate petals and sepals forming the hood. The hood is mauve in color and covers the column. Each flower is subtended by a lanceolate foliar bract. Three to 5 flowers per scape are born in a raceme. The labellum is white, entire, and broadly rounded at the apex.

Sexual reproduction is accomplished by the complex pollination mechanism previously described. Tests of this mechanism using a pencil yielded 29% position changes capable of effecting pollination. The pollinator, *B. vagans*, observed removing pollinia, had 4 pollinia previously attached medially to the frons area inferior to the ocelli. Upon exiting, the bee removed both pollinia which became attached to same area. The pollinia immediately moved anteriorly and drooped with-

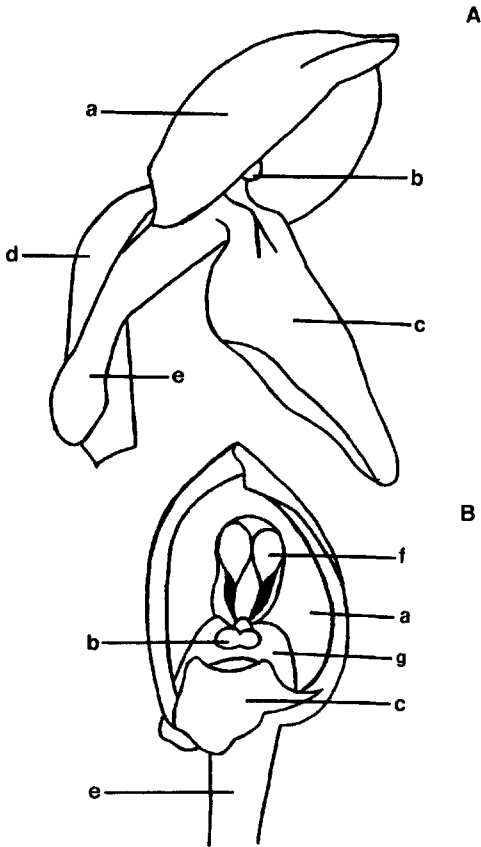


FIGURE 2. Flower of *Orchis spectabilis*. A. Side view. B. Front view. a = hood, b = rostellum, c = labellum, d = ovary, e = nectar spur, f = pollinia developing sheaths, g = stigmatic surface. 3 $\times$ .

out extending horizontally. Tests using a pencil, therefore, may give false results.

Other anomalies related to pollination included the removal of only the caudicles with the pencil, the pollen sacs remaining within the developing sheaths. Some nectar spurs had ants, spiders, and slugs blocking the entrance as well as spider webs and fungus. These obstacles might effectively eliminate pollinator access.

At site II there were 90 plants producing 54 flowers in 1979 and 55 in 1980. At site III there were 280 plants producing 143 flowers in 1979 and 122 in 1980. Open pollination of *Orchis* at site II was possible with 17% of the plants flowering

A in both years. Fruit production was 9% in 1979 and 11% in 1980. Open pollination at site III was possible with 21% of the plants flowering in 1979 and 15% in 1980. The corresponding fruit production was 1% and 0%.

Of the caged flowers left untouched, none fruited, suggesting that an animal pollen vector is required. Artificial autogamous pollination of 25 flowers yielded one fruit indicating a 4% self-compatibility of the flowers tested. Artificial geitonogamous pollination of one flower produced no fruit, but artificial xenogamous pollination of one flower produced a fruit.

Seed production using the 1979 method averaged 3485 seeds/fruit while the 1980 method averaged 6656 seeds/fruit. Both methods indicate more than adequate seed set to replace the existing populations even if only one fruit/season were produced. The artificially self- and cross-pollinated fruits averaged 7468 and 6954 seeds, respectively. These values are comparable to those produced under open pollination using the 1980 method.

## DISCUSSION

If a comparison is made of the number of bees captured per total observation time for each plant species studied, *Orchis spectabilis* received the fewest bees captured/hr while the introduced *Iris pseudacorus* had the highest. It seems that *Orchis* may be receiving relatively few pollinators compared to other plant species of the local flora. There are 2 possible explanations for this. First, ultraviolet reflectance of *Iris* may effectively reduce pollinator frequency of *Orchis*. The fall of *Iris* is highly reflective in ultraviolet light and more importantly has a sharp contrasting pattern and large surface area for attraction. Second, during the blooming period of *Orchis*, which has a relatively short inflorescence height, both *Geranium* and *Iris* inflorescences are at a height comparable to the surrounding vegetation. *Geranium* and *Iris* may, therefore, maintain a greater frequency of bumblebees foraging consistently at this

level. Heinrich (1975) has suggested that insects discover food sources first which possess conspicuous flower signals. Levin and Kerster (1973) have observed that honeybees tend to exhibit horizontal flight while foraging.

Corbicular pollen collection, visible color reflectance, and tongue length correspondence to corolla tube length are not directly related to maintaining an adequate pollinator frequency. The majority of *Bombus vagans* visit flowers with short nectar tubes. Since the spur of *Orchis* had nectar of various depths, spur length itself would not eliminate bees with short tongues.

*Orchis* maintains a potential for self-pollination when a bumblebee enters, removes pollinia, and reenters the same flower. A similar situation has been documented with *Cypripedium acaule* (Wright 1975). The small and disjunct populations combined with the capacity for self-pollination leaves a potential for inbreeding.

If pollinator frequency is the main factor involved in the limited occurrence of *Orchis* in northeast Ohio then seed set would be inadequate to maintain the populations. This does not seem to be the case. One seed capsule/season would produce 12× the number of seeds necessary to replace the largest population studied (280 plants) assuming the smaller number of seeds/capsule measured. Pollinator frequency, and hence seed set, seems to be more than adequate to maintain and even increase the existing populations.

Macior (unpubl. data) has recorded *Bombus fervidus*, *B. nevadensis auricomus*, and *B. pennsylvanicus* queens having *Orchis spectabilis* pollinia attached to their heads. These 3 *Bombus* spp. plus *B. griseocollis* are considered to be open field- or meadow-dwelling and possess tongues long enough to suck nectar from *Orchis* (Macior 1968, 1978a). *Bombus vagans* is considered a woodland-dwelling species. It seems that *Orchis* is a woodland inhabiting plant species depending on both open field- and woodland-dwelling *Bombus* queens for

chance pollination. Competition from introduced plants such as *Iris pseudacorus* and pollinator activity at different vegetational levels may be factors reducing pollination. This low frequency pollination results in an adequate seed production and is probably not responsible for the locally limited occurrence of *O. spectabilis*. Further study into habitat disturbance, seed viability, and seed germination might provide more useful information.

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