Pollination Ecology of the Simultaneously Flowering

Impatiens capensis and I. pallida

by

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(ABSTRACT)

In mixed stands of the annual *Impatiens capensis* and *I. pallida*, bumblebees visit *I. pallida* almost exclusively even when the nutritional reward of *I. pallida* is removed. This phenomenon contradicts certain models proposed for foraging behavior in simultaneously flowering species and those dealing with optimal foraging. Such foraging behavior could have potentially devastating effects on the nonpreferred species, especially if the plant is an annual.

Both *Impatiens* species share the same primary pollinators when they grow allopatrically, but when they grow sympatrically *I. pallida* continues to attract bumblebees while *I. capensis* depends on hummingbirds and small bees for pollination. Thus competition for pollinators between these *Impatiens* represents an important plant-plant and plant-pollinator interaction with potential consequences. When *I. capensis* is less frequented by bumblebees when sympatric with *I. pallida*, *I. capensis* exhibits reduced fecundity.

Mechanisms of interference through improper pollen transfer are described for the *Impatiens* species. Pollen of *I. pallida* adheres to and germinates on the stigmas of *I. capensis* and the pollen tubes reach the ovules. In contrast, pollen of *I. capensis* adheres poorly to *I. pallida* stigmas and fails to germinate. No hybridization occurs between the species. Scanning electron micrographs of pollen and stigma surfaces of the two species revealed no morphological differences.

Among the floral characteristics examined, only flower color appeared to influence bumblebee preference for *I. pallida*. Differences between the species in ultraviolet absorbance patterns, perianth size and shape, odor, and nectar quantity and quality did not affect the pollinator preference of bumblebees. Differences in nectar characteristics and production rate were found

among *Impatiens* populations. However, nectar quantity, quality, and production rates did not differ within allopatric or sympatric populations, but both species had higher nectar volumes with lower solute concentrations in the morning, and lower nectar volumes with higher solute concentrations in the late afternoon. Differences in nectar volumes among populations appeared to be associated with environmental factors affecting population sites.

I. capensis avoids competition and maintains itself in sympatric populations with *I. pallida* by depending on hummingbirds and insects other than bumblebees as pollinators, and has the ability to produce cleistogamous flowers and their subsequent progeny to ensure sexual reproduction. However, inbred progeny of *I. capensis* are competitively inferior to outcrossed stock. Nevertheless, *I. capensis* does persist in mixed stands of *I. pallida* despite the absence of bumblebee pollination and the presence of lower seed set.

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We, as individuals, are the result of a historical progression from infancy to the present. So too is the completion of this dissertation an amalgamation of input from the many individuals influencing my development.

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Table of Contents

List of Illustrations

List of Tables

Chapter I

Introduction

Flowering plants depend on the external agents of wind, water, and animals for the transfer of pollen. These dispersal agents require that plants possess precise morphological adaptations for dispersing and receiving pollen, but animal pollination includes the additional element of influencing animal behavior. In general, plants that utilize animals as pollen vectors use an attractant or "advertisement" and then offer a reward to the pollinator. Pollinator fidelity often depends on the advertisement and reward which then influence the accuracy of conspecific pollen transfer. Simultaneously flowering species often share the same pollinators. If one species is more abundant or attractive than others it may receive a greater proportion of pollinator visits. In addition, simultaneously flowering species sharing the same potential pollinators risk receiving heterospecific pollen and/or losing pollen to improper stigmas. The results of receiving heterospecific pollen and/or the general loss of pollen may be detrimental, or these interactions may be tolerated by the plant. In this dissertation I investigate various levels of interaction between the simultaneously flowering *Impatiens capensis* and *I. pallida*.

Three general questions addressed in this investigation are:

I. Do the two *Impatiens* species compete for bumblebee pollination when they co-occur?

> *Impatiens capensis* and *I. pallida* are reported to share the same primary pollinators, namely bumblebees. Levin and Anderson (1970) and Straw (1972) presented theoretical

1

models showing that plant species should be visited in in proportion to their frequency. Models designed to test this premise show that the less visited species will become extinct in only a very few generations. Thus it follows that extinction is the fate of a less prefered species, regardless of its frequency. Pollinator service may, therefore, be an important component in the persistence of sympatric plant species.

II. If the two *Impatiens* species share pollinators, even if infrequently, what are the consequences?

The consequences of pollinator sharing might include a general loss of pollen to improper stigmas and/or interference from foreign pollen. Both cases can reduce fecundity in one or all interacting species. However, pollinator-sharing might not reduce plant fitness if the interacting plant species can tolerate the presence of foreign pollen or show some other mechanism of avoidance.

III. If one *Impatiens* species is more "attractive" to pollinators than the other, what floral characteristics of the *Impatien s* species make it more attractive?

Most entomophilous species possess colorful flowers with ultraviolet absorbance-reflectance patterns (nectar guides), various flower sizes and shapes, and odors, which attract pollinators. The differences among these characters are evident in the diversity in flower morphology seen in today's flora. Some of these, or a combination thereof, may be more influencial to one group of pollinators over another. The relative attractiveness of floral traits, singularly or in combination, represents a means by which flowering plants might differentially attract pollinators. The differential attraction of pollinators may also represent a mechanism by which flowering plants have diversified in order not to compete for pollination.

Species biology

Impatiens, one of two genera in the Balsaminaceae, comprises about 450 species centered mainly in tropical Asia and Africa, with a few taxa in temperate areas of the Old and New Worlds (Cronquist 1984). Ornduff (1967) recognized five species in North America, four of

2

which occur in the Pacific Northwest, with two in Eastern North America. Our study covers only the eastern species.

Impatiens capensis and *I. pallida*, jewelweeds or touch-me-nots, are similar in vegetative and floral morphology, with orange and yellow flowers, respectively. The flower of *Impatiens pallida* has somewhat larger petals and a shorter spur than that of *I. capensis*. Both species usually inhabit wet places, although they can occasionally be found in drier sites. *Impatiens capensis* is widespread, bridging the northwestern and eastern distribution of the genus from Alaska, south to British Columbia, east to Newfoundland, south to Florida, Alabama, Arkansas, and Oklahoma (Wood 1975) (Figure 1 on page 5). *Impatiens pallida* is found primarily in eastern North America from Newfoundland to Georgia, but does extend into Saskatchewan; it occurs primarily in the higher elevations in the southern part of its range (Fernald 1950). Both species occur in mixed populations over much of their range.

The two species are identical phenologically. Flowering begins in July and continues until the first killing frost, typically late September in southwest Virginia. Personal observations suggest that senesence may be photoperiodically triggered. Both species are strongly protandrous, spending approximately three days in the male phase and one day in the female phase (Rust 1977). The androecium is fused into a single unit that completely encloses the pistil. As the maturing pistil elongates, the androecium is pushed off exposing the receptive stigma. This mechanism insures outcrossing, although geitonogamy may still take place and was estimated to be 10% (Waller 1980). Cleistogamous (CL) flowers are produced in both species. These may appear early in the season when the plants are less than 15 cm tall (personal observation), but they are more typically produced late in the season after the peak in chasmogamous (CH) flowering (Waller 1984, personal observation). Rust (1977), Schemske (1978), Waller (1984), and Simpson et al. (1985) indicated that the major reproductive component for *I. capensis* comes from CL flower production. Rust (1977) found this also to be true for *I. pallida*. Simpson et al. (1985) indicated that one of their four New Jersey populations did not produce CH flowers. This is contrary to our observations in Virginia, where CH flower and CH-derived fruit production is considerably greater than CL flower and fruit production (Randall 1988). Since CL flowers occur primarily in September, many CL-derived fruits never mature due to frost. The differences in CH and

3

CL numbers between northern and southern populations may be the result of habitat differences and/or racial variation.

Previous pollination biology studies of *I. capensis* have shown that rates of nectar production are essentially constant from morning until dusk (Marden 1984), and that nectar solute concentrations are constant within and among individuals (Zimmerman 1981, Bell et al. 1984). Zimmerman examined only one population for a one-day period while Bell and collaborators sampled two populations over several days. Rust (1977) reported that both species share the same primary pollinators (bumblebees), produce the same amounts of nectar, and contain similar nectar solute concentrations. However, he detected several amino acids in *I. capensis* not found in *I. pallida*. Rust concluded that there was little or no pollinator preference for either species when growing in mixed stands. However, his conclusion was based purely on visual observations without any supportive data. Many workers have indicated that both *Impatiens* species share many of the same pollinators, primarily bumblebees (Robertson 1928, Rust 1977, Heinrich 1979, Bell et al. 1984, Schmitt et al. 1985). Robertson also noted that the ruby-throated hummingbird (*Archilochus colubris*) visited *I. capensis* regularly and *I. pallida* occasionally. We sought to determine the interspecific pollination dynamics between the two *Impatiens* species.
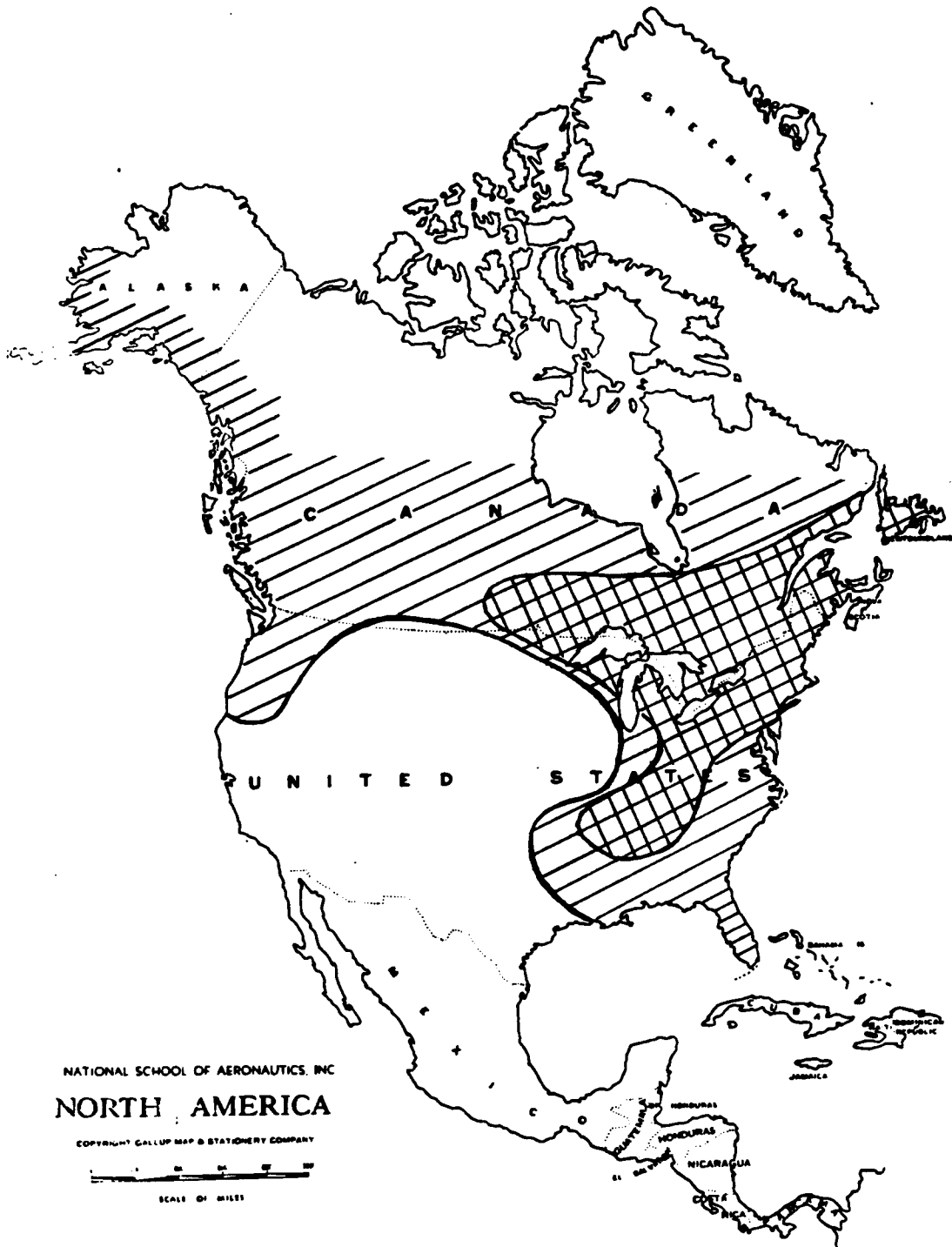
(see chapter 2 for references)

Figure 1. Distribution of Impatiens capensis and I. pallida: Cross-hatched area indicates the distribution of *I. pallida* in addition to the area of overlap between the two *Impatiens* species.

5

Chapter II

Competition for pollination in mixed stands of

Impatiens capensis and I. pallida

Introduction

Competition for pollination between species proximal in time and space might lead to

reduced reproductive success in one or both species if the more attractive species lures a

significant proportion of available pollinators away from the other species (Clements and

Long 1923, Mosquin 1971, Straw 1972, Reader 1975). Models developed by Levin and

Anderson (1970) and Straw (1972) imply that pollinators vist plant species in proportion to

plant frequency, and that competition between simultaneously flowering species would lead

to elimination of the less abundant species in a very few generations. Thus, competition for

pollinators between simultaneously flowering species could represent an important

plant-plant interaction.

Simultaneously flowering species provide a unique opportunity for identifying such

competitive interactions since they frequently utilize the same pollinators (Mosquin 1971,

Heinrich 1975). As early as 1895, Robertson recognized that competition among plants for

pollination represented a means by which plants might have diverged in flowering time and other characters. Levin and Anderson (1970), Mosquin (1971), Reader (1975), and Levin (1979) attribute the divergence in flowering time, flower morphology, color, odor, nectar quality and quantity, pollen amounts, and height in plants to their differential attraction of pollinators. Waser (1983), reviewing the evidence for competition between simultaneously flowering species, concluded that studies of plant-pollinator interactions have been primarily descriptive and that the exact mechanisms and possible evolutionary outcomes of these interactions remain controversial. However, there appear to be two broad categories of competitive interactions for pollination in simultaneously flowering species. First, one plant species may effectively attract pollinators away from another species (Waser 1983). Second, foreign pollen may occupy receptive stigma sights, causing clogging (Rathcke 1983, Waser 1983); this also reduces effective pollinator movements and consequently lowers reproductive success (Lewis 1961, Levin 1971, Waser 1978, 1983).

Levin and Anderson (1970) and Straw (1972) presented models predicting foraging behavior in simultaneously flowering species. These models have not been tested experimentally. Our study examines foraging behavior and reproductive success in controlled and natural allopatric and sympatric populations of two congeneric annuals, *Impatiens capensis* Meerburgh and *I. pallida* (L.) Nuttall. The species flower simultaneously and share essentially the same ecology, including pollinators.

The study addresses two questions: 1) is there competition for pollination between *Impatiens* species? 2) does simultaneous flowering in *Impatiens* affect fecundity as measured by fruit set? Similarity in habitat requirements, phenologies, floral morphologies, and pollinators, as well as occurrence in allopatry and sympatry make these *Impatiens* species well suited to test the interactions between two simultaneously flowering species.

Materials and Methods

Frequency-dependent foraging behavior

Controlled foraging of bumblebees on the *Impatiens* species was tested using a replacement
series design (Kahn and Bradshaw 1975) in order to check both inter- and intraspecific
interactions, and to determine the relative attractiveness of the two simultaneously flowering
species at equal densities. Fifty seedlings of each *Impatiens* species were collected from the
wild in the spring of 1986 and 1987. Two plants were grown in each of 50 three-gallon plastic
containers and the *Impatiens* species were arranged in random order on a square grid within
a 6 m x 6 m x 3 m screened pollinarium. The experiment was conducted in this exclosure
to ensure that the standing nectar crop was not depleted by foraging insects.

In order to create different ratios of *Impatiens* flowers while maintaining the same patch
density, flower numbers were manipulated by pinching the flowers off to the desired
proportions. This procedure eliminated the moving of fragile plants. The ratios of *I. capensis*
: *I. pallida* flowers used in this experiment were: 0:1, 1:1, 1:2, 2:1, 3:1, 6:1, and 1:0.

Bumblebees inexperienced to the flower handling and reward expectations of the
respective species were used to test initial responses. Bumblebee species used in this study
were *Bombus impatiens, B. vagans, and B. pennsylvanicus*. All of these bumblebees are known
to forage on *Impatiens* (Mitchell 1962). Bumblebee colonies were collected in the spring and
summer of each field season, either before the flowering season of *Impatiens* or in areas devoid
of *Impatiens* after flowering had started. Only two colonies were collected in the latter case;
they were isolated from *Impatiens* populations by several kilometers. Bees were numbered
for visual identification by gluing small, colored, numbered tags (Opalithplättchen, Germany)
on the backs of their thorax. Voucher specmens are located in the Department of
Entomology, VPI&SU.

Bumblebees were housed in wooden "colony boxes" which were arranged around the
pollinarium perimeter. The colony boxes were connected to the pollinarium by clear plastic

tubing and PVC plastic fittings that allowed access to both the pollinarium and to the outside (Figure 2 on page 18). Bees were allowed to forage naturally on the surrounding vegetation until they were required for a controlled foraging bout on the enclosed *Impatiens*. No *Impatiens* grew within three km of the pollinarium, outside the two km foraging range of bumblebees (Heinrich 1979). This ensured that the bumblebees used in foraging experiments were unfamiliar with *Impatiens*.

Foraging bouts within the pollinarium were initiated by closing the passage to the outside and opening the passage leading into the pollinarium. Only one bee was allowed to forage at a time. Bumblebees used in a foraging bout, identified by their tag color and number, were used only once for initial response tests. Foraging bouts were not initiated until peak flowering in late August.

Pollinator surveys:

A pollination study was also conducted in field sites to compare with results obtained under the controlled conditions of the pollinarium. Nine natural populations of *Impatiens* (three *I. capensis*, three *I. pallida*, and three sympatric), were located in the Blacksburg area, Montgomery County, Virginia. All populations were selected to be isolated from one another by at least 2 km from each other to render them outside the normal foraging range of bumblebees. This criterion was important since the study required pollinators unfamiliar with the other *Impatiens* species. The nine populations were comparable in canopy cover, aspect, and soil moisture levels. These populations were identified as C1, C2, C3 (for *I. capensis*), P1, P2, P3 (for *I. pallida*), and S1, S2, S3 (for sympatric populations). Since bumblebees are known to switch species on sequential foraging bouts (Heinrich 1975), the bumblebees in mixed stands were captured and marked for identification (as described above), and released to observe the behavior of individual bees over the course of the day.

Observations and collections were made for all of the different pollinators of *Impatiens* by capturing them directly from the flower they were foraging on. Night pollination was also checked in field sites C1, C2, and S2.

Pollinator exclusion experiments:

Since bumblebees are considered as the primary pollinators of *Impatiens* species (Rust 1977, Heinrich 1977, Bell et al. 1984, Schmitt et al. 1985), and the ruby-throated hummingbird is reported to be an important pollinator of *I. capensis* (Robertson 1928, Rust 1977, Laverty and Plowright 1985), exclusion experiments were designed to test: 1) the importance of hummingbirds as pollinators of *I. capensis* when growing sympatrically with *I. pallida*, and 2) the pollination effectiveness of insects other than bumblebees.

The hummingbird exclusion experiment was located in a natural *I. capensis* population with active hummingbird pollinators. Two mixed populations of 10 potted individual plants of each species (n = 20) were placed in a shallow stream. One "population" was established within a cylindrical exclosure (2 m in diameter x 1.5 m high) constructed of 3 cm mesh chicken-wire. An uncaged control population was located adjacent to the exclosure. Twenty flowers of each species, inside and outside the exclosure (for a total of 80 flowers), were marked on their pedicels with acrylic paint for identification. All flowers were in the male phase when marked (where the androecium covers the stigma) to ensure that they had not yet been pollinated. This experiment was replicated three weeks later to check for temporal changes in hummingbird foraging behavior. Successful pollination was assumed to have occurred if capsules were produced, because unfertilized ovaries abort shortly after flower drop (Rust 1977, personal observation).

In order to test the effectiveness of insect pollinators other than bumblebees, an exclosure similar to the one used in the above experiment was constructed out of 5 mm mesh hardware cloth in a pure population of *I. capensis* with an adjacent control population.

Results and Discussion

Frequency-dependent foraging behavior:

In the 1986 pollinarium study bumblebees preferred *I. pallida* over *I. capensis* regardless of plant species ratio. The proportion of visits of 20 different bumblebees to *I. pallida* ranged

10

between .96 and 1.0 (Figure 3 on page 19). Bumblebees rarely approached *I. capensis* in the pollinarium (< 5%), and even more rarely entered the flower. During the 1987 pollinarium experiments thrips interferred with the experiment by depleting nectar. These insects depleted nectar from only *I. pallida*, possibly because it is more accessible to them because of the shorter spur of the *I. pallida* flower compared to that of *I. capensis*. Subsequently, flowers of *I. pallida* in the pollinarium were bagged to prevent nectar thievery. After bagging, the proportion of visits to *I. pallida* never dropped below .89 regardless of species ratios. The results of the 1987 foraging experiment essentially duplicated those of 1986.

Before *I. pallida* flowers were bagged to discourage thrips, some foraging bouts were run between *I. capensis* with full nectar volumes and *I. pallida* with depleted nectar volumes. This altered the sequences and proportion of visits to the different *Impatiens*. Bumblebees initially chose *I. pallida*; however, after making several visits without reward, bumblebees switched to *I. capensis* which was full of nectar. Thus a reward was now available in the less preferred species that was not present in the more preferred species. This set up a foraging sequence which consisted of first choosing *I. pallida*, then switching to *I. capensis*. However, bees continued to switch back to *I. pallida* (Figure 4 on page 20). Despite the high food value of *I. capensis* compared with *I. pallida*, the proportion of visits to *I. pallida* never fell below .49 (2:1 ratio, *I. capensis* : *I. pallida*).

These data contradict the models predicting foraging behavior in simultaneously flowering species, where the plant species in greatest abundance is supposed to be most often pollinated. In addition, optimal foraging models (MacArthur and Pianka 1966) do not seem to apply in this case since they predict that a foraging animal will maximize its fitness by choosing the most energy efficient resource. Furthermore, experimental studies on the foraging behavior of bumblebees show that a constant nectar volume is prefered over a variable volume even when the mean amounts are equal (Real et al. 1982, Real and Caraco 1986). The two *Impatiens* species not only had variable nectar volumes, but they differed drastically in nectar volumes in 1987. Thus bumblebees tended to choose *I. pallida* over *I. capensis* regardless of its proportion in the population or its energy reward.

Thomson (1978) demonstrated in two-species mixtures of simultaneously flowering *Hieracium*, with orange and yellow flowers, that the orange-flowered species is prefered by

11

pollinators over the yellow-flowered species. In fact, pollination of the yellow-flowered species is "facilitated" by the presence of the orange-flowered species (Rathcke 1983). Thus not all simultaneously flowering species that share the same pollinators in mixed stands necessarily compete for pollination. Facilitation for pollination is a new concept among pollination biologists (Rathcke 1983).

Two levels of plant-pollinator attraction exist: primary (visual attraction) and secondary (olfactory recognition) (Kevan 1983, Waser 1983). Bees may discriminate among a variety of characters, giving more weight to some than others (Bateman 1952). Our data strongly suggest that some primary recognition component takes precedence over the secondary component in the choice of *I. pallida* over *I. capensis* in bumblebees (Randall and Hilu in preparation).

Pollinators of *Impatiens*:

In pure stands, both *Impatiens* species were primarily pollinated by the same bumblebee species, *Bombus impatiens, B. pennsylvanicus,* and *B. griseocollis*. Other foragers were also observed, although they made up a small percentage of the pollinator fauna. Secondary pollinators included honeybees (*Apis mellifera*), andrenid and megachilid bees, *Halictus* sp., yellow jackets (*Vespula* spp.), and soldier beetles (Cantheridae). The *Halictus* was also a nectar thief on *I. pallida*, and the soldier beetle was believed to be a flower predator rather than a pollinator. The ruby-throated hummingbird was the most prominent secondary pollinator of *I. capensis*. Hummingbirds were never seen foraging on *I. pallida*, which is almost exclusively bumblebee pollinated, although it was reported to pollinate *I. pallida* by Robertson (1928).

Twelve hours of observation were spent in each mixed population (four hours per population on three consecutive days at different times). Plant ratios of *I. capensis* : *I. pallida* in these populations were approximately 1:1 in S1, 20:1 in S2, and 2:1 in S3. Seven bumblebees were numbered in population S1, five in S2, and seven in S3. As in the pollinarium, bumblebees chose to concentrate on *I. pallida* in these mixed stands and would occasionally approach, but very rarely enter, an *I. capensis* flower. Data on these floral visitation sequences were not tabulated since it was clear that the same bumblebees were

12

returning over the course of the day to forage exclusively on *I. pallida*. The same behavior in the marked bees was observed in these populations on three consecutive days.

During this study other pollinators of *Impatiens* were collected (except for hummingbirds). Of these alternative pollinators, the andrenids, megachilids, and yellow jackets foraged on both *I. pallida* and *I. capensis*. In population S2 during both 1986 and 1987 seasons, and population S3 in 1987, where hummingbirds were especially abundant, the bumblebee *B. griseocollis* was a legitimate pollinator on *I. pallida*, but behaved as nectar-robber on *I. capensis*. Other insects (*Halictus* and yellow jackets) took advantage of the hole in the spur produced by the nectar-robbing bumblebees and acted as secondary nectar-robbers.

Nectar-robbing was not prevalent in other populations. Laverty and Plowright (1985) demonstrated that resource partitioning, as the result of competition, occurred between bumblebees and the ruby-throated hummingbird in an *I. capensis* population. They concluded that hummingbirds tend to forage on the population perimeter and toward the outside branches of the plant while bumblebees tend to forage within the population and on the innermost flowers of the plant. They did not report nectar-robbing by the bumblebees in their study.

To further test the idea that *I. pallida* was preferred over *I. capensis*, a single potted individual of *I. pallida* with five flowers was placed in each of two separate pure stands of *I. capensis* giving an approximate ratio 1000:1, *I. capensis* : *I. pallida*. These pure stands were several km away from any *I. pallida* and thus the bumblebees in these *I. capensis* populations were necessarily unfamiliar with *I. pallida*. Almost the instant that the *I. pallida* was placed in the *I. capensis* population, bumblebees switched to the *I. pallida*. This switch was so dramatic that three of the five *I. pallida* flowers on the single individual were occupied at once. At least ten different bumblebees switched from *I. capensis* to the *I. pallida* during an hour's observation; as the potted *I. pallida* was carried out of the *I. capensis* stand, bumblebees continued to alight on the flowers.

13

Pollinator exclusion experiments

The hummingbird and bumblebee exclusion experiments indicated that neither pollinator is essential for *I. capensis* pollination. In the hummingbird exclusion experiment where mixed populations were used, bumblebees again preferred *I. pallida* over *I. capensis*, but there was no significant difference between the pollination success within caged and uncaged conspecific *I. capensis* ($X^2_{(1)}$ = 1.1, p > .20), or between caged and uncaged *I. pallida* ($X^2_{(1)}$ = .02, p > .90). However, there was a significant difference in the pollination success of the two *Impatiens* species ($X^2_{(1)}$ = 8.09, .001 < p < .01). Fruit set was 97% for *I. pallida* and 77% for *I. capensis*. These data reflect the influence of *I. pallida* on the fecundity of *I. capensis* when they grow in sympatry.

In the exclusion experiment for both hummingbirds and bumblebees (pure populations) there was no significant difference between caged and uncaged *I. capensis* ($X^2_{(1)}$ = 1.1, p > .20), which had 85% and 95% fruit set, respectively. *Impatiens capensis* had 77% fruit set when it was sympatric with *I. pallida* compared to 90% pollination success when allopatric. This provides further evidence for the interspecific influence of *I. pallida* on *I. capensis*.

Despite the lower fecundity of *I. capensis* when sympatric with *I. pallida*, it reproduces successfully, even without hummingbird or bumblebee pollination. In addition, CL flowers ensure additional seed production. For example, in 1986 a single large (> 2m tall) *I. capensis* in population S2 produced over 900 CH and 150 CL flowers over the season. Plants of this size are not uncommon in most *I. capensis* populations. This one individual probably produced enough propagules (from both CH and CL flowers) to maintain *I. capensis* in the population in spite of the presence of *I. pallida*.

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Figure 2. **Bumblebee colony boxes:** Colony boxes were disigned to allow bumblebees to come and go freely or to be directed into the pollinarium. To direct bumblebees into the pollinarium, passage 'A' to the outside was closed and passage 'B' to the pollinarium was opened. Bees leaving the colony box would thus exit into the pollinarium.
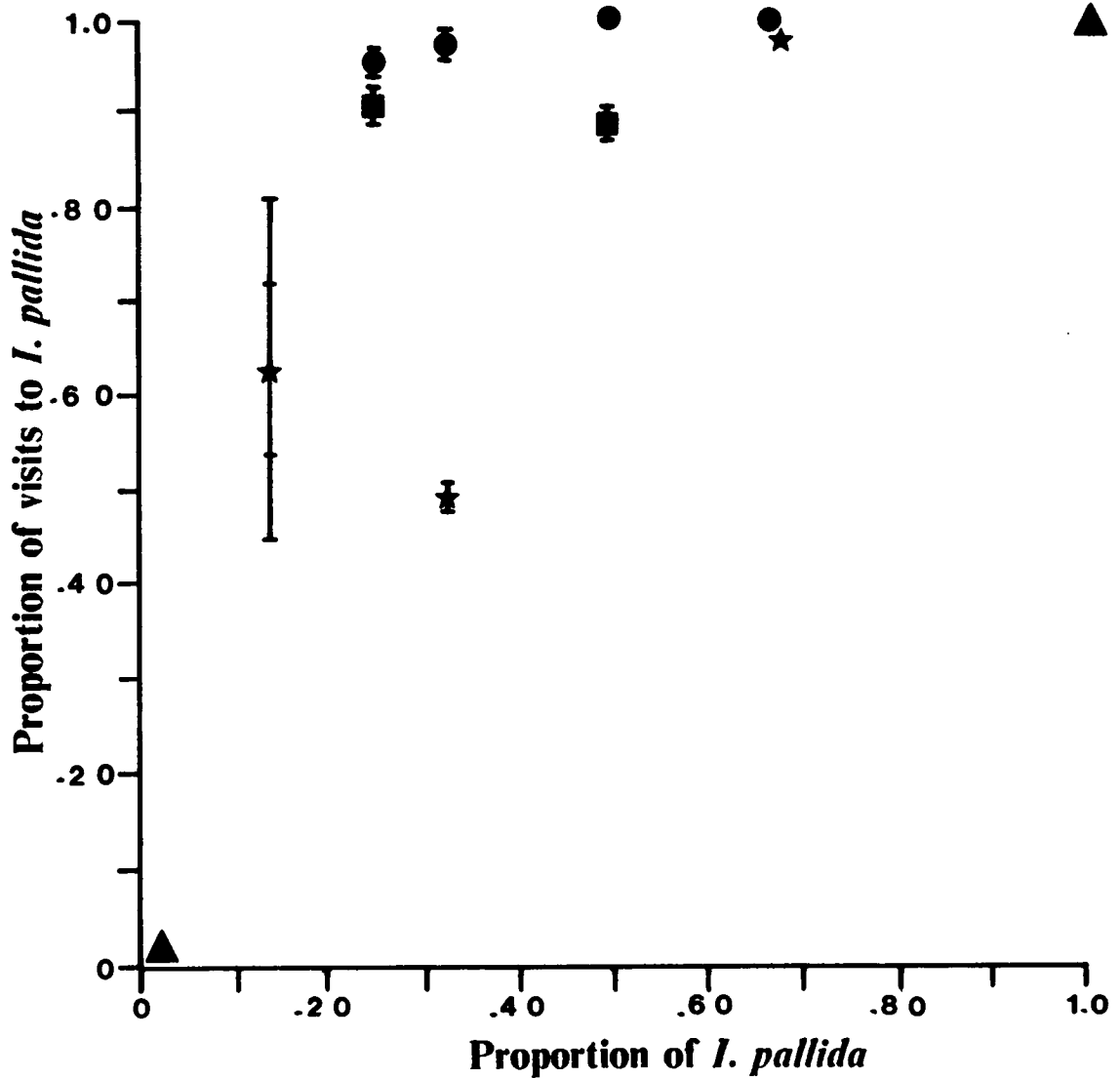
Figure 3. The proportion of bumblebee visits to I. pallida: The proportion of bumblebee visits to *I. pallida* with respect to its proportion with *I. capensis.* ● = 1986; ■ = 1987 (bagged flowers); ★ = 1987 (unbagged flowers); ▲ = the proportions of visits to *I. pallida* when its proportions to *I. capensis* are zero and 1.0. Vertical lines represent standard errors.
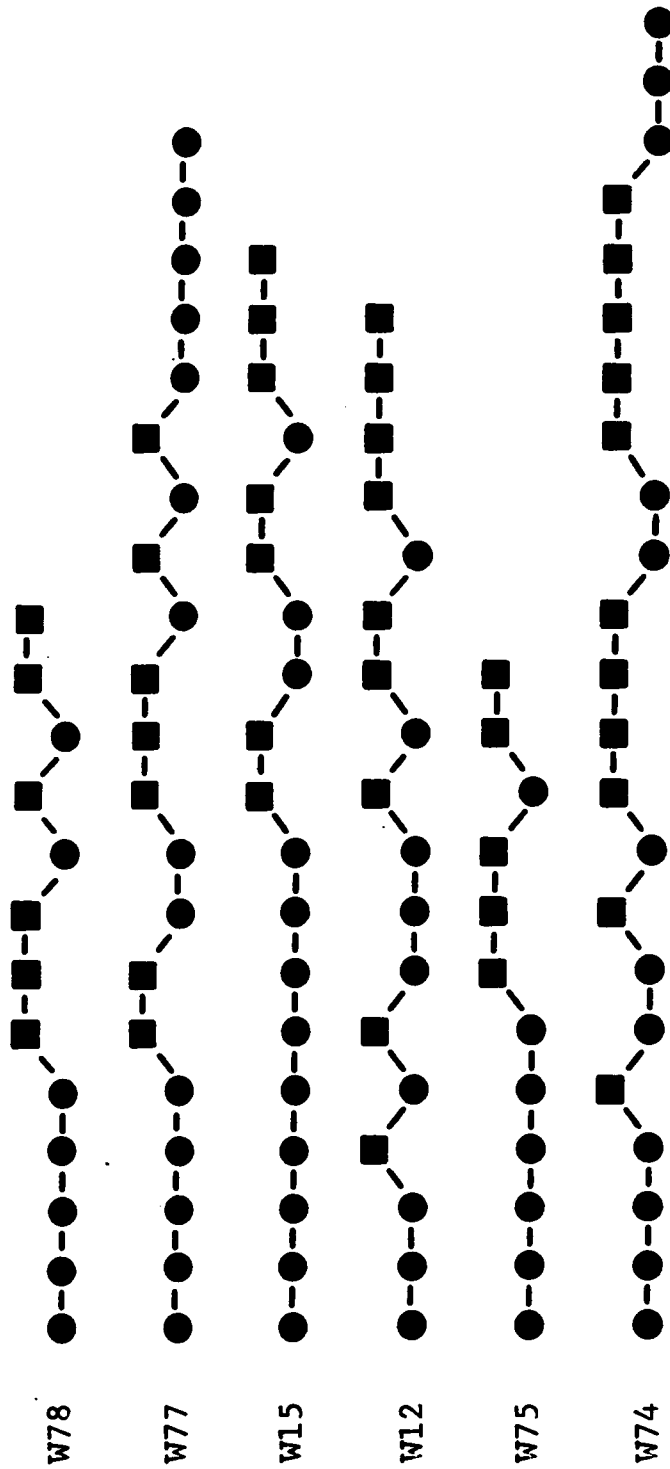
Figure 4. Sequence of foraging bouts between the Impatiens species: Sequence of foraging bouts between nectar-depleted *I. pallida* (●) and nectar-full *I. capensis* (■) at a ratio of 2:1 (*I. capensis* : *I. pallida*). Bumblebees used are identified at the beginning of each foraging bout.

20

Chapter III

Interference through improper pollen transfer

Introduction

Much interest has been paid to the mechanisms of competition between simultaneously flowering species. After reviewing the literature, Waser (1983) purports competition to be the mechanism that led to much of today's diversity in flower morphology and flowering times. Pleasants (1980) cited competition for pollination as an important force directing the structure of flowering phenologies within "guilds" of plants, and indicated that communities might be "structured" by species number and interactions with pollinators.

Two mechanisms of competition have been proposed. First, competition through pollinator preference can operate when one plant species effectively attracts pollinators away from another species (Waser 1983). Second, competition through improper pollen transfer can occur and has the following levels of interaction: 1) reduction in pollen amounts among species sharing pollinators; 2) displacement of receptive stigma sites by heterospecific pollen; 3) clogging styles with foreign pollen tubes; and 4) production of maladapted hybrids (Lewis 1961, Levin 1971, Waser 1978, 1983, Rathcke 1983). Competition through pollinator preference could lead to evolutionary changes in floral attractiveness (Rathcke 1983 Waser 1983, Campbell and Motten 1985). On the other hand,

21

competition through improper pollen transfer could result in character displacement, including divergence in flowering time (Rathcke 1983, Waser 1983, Brown and Kodric-Brown 1979). Few studies have addressed these issues (Waser 1978, Brown and Kodric-Brown 1979, Pleasants 1980). Randall and Hilu (in preparation) showed that *Impatiens capensis* incurs a statistically significant reduction in chasmogamous seed set ($X^2_{(1)}$ = 8.09, .001 < p < .005) when it occurs with *I. pallida*. Campbell (1985) and Campbell and Motten (1985) tested both mechanisms of competition and quantified their outcomes.

Competition through improper pollen transfer can be determined through examination of gametophytic interactions of selfed, conspecific, and heterospecific crosses (using microscopy) and measurement of offspring vigor (e.g. Mulcahy 1971, 1974, Mulcahy and Mulcahy 1975). In addition, the functional aspects of improper pollen transfer can be assayed from fruit and seed production in natural systems (e.g. Campbell 1985, Campbell and Motten 1985, Motten 1986). In this study, both approaches were employed to determine 1) whether improper pollen transfer occurs between the simultaneously flowering annuals *Impatiens capensis* Meerb. and *I. pallida* (L.) Nuttall (jewelweeds or touch-me-nots), and if so, 2) the consequences of this interaction.

Methods and Results

Stigma receptivity and pollen germination rates

In order to estimate the effectiveness of pollination and to compare the plausibility of heterospecific crosses, it was necessary to establish times of stigma receptivity and pollen germination rates for both *Impatiens* species.

Stigma receptivity time was determined by removing the nearly disarticulating androecia of 45 flowers from each *Impatiens* species. Instead of bagging, the perianths of these flowers were pulled from the pistil to prevent insect visitation. Bags produce unnatural conditions of light and

22

humidity, especially in the rain. Furthermore, since the flower may contain up to 30 μl of nectar, it is difficult to exclude small insects (i.e. ants and thrips), which may contaminate crosses.

In this experiment, five pistils were hand-pollinated during each of nine time periods: 0, 15, 30, and 45 min, and at 1, 2, 5, 24, and 48 hours following androecial removal. One hour after each pollination, the pistils were removed and fixed in 70 % ethanol. In addition, ten flowers were hand-pollinated once after the perianth dropped naturally. These flowers had been bagged to prevent insect visitation. To estimate the potential of apomixis, ten flowers were also bagged and left until maturity.

Pollen germination rates were determined by removing the androecia from 40 flowers of both *Impatiens* species. Again, perianths were removed rather than bagged. All pistils were hand-pollinated sequentially and collected after 15, 30, and 45 min, and 1, 2, 5, 24, and 48 hours had elapsed.

In order to observe pollen germination and pollen tube growth, the pistils were softened and cleared in 8N sodium hydroxide at 60° C, stained with decolorized aniline blue, and examined by fluorescence microscopy following the procedures of Mulcahy (1983).

Stigmas in both *Impatiens* species were receptive to conspecific pollen from the time of androecium disarticulation until after perianth drop (Table 1 on page 33). Pollen germinated on the stigmas within 15 minutes and continued to germinate until 48 hours after androecium disarticulation (Table 2 on page 34). A single hand-pollination induced capsule formation in all 10 replicates. Each capsule produced 3 to 5 seeds. Bagged, unpollinated pistils did not produce capsules, implying lack of apomixis. All pistils tested for apomixis aborted within five days of perianth drop.

Seed set through heterospecific crosses

To determine whether hybridization was possible, or the pollen of one *Impatiens* species could germinate on the stigmas of the other, heterospecific crosses were made. Stigmas were pollinated with heterospecific pollen in addition to pollen mixtures from the two *Impatiens* species to

23

determine the effects of interspecific pollination, the influence of foreign pollen on conspecific pollen, and the amount of fruit set.

A total of 130 hybridizations were made between *I. capensis* (C) and *I. pallida* (P) in 1986 and 1987. The following cross pollinations were performed (parentheses indicate pollen mixtures): CxC, CxP, Cx(P + C), PxP, PxC, and Px(C + P). Crosses were made 30 min after manual removal of nearly disarticulating androecia. Perianths were removed from the pistils to prevent insect visitation. Ten stigmas from each interspecific pollination were collected after one hour, fixed in 70% ethanol, and examined by fluorescence microscopy for pollen germination; the remaining stigmas were left to mature.

Interspecific hybridization was never successful since none of the CxP or PxC crosses produced capsules, and all ovaries aborted within five days of pollination (Table 3 on page 35). *Impatiens capensis* pollen failed to adhere to the stigmas of *I. pallida*. However, *I. pallida* pollen adhered well to *I. capensis* stigmas and germinated (Figure 5 on page 30), and the pollen tubes reached the ovules. The mixed-pollen crosses indicated pollen/pistil interaction. When *I. capensis* was used as the maternal parent, one capsule formed in 15 trials. In contrast, when *I. pallida* served as the maternal parent, and the same pollen mixtures were used, eight capsules formed in 16 trials. This difference in capsule formation is statistically significant ($.01 < p < .05$) (Table 4 on page 36). These findings demonstrate experimentally that the presence of *I. pallida* pollen can reduce the fecundity of *I. capensis*.

Seed set from one pollination event

Since *I. capensis* appears to be pollinator limited, with respect to bumblebees, when it occurs sympatrically with *I. pallida*, the success of one natural pollination event in *I. capensis* was tested. Androecia were removed from ten flowers in a pure *I. capensis* population. The pedicels of these flowers were then marked with acrylic paint and tagged to differentiate them from flowers with naturally disarticulated androecia. Following one bumblebee visit, perianths were removed to prevent additional visitations.

All ten flowers visited by bumblebees initiated capsules. These were observed for 12 days (until first frost), during which time it was possible to see the developing ovules within each capsule. The 12 day period was sufficient since ovary abortion occurs within five days of perianth drop in unfertilized flowers. Therefore, one pollination event by a bumblebee was sufficient to initiate capsule development and subsequent seed set.

Pollen / stigma interactions

The heterospecific pollinations indicated that pollen from *I. pallida* adheres to and germinates on the stigmas of *I. capensis*, but *I. capensis* pollen does not adhere well to the stigmas of *I. pallida* and fails to germinate. To determine if there is a morphological basis for this phenomenon, the pollen/stigma interactions between the two *Impatiens* species were examined by scanning electron microscopy (SEM). Conspecific and interspecific pollinations were made by hand one hour after manual androecium disarticulation. As in the above designs, perianths were removed to prevent insect visitation. Pistils were collected at one and two hour intervals, fixed in absolute ethanol, and later dessicated, mounted, and observed under SEM.

The pollen and stigmas of the two *Impatiens* species did not appear to differ morphologically (Figure 6 on page 31). As was seen using fluorescence microscopy, *I. capensis* pollen adhered poorly to *I. pallida* stigmas while *I. pallida* pollen adhered well and germinated on stigmas of *I. capensis* (Figure 7 on page 32).

Discussion

The modes of competition between *I. capensis* and *I. pallida* appear to be multi-leveled. It is apparent that pollen from *I. pallida* interferes with that of *I. capensis* by improper pollen transfer because of one or all of the following mechanisms: a) *I. pallida* pollen adheres to and germinates

on the stigmas of *I. capensis*, occupying receptive stigma sites, b) *I. pallida* pollen tubes may clog the style of *I. capensis*, and c) *I. pallida* pollen tubes actually reach the ovules apparently without fertilizing them. Since *I. capensis* stigmas remain receptive throughout their flowering period and depend on indiscriminate pollinators, the risk of receiving improper pollen transfer from *I. pallida* may be high. As a result of interspecific pollen movement and/or pollinator preference, *I. capensis* incurs a statistically significant reduction in chasmogamous seed set ($X^2_{(1)}$ = 8.09, .001 < p < .005) when it occurs with *I. pallida* (Randall and Hilu in preparation).

Nevertheless, one pollination event is sufficient for seed set in *I. capensis*. Ovaries of both *Impatiens* species typically contain "three to many ovules" (Wood 1975). Our hand-pollinations resulted in the production of three to five seeds per capsule. Similarly, single bumblebee visits to *I. capensis* result in three to five seeds per capsule; the same number of seeds are produced under natural conditions (Wood 1975, Cronquist 1981, J. Randall personal observation).

Conspecific adhesion and germination of pollen grains to their respective stigmas was clearly observed through fluorescence microscopy and SEM. The adhesion of *I. pallida* pollen to *I. capensis* stigmas, but not the opposite, does not appear to have a mechanical basis, since the pollen and stigmas of the two *Impatiens* species did not differ morphologically. Perhaps a chemical component controls the degree of adhesion and germination of pollen on stigmas in *Impatiens*.

The similar pollen morphologies also prevented the measurement of pollen tube growth-rates of pollen mixtures on a common stigma. Thus it could not be determined whether "pollen tube competition" (or "female choice") was operating as a component of genotype selection (Stephenson and Bertin 1983, Mulcahy 1983, Mulcahy et al. 1983). Mulcahy (1983) indicated that in the taxa he investigated, conspecific pollen is competitively superior to interspecific pollen. If foreign pollen reaches the stigma first however, this competitive edge may be of little importance. Campbell (1985) and Campbell and Motten (1985) showed that "competition through improper pollen transfer" occurred between *Claytonia virginica* (Portulacaceae) and *Stellaria pubera* (Caryophyllaceae). Bee-flies (*Bombycilis*) indiscriminately foraged between these two species causing a reduction in seed set in *Stellaria* because of the general loss of pollen to improper stigmas. In this case, there was no stigmatic interference since *Claytonia* pollen is too large to adhere to the stigmas of *Stellaria*.

26

Improper pollen transfer is not necessarily an important competitive mechanism in all simultaneously flowering systems. Motten (1986) observed no reduction in fecundity for 12 vernal species in North Carolina despite rampant improper pollen transfer by bee-flies. His hand-pollinations induced as much seed set as did natural pollinations. The detrimental effects of improper pollen may have been an important selective force in directing the non-competitive interactions now seen among these 12 vernal taxa, according to the arguments of Brown and Kodric-Brown (1979), Rathcke (1983), and Waser (1983). This is the only study that shows experimental evidence of pollinator sharing without plant/plant competition.

Despite the disadvantages to *I. capensis* when it grows with *I. pallida*, it appears to maintain itself in sympatric stands. Randall and Hilu (in preparation) showed that a significant reduction in seed set occurs in *I. capensis* when growing sympatrically with *I. pallida*. Yet, *I. capensis* still produced a considerable number of capsules (ca. 6 capsules/plant/month). The nature of competition may not be as severe as theory suggests, or a different mode of competition may be directing survivorship in this particular system. Competition at the seedling level may dictate survivorship; perhaps *I. capensis* seedlings, though presumably fewer in number, are more vigorous.

The above postulates remain to be tested. This study and that of Randall and Hilu (in preparation) suggest that *I. capensis* does persist in mixed populations with *I. pallida* by 1) depending on hummingbirds and small bees for pollination, 2) having the ability to produce a full seed complement via one pollination event, and 3) possessing cleistogamous flowers to insure sexual reproduction.

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28

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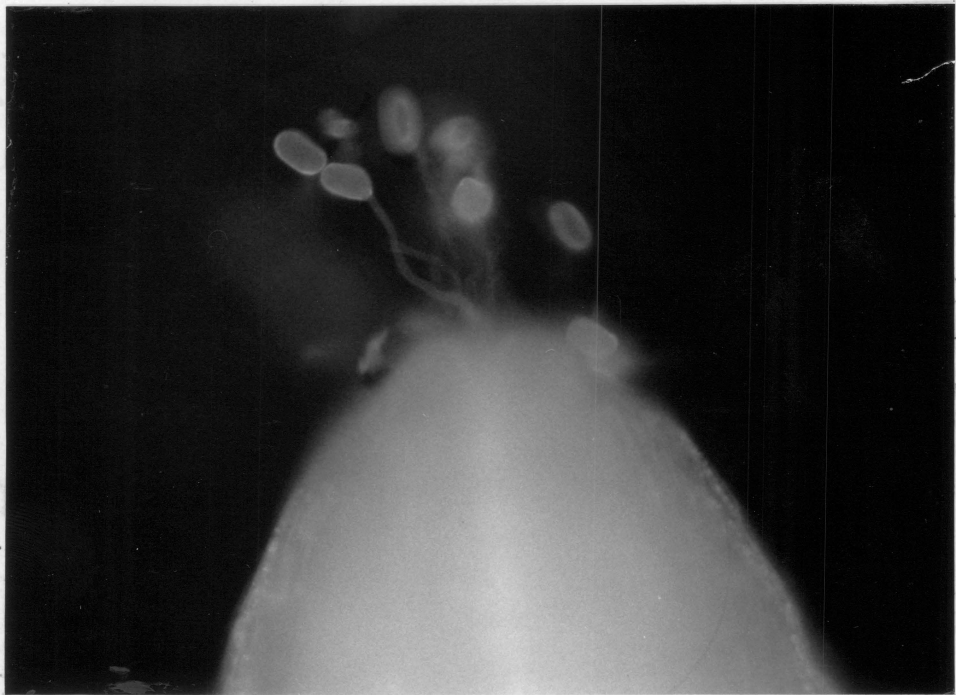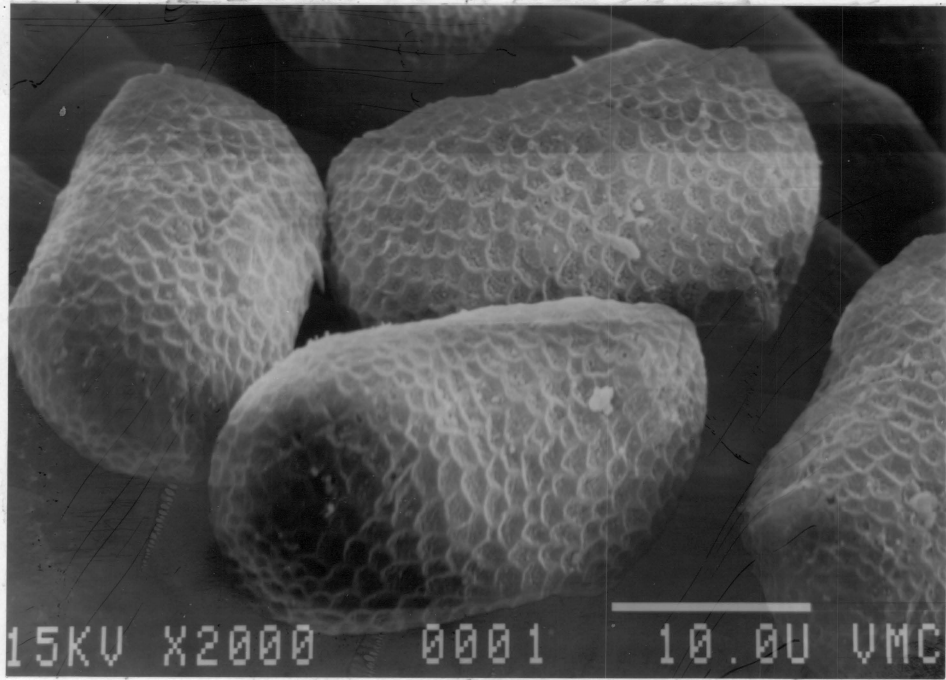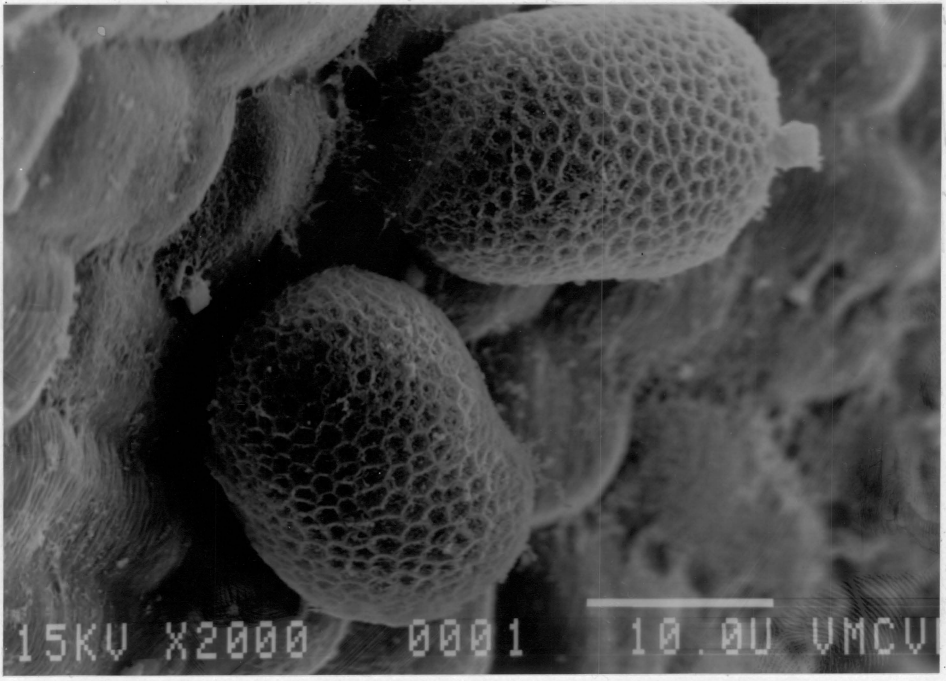Wood, C. E. 1975. The Balsaminaceae in the southeastern United States. *J. Arnold Arboretum* 56:413-426.

Figure 5. Impatiens pallida pollen germinating on I. capensis stigmas

Figure 6. **Impatiens pollen and stigma morphology.:** A. *I. capensis* pollen morphology. B. *I. pallida* pollen morphology.
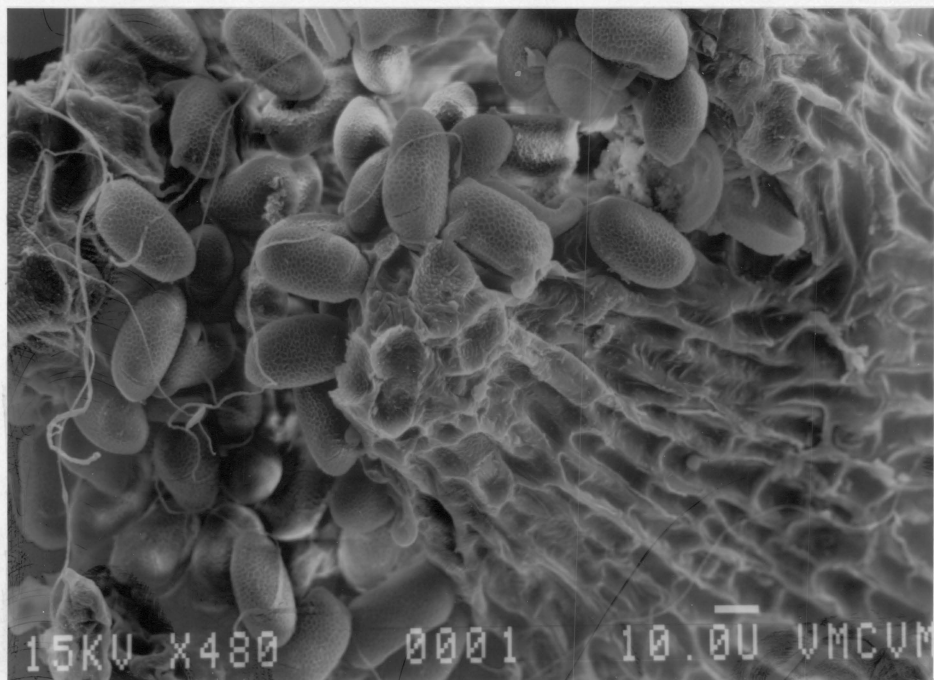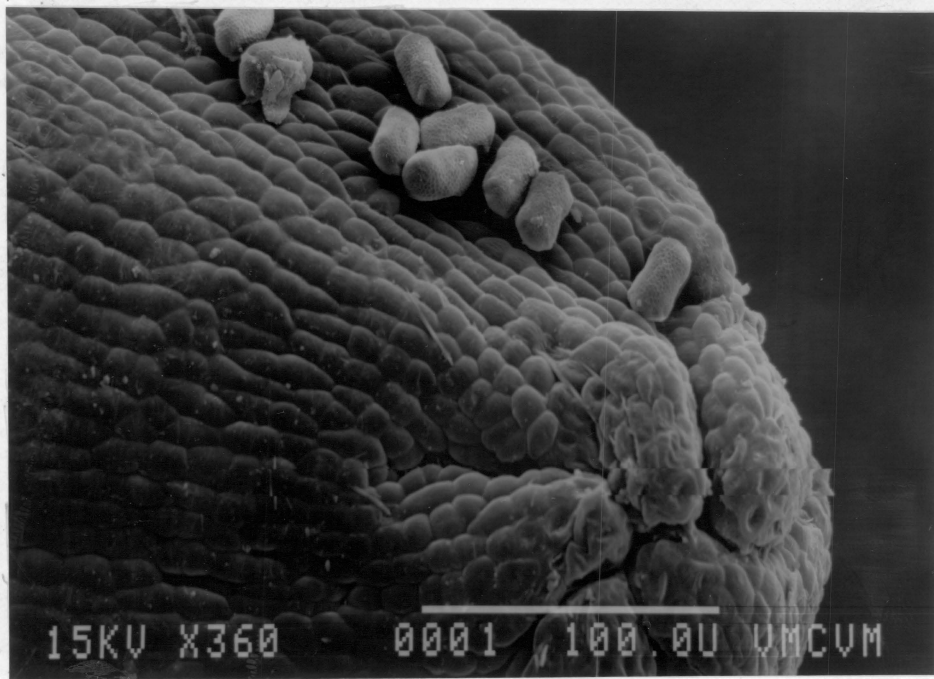
Figure 7. Impatiens pallida pollen on I. capensis stigmas: A. *I. pallida* pollen adhering to the stigmas of *I. capensis*. B. *I. pallida* pollen germinating on the stigmas of *I. capensis*.

32

Table 1: Stigma receptivity times for *Impatiens capensis* and *I. pallida*; (+) = pollen germination. Each time contains five replicates.

Species	Minutes				Time		Hours			
	0	15	30	45	1	2	5	24	48
I. capensis	+	+	+	+	+	+	+	+	+
I. pallida	+	+	+	+	+	+	+	+	+

Table 2: Pollen germination rates for *Impatiens capensis* and *I. pallida*; (+) = pollen germination. Each time contains five replicates.

Species	Minutes			Time			Hours		
	15	30	45	1	2	5	24	48
I. capensis	+	+	+	+	+	+	+	+
I. pallida	+	+	+	+	+	+	+	+

Table 3: Seed set via hybridization. C = *I. capensis*, P = *I. pallida*.

Test	N	Capsules	No Capsules
C x P	65	0	65
P x C	65	0	65

Table 4: Seed set via pollen mixture pollinations. C = *I. capensis*, P = *I. pallida*, (C + P) = pollen mixture.

Test	N	Capsules	No Capsules
C x P(C + P)	15	1	14
P x C(C + P)	16	8	8

Chapter IV

Floral Characteristics Influencing Bumblebee

Preference for Impatiens pallida over I. capensis

Introduction

Bumblebees respond to primary and secondary sensory information on approaching and entering

a patch of flowering plants (Barth 1985). Primary attractants are visual and include perianth color

and ultraviolet absorbance-reflectance patterns, flower size and shape, plant size, and patch density

(Kevan 1983, Waddington 1983, Waser 1983, Barth 1985). Secondary attractants are associated

with floral reward and include floral odors (von Frisch 1967, Kevan 1983, Barth 1985).

Bumblebee foraging behavior results from innate and learned information "superimposed on

an environmental background" (Waddington 1983 p. 215). To process information, foraging

bumblebees rely upon innate response (influenced by the organism's evolutionary history), sensory

information acquired from learning (e.g. expected reward perceived from a particular flower shape),

as well as primary and secondary sensory information received from the environment (Waddington

1983). No studies, however, have taken a naive pollinator response and broken it down into the

various components associated with floral characteristics.

37

Using controlled experiments, Randall and Hilu (in preparation) determined that in the simultaneously flowering *I. capensis* Meerb. and *I. pallida* (L.) Nuttall, inexperienced bumblebees show a preference for *I. pallida* over *I. capensis* regardless of plant species ratios. Both *Impatiens* species share the same bumblebee pollinators when they grow allopatrically. In sympatric populations, however, *I. pallida* attracts bumblebees away from *I. capensis*, leaving *I. capensis* to depend on hummingbirds and small bees for pollination. The objective of this study was to determine which specific floral character(s) influences the pollinator preference of bumblebees toward *I. pallida*. Floral characters examined were: perianth ultraviolet absorbance-reflectance patterns, flower color, petal size and shape, odor, and nectar production rate and quality.

Methods and Results

Study sites

Nine natural populations of *Impatiens* (three *I. capensis*, three *I. pallida*, and three sympatric) were located in Montgomery County, Virginia. The nine sites were comparable in canopy cover, and had similar aspect and soil moisture levels. These populations were identified as C1, C2, C3 (for *I. capensis*), P1, P2, P3, (for *I. pallida*), and S1, S2, S3 (for sympatric populations).

Nectar characteristics

To determine if the preference of bumblebees for *I. pallida* over *I. capensis* is a response to experience with nectar rewards in natural sympatric populations, the nine populations were surveyed to determine whether flowers of the *Impatiens* species differ in nectar quantity, quality, and production times.

Twenty individual plants in each population (of each species in sympatric populations) were selected for study. Flowers were chosen by walking a transect and selecting every 1 m the nearest flower due to open the following day. Flowers were covered with a fine-mesh pollination bag to prevent foraging. The next day, nectar samples were collected in 5 μl micropipetts from each bagged flower at 0800, 1300, and 1800 in populations C1, C2, P1, P3, S1, and S2. However, in populations C3, P2, and S3 the nectar was collected at 0800 and 1300 only, because the nectar-containing spurs had become damaged by previous sampling. Nectar samples were taken in August and September, 1986. Sample volumes were recorded, and each was analyzed for solute concentration with a Reichert hand-held refractometer. For nectar sugar analysis, a second set of samples was collected, placed on ice in the field, and later frozen and stored at -18° C. Sugar constituents and their quantities were determined by high performance thin layer chromatrography (HPTLC) and quantified by a CAMAG TLC scanning densitometer using the procedures of Fell (1988). The study showed that nectar quantity, solute concentration, and time of production were significantly different among populations. However, there were no significant differences in nectar characteristics within allopatric and sympatric populations (p > .05). All populations but C3 exhibited a trend toward high nectar volumes with low solute concentrations in the morning, and low nectar volumes with high solute concentrations in the late afternoon (Figure 8 on page 47, Figure 9 on page 48, Figure 10 on page 49, Figure 11 on page 50). Nectar volumes among populations ranged from 0 to 13 μl ($\bar{x} = 3.3$ μl) in *I. capensis* and from 0 to 14 μl ($\bar{x} = 2.3$ μl) in *I. pallida*. Nectar concentrations among populations ranged from 14 to 48 brix (g solute/100g solution) ($\bar{x} = 34.9$ brix) for *I. capensis* and from 23 to 50 brix ($\bar{x} = 38.2$ brix) for *I. pallida*. The content and quality of nectar sugars did not differ between the *Impatiens* species. Nectars of both species were composed of 91% sucrose and 9% fructose.

Floral ultraviolet absorbance-reflectance patterns

To determine if *I. capensis* and *I. pallida* have UV patterns, UV photographs were taken of flowers of both species using a UV light source and a Kodak/Wratten 18A filter, allowing only UV wavelengths (300-400 nm) to pass through the lens.

39

The two *Impatiens* species exhibit opposite UV patterns (Figure 12 on page 51). Flowers of *Impatiens capensis* are essentially UV absorptive, except for a UV reflective inner margin of the petals (Fig. 12-C). In contrast, the flowers of *I. pallida* are UV reflective, except for the UV absorptive inner margins of the petals (Fig. 12-D).

Effects of ultraviolet absorbance-reflectance patterns on foraging behavior

If characteristic UV patterns were eliminated, bumblebees would have to respond to visual characters other than UV patterns to discriminate between the two *Impatiens* species. To achieve this situation, orange and yellow acrylic paints were applied to 20 flowers of each *Impatiens* species in sympatric population S1, and was repeated in S2 the following week. Since Bell et al. (1984) determined that there is a partial preference by bumblebees for the male flowers of *I. capensis*, flowers were tested in the male phase. The day following the application of paint, the painted flowers were uncovered at approximately 0700 and observed for bumblebee visits for 5 hours. To verify the effectiveness of acrylic paint in disguising the UV patterns, UV photographs were taken of painted *Impatiens* flowers.

Ultraviolet photographs of painted *Impatiens* flowers showed that the paint completely absorbed all UV light, indicating that the the UV patterns of both *Impatiens* species is disguised (Fig. 12-E,F.). Consequently, when painted, *I. pallida* flowers resembled *I. capensis* flowers with respect to their UV patterns. In spite of masking the UV patterns, bumblebees continued to prefer *I. pallida* in both populations S1 and S2. Bumblebees foraged on the painted and unpainted flowers of *I. pallida* but avoided painted as well as unpainted flowers of *I. capensis*.

Effects of flower color on foraging behavior

Since UV patterns alone do not direct bumblebee foraging behavior, the effects of flower color were examined. Flower color perception in bumblebees was tested by trimming 20 flowers (in the male

phase) of each *Impatiens* species to be similar in shape, thus eliminating the effects of shape. This experiment was tested in sympatric population S1 and repeated in population S3 one week later. Flowers were trimmed in the evening and bagged to prevent foraging. The trimmed flowers were uncovered at approximately 0700 and the frequency of visitation was observed for six hours.

Flowers of *I. pallida* were visited exclusively despite their similarity in size and shape to those of *I. capensis*. Therefore, trimming flowers had no effect on the foraging behavior of bumblebees. These results indicate that flower color is recognized by bumblebees and affects their foraging behavior in sympatric patches of the two *Impatiens* species.

Effects of flower shape on foraging behavior

To isolate the possible relationship between *Impatiens* flower shape and bumblebee foraging behavior, the influence of flower color and UV patterns were removed by painting flowers of both *Impatiens* species the same color. Blue acrylic paint was applied to 20 flowers of each *Impatiens* species in population S1, and the painted flowers were then bagged. The painted flowers were uncovered the following day at approximately 0700, and bumblebee visitations were observed for six hours.

Bumblebees did not visit painted flowers of either *Impatiens* species; they did, however, visit unpainted *I. pallida*, but not unpainted *I. capensis*. At least 12 different bumblebees were observed on *I. pallida* flowers surrounding the 20 painted flowers during the six hour period. After one hour of bumblebee foraging, the surrounding *Impatiens* flowers being foraged on were pulled off in an attempt to encourage foraging on the painted flowers. This resulted in bumblebees extending their flights to forage on *I. pallida* further away from the painted flowers. To further examine bumblebee shape perception, all of the unpainted flowers of both *Impatiens* species were removed, leaving only painted flowers. Bumblebees continued to visit the population but did not forage on the painted *Impatiens* flowers.

Effects of flower odor on foraging behavior

Odor differences were tested by placing flowers of each *Impatiens* species in 1-quart Mason jars and sealing them overnight to allow the acculmulation of floral odors. The jars were then connected to bumblebee colony boxes by clear PVC plastic tubing and a "T" coupling device (Figure 13 on page 52). The design of this experiment was in principle similar to that used by Martin (1964) (as described by Barth 1985) for testing close-range orientation by bees in an odor field. The T-junction created by the two opposing plastic tubes forced bees to choose between turning toward one or the other jars containing the floral odors of *Impatiens* species flowers. Since insects are known to show "handedness" (Jander 1975), jar positions were switched after one half of the trials. Colony boxes of two different bumblebee species (*B. pennsylvanicus* and *B. impatiens*) were used in the experiment. The experiment showed that bumblebees exhibit no preference for the odor of either *Impatiens* species. Six bees made their first turn toward *I. pallida* while four bees made their first turn toward *I. capensis*. The overall turning directions were 29 toward *I. pallida* and 31 toward *I. capensis* (Figure 14 on page 53). Thus turns were made toward each *Impatiens* species in approximately equal numbers.

It is possible that forcing bees to chose between moving right or left might have induced unnatural responses. Therefore, another experiment was designed to test the effects of *Impatiens* flower odor. Twenty sterile, 1 x 1.5 cm, polyurethane foam plugs were placed overnight in each of two 1-quart Mason jars containing flowers of both *Impatiens* species to absorb floral odors. The plugs were hung among the flowers in natural allopatric populations C2 and P2. This experiment was duplicated the following week. Bumblebees showed no positive response to the *Impatiens*-scented polyurethane foam plugs, indicating that flower odor was not an attractant.

Discussion

Since the secondary stimulus of odor did not influence bumblebee foraging behavior, the primary stimulus of vision is considered to be the important element to which bees respond on entering a sympatric population of *Impatiens*. Among the suite of visual stimuli, floral color above 400 nm appears to be the characteristic that most influences bumblebee foraging behavior on *Impatiens*. Bumblebees always preferentially visited the yellow-flowered *I. pallida*, even when flowers were trimmed to resemble *I. capensis*, or painted to disguise UV patterns. The individual effects of flower UV patterns, size and shape, and odor, did not affect bumblebee preference for *I. pallida* over *I. capensis*.

Flower color, particularly highly reflective color, has been recognized as a primary signal for bees searching for food (Levin 1968, 1972, Jones and Buchmann 1974, Jones 1977, Barth 1985). Barth (1985) described the 1943 experiments of Kugler which showed that the sight of an inflorescence elicited approach behavior in bees. This behavior was shown to be primarily dependent upon color, rather than on shape or odor. Mulligan and Kevan (1973) determined that pollinator visitation frequencies among some weeds in Canada were more influenced by strong flower reflectance than by flower size, color, UV patterns, or a combination of these. Levin (1968) noted that bumblebees prefer a light-colored, UV-reflective target over a dark-colored, UV absorptive one. Our results indicated that reflection in the UV wavelengths was not crucial, since bumblebees continued to visit the UV-absorptive *I. pallida* flowers after they were painted yellow. However, the yellow pigments of painted flowers remained highly reflective above 400 nm even though the UV wavelengths were absorbed.

Nectar guides have been shown to influence the foraging behavior of bees in several studies. Jones (1977) cites UV patterns as an important orientation cue for a pre-pollination isolating mechanism between sympatric species of *Cercidium* (Fabaceae). Waser and Price (1981) have shown that "albino" flowers of *Delphinium nelsonii* (Ranunculaceae), which lack color and nectar guides, are visited less frequently than purple flowers, and require a longer handling time. Jones and Buchmann (1974) identified UV patterns as an isolating mechanism utilized by bees to differentiate between co-flowering species. In addition to rendering *I. pallida* UV-absorptive by

43

painting, UV patterns serving as nectar guides were also eliminated. The absence of nectar guides does not seem to affect bumblebee foraging behavior to the extent that has been reported in past studies.

Diurnal variation in nectar measurements suggests that there is an environmental influence on nectar production. In early morning, when humidity is highest and temperatures are lowest, nectar is in greater quantity and is more dilute. In the afternoon, when humidity is lowest and temperatures are highest, nectar volumes are lower and more concentrated. This phenomenon is due perhaps to evaporation and possible readsorption of water by the plant (Bertsch 1983). Potted plants kept in water-saturated soil maintained essentially constant nectar volumes and solute concentrations throughout the day (J. Randall unpublished data), indicating that nectar quantity and solute concentrations are environmentally based. Similarly, Zimmerman (1983) found that nectar production in *Delphenium nelsonii* was increased by supplemental watering.

Marden (1984) demonstrated in *I. capensis* that intrapopulation variation in nectar production appeared to be caused by soil moisture levels, but was not correlated with time of day, as our data suggest. In contrast, Zimmerman (1981) noted that nectar dispersion patterns in a population of *I. capensis* were variable due to "patches" of "cold" and "hot" flowers. Zimmerman's study suggested that nectar production rate has a genetic rather than an environmental basis since his population site had a constant water level. However, his data were the result of only one day's sampling in one population.

Our results show that flower color is the primary stimulus for attracting bumblebees. The *I. capensis* flower is obviously recognized by bumblebees, since they act as its primary pollinator in allopatric populations. Therefore, bumblebees are attracted to *I. capensis* by its color, and perhaps by other traits as well. However, bumblebees seem to respond to the visual stimulus of *I. pallida* color to the extent that a valuable food source goes unrealized since sympatric *I. capensis* exhibits comparable nectar volumes and concentrations.

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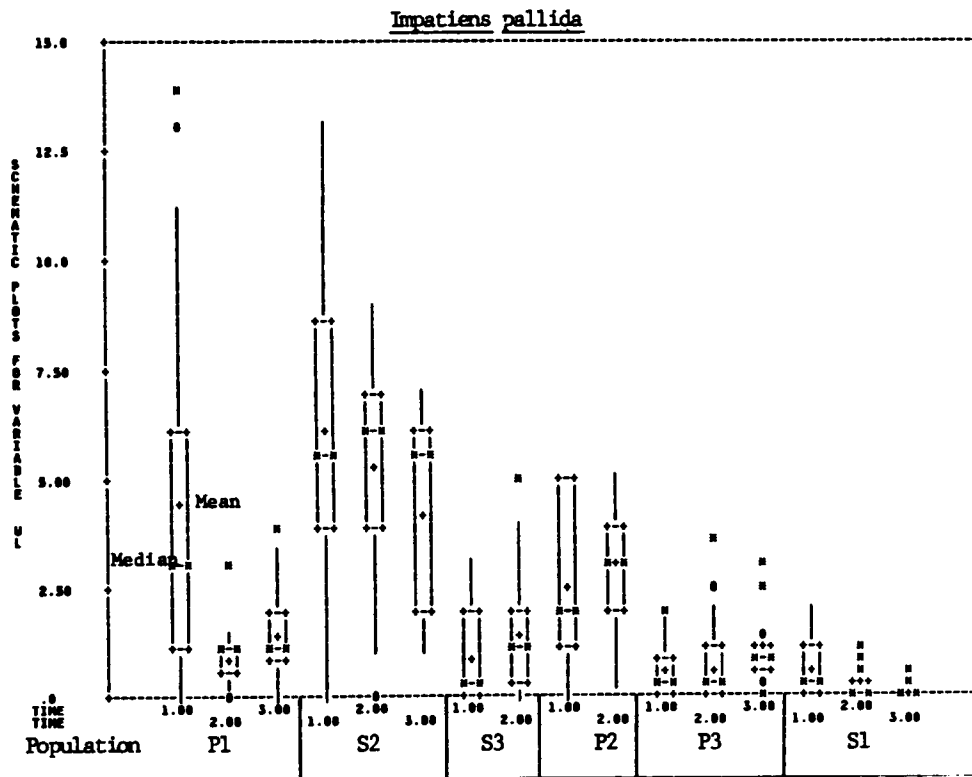
Figure 8. Impatiens pallida nectar volumes for allopatric and sympatric populations
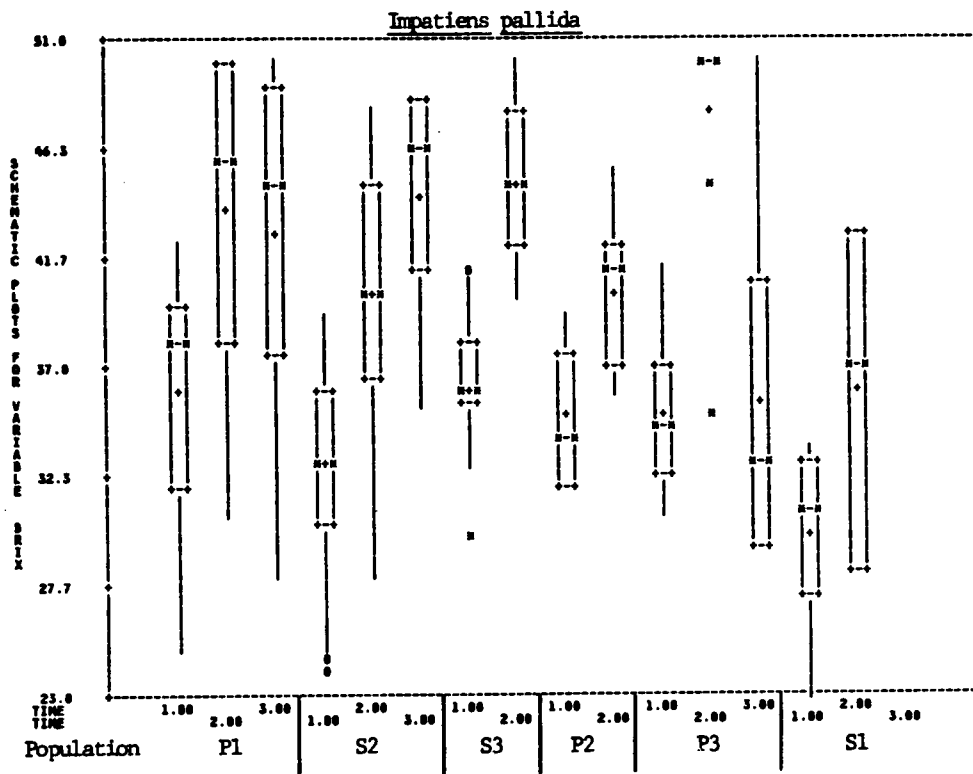
Figure 9. I. pallida nectar concentrations for allopatric and sympatric populations
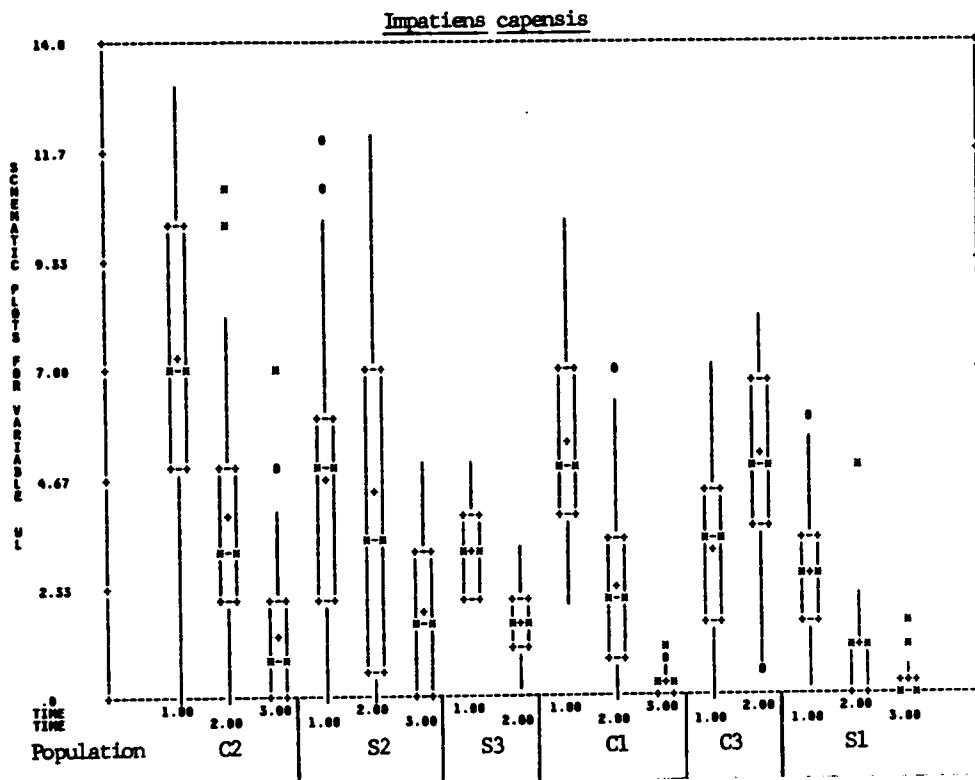
Figure 10. I. capensis nectar volumes for allopatric and sympatric populations
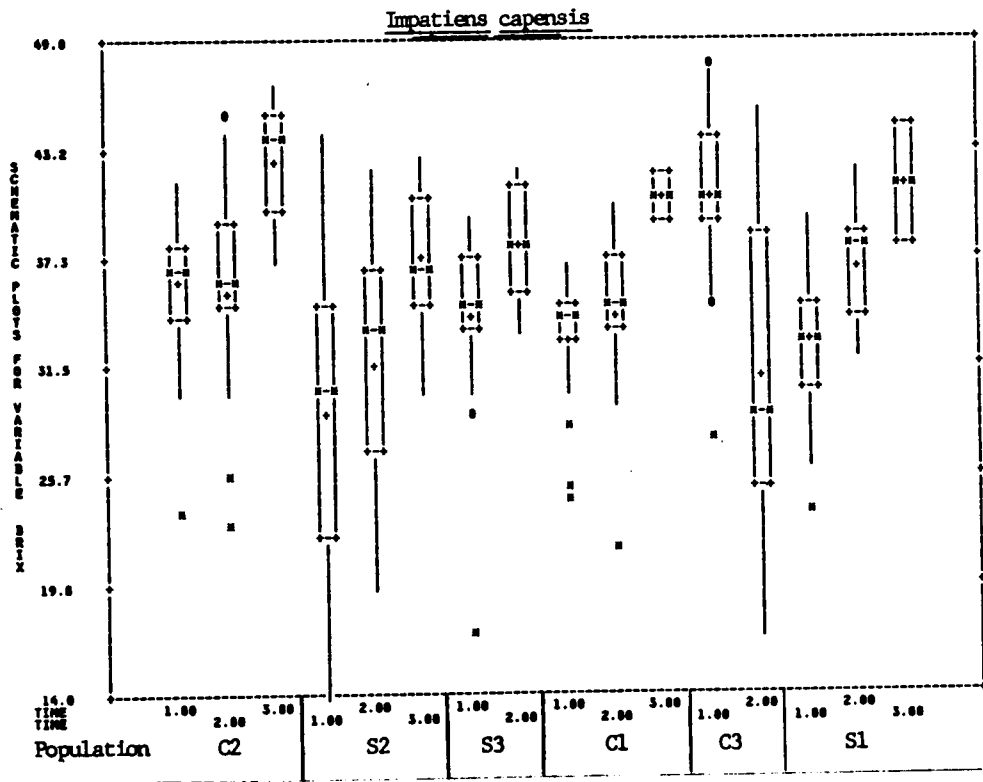
Figure 11. I. capensis nectar concentrations for allopatric and sympatric populations
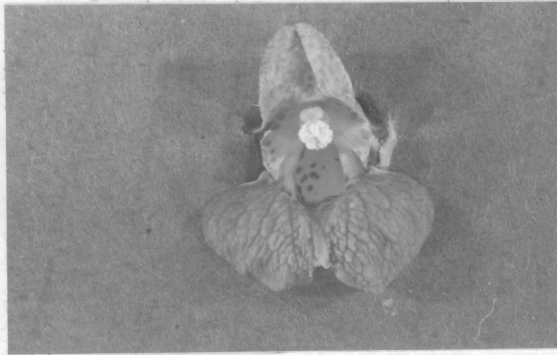
A. Impatiens capensis – visible lt. B. Impatiens pallida – visible lt.
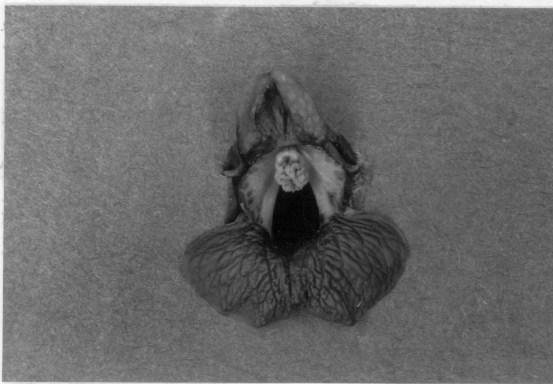
C. I. capensis – UV lt. D. I. pallida – UV lt.
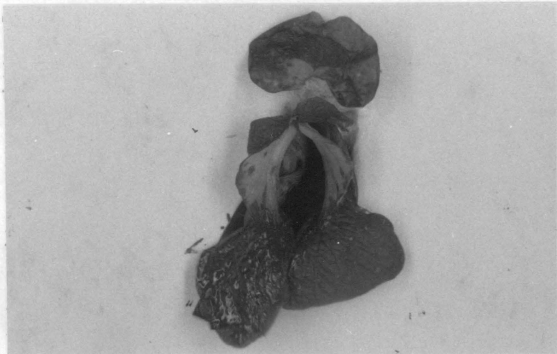
E. I. capensis – painted petal, UV F. I. pallida – painted petal, UV

Figure 12. Impatiens flowers under "visible" and UV light

Figure 13.   Bumblebee colony box fitted with nectar odor apparatus.

Colony One, Bombus pensylvanicus

Bee number
  R5   p-b-p-c-b-c-b-p-c-b-p
  R8   c-b-c-b-p-c-p-c-b
            switched jar position
  R20   p-c-p-c-b-c-b-p-b-c

Colony Two, Bombus impatiens
  B19   p-b-c-b-p-b-p-b-p-c-b-c-b
  B12   c-b-c-b-p-b-p-b-c
  B4   p-c-p-c-p-c-b
  B20   p-c-p-b-p-c-b
            switched jar position
  B1   c-b-c-b-p-b-c-b-p-b-p-b
  B22   c-b-p-b-c-b-p-c-p-c-b
  B24   p-c-p-c-b

Figure 14.   Bumblebee odor preference turning sequences: P = *Impatiens pallida*, C = *Impatiens capensis*, B = returned to box.

Chapter V

Conclusions

From these studies, several conclusions can be drawn. First, bumblebees show a strong preference for *Impatiens pallida* over *I. capensis*. Inexperienced bumblebees visit *I. pallida* almost exclusively, regardless of *Impatiens* species ratios or nectar rewards. Second, when heterospecific pollen transfer occurs between the *Impatiens*, *I. pallida* pollen reduces the fecundity of *I. capensis* through interference from one or both of the following: *Impatiens pallida* pollen a) adheres to and possibly clogs the stigmas of *I. capensis*, and/or b) germinates on the stigmas and pollen tubes grow to the ovules of *I. capensis*, but without fertilizing them. Third, the preference for *I. pallida* over *I. capensis* by bumblebees appears to be influenced primarily by flower color, although the combination of several floral characteristics may also contribute to this. The flower of *I. pallida*: 1) is more light reflective, especially in the UV and yellow wavelengths, making for a brighter target, whereas *I. capensis* is UV absorptive; 2) displays a distinctive UV absorbance-reflectance pattern which serves as a nectar guide; and 3) possesses somewhat larger flowers with easier access to the nectar-containing spur, perhaps requiring less handling time than *I. capensis*.

Despite its lesser attraction for bumblebees and pollen interference, *I. capensis* avoids competition with *I. pallida*, through one or all of the following: 1) *I. capensis*, like *I. pallida*, produces cleistogamous flowers, ensuring sexual reproduction even in the absence of pollinators.

2) *I. capensis* is capable of producing a full seed complement via one pollinator visit. 3) *I. capensis* attracts hummingbirds and small bees for pollination in the absence of bumblebee visitation.

These studies have demonstrated that models predicting foraging behavior and optimal foraging provide null hypotheses for experimental work, and not necessarily a view of reality. Also, competition may not represent as strong a force influencing population structure as has been reported in the literature. The occurrence of *I. capensis* and *I. pallida* in sympatric populations wherever their ranges overlap indicates that these two species are able to coexist despite the specter of competition. This investigation has reminded me, once again, that *Natur complexus est.*

Appendix A

1985 Nectar data.

mm nectar (u1=32-5)

brix

humidity

temperature

time

flower number

plant number

species

population

Appendix B

1986 nectar data.

population	plant number	flower number	time	microliter	brix
652P	2	1	1	6	42
652P	2	2	1	6	40.5
652P	4	1	1	5	35.5
652P	4	2	1	6	38.5
652P	6	1	1	6	38
652P	5	1	1	0	.
652P	5	2	1	0	.
652P	7	1	1	6	37
652P	7	2	1	3	41
652P	7	3	1	2.5	39
652P	13	1	1	0	.
652P	16	1	1	1	40
652P	16	2	1	.	.
652P	18	2	1	5	40
652P	2	1	1	.3	.
652P	2	2	1	.5	33
652P	4	1	1	8	32
652P	6	1	1	11	27
652P	5	1	1	0	.
652P	5	2	1	7	25
652P	7	1	1	11	32
652P	7	2	1	14	32
652P	7	3	1	11	31.5
652P	16	1	1	13	28
652P	16	2	1	11	37
652P	18	2	1	8	32
652P	1	1	1	1	40
652P	2	1	1	1	.
652P	3	1	1	2	35
652P	4	1	1	0	.
652P	6	1	1	2	38
652P	7	4	1	2	38.5
652P	7	5	1	3	38.5
652P	8	1	1	4	38
652P	11	1	1	5	38
652P	12	1	1	2	39.5
652P	14	1	1	0	.
652P	17	1	1	1	40
652P	18	2	1	1	36
652P	1	1	2	1	50
652P	2	1	2	1	31
652P	3	1	2	.5	.
652P	4	1	2	0	.
652P	6	1	2	1	50
652P	7	4	2	1	50
652P	7	5	2	.6	.
652P	8	1	2	.2	.
652P	11	1	2	1	38
652P	12	1	2	.8	.
652P	14	1	2	.8	41
652P	17	1	2	1	.
652P	18	2	2	3	46
652P	1	1	3	1.5	50
652P	2	1	3	.8	28
652P	3	1	3	1	45
652P	4	1	3	0	.
652P	6	1	3	2	36.5
652P	7	4	3	2.5	43
652P	7	5	3	1	.
652P	8	1	3	4	50
652P	11	1	3	2	37.5
652P	12	1	3	2.5	49
652P	14	1	3	0	.
652P	17	1	3	1	.
652P	18	2	3	.8	47
652C	2	1	1	11	38.5
652C	3	1	1	7	38
652C	3	2	1	10	34
652C	4	1	1	6	30
652C	5	1	1	5	35
652C	5	2	1	10	36
652C	6	1	1	10	39
652C	7	1	1	3	37.5
652C	8	1	1	5	24
652C	8	2	1	9	37.5
652C	9	1	1	11	31
652C	9	2	1	0	.
652C	9	3	1	10	36
652C	10	1	1	1	41
652C	10	2	1	5	37
652C	11	1	1	6	35
652C	11	2	1	11	32
652C	12	1	1	5	39
652C	13	1	1	10	37
652C	13	2	1	4	36
652C	13	3	1	13	36
652C	14	1	1	7	34
652C	15	1	1	6	33.5
652C	17	1	1	7	38
652C	17	2	1	3	37
652C	18	1	1	6	36.5
652C	18	2	1	6	37.5
652C	19	1	1	13	38
652C	20	1	1	10	38
652C	20	2	1	9	39
652C	21	1	1	10	39
652C	22	1	1	7	33
652C	2	1	2	3	35
652C	3	1	2	5	30
652C	3	2	2	7	23
652C	4	1	2	1	34.5
652C	5	1	2	2.5	39
652C	5	2	2	1	36
652C	6	1	2	1	39.5
652C	6	2	2	1	32
652C	7	1	2	4	40
652C	8	1	2	3	37
652C	8	2	2	5	25.5
652C	9	3	2	6	35.5
652C	10	1	2	11	35
652C	10	2	2	10	35
652C	11	1	2	3	37.5
652C	11	2	2	4	37
652C	12	1	2	2.5	36
652C	13	1	2	4	38
652C	13	2	2	11	34.5
652C	14	1	2	7	32
652C	14	2	2	5	34.5
652C	15	1	2	5	32
652C	17	1	2	0	.
652C	17	2	2	1	37.5
652C	18	1	2	3	39
652C	18	2	2	1	40
652C	19	1	2	2	38
652C	20	1	2	2	34
652C	20	2	2	3	45
652C	21	1	2	2.5	39
652C	22	2	2	2	40
652C	2	1	3	0	.
652C	3	1	3	1.5	40
652C	3	2	3	2.5	40.5
652C	4	1	3	.5	.
652C	5	1	3	0	.
652C	6	1	3	0	.
652C	6	2	3	0	.
652C	7	1	3	0	.
652C	8	1	3	1.5	45
652C	8	2	3	2	38.5
652C	9	3	3	.8	45
652C	10	1	3	1.5	46.5
652C	10	2	3	3	46
652C	11	2	3	.5	.
652C	12	1	3	2.5	45
652C	13	1	3	7	37.5
652C	13	2	3	.5	.
652C	13	3	3	0	.
652C	14	1	3	0	.
652C	14	2	3	5	38
652C	15	1	3	.5	40
652C	17	1	3	0	.
652C	18	1	3	.2	.
652C	18	2	3	3	45
652C	19	1	3	2	44
652C	20	1	3	2.5	45
652C	20	2	3	1	42
652C	21	1	3	.3	.
652C	22	1	3	1	.
NRJP	1	1	1	5	33
NRJP	1	2	1	7	35
NRJP	2	1	1	11	38
NRJP	2	2	1	13	38
NRJP	3	1	1	12	35.5
NRJP	3	2	1	7	33
NRJP	4	1	1	2	34
NRJP	5	1	1	5	39
NRJP	9	1	1	.5	.
NRJP	10	1	1	3	36
NRJP	11	1	1	4	33
NRJP	1	1	2	7	44.5
NRJP	1	2	2	7	40
NRJP	2	1	2	4	48
NRJP	2	2	2	4	48
NRJP	3	1	2	5	41
NRJP	3	2	2	4	46
NRJP	4	1	2	6	45
NRJP	5	1	2	4	48
NRJP	9	1	2	0	.
NRJP	10	1	2	5	44
NRJP	11	1	2	0	.
NRJP	1	1	3	6	48.5
NRJP	1	2	3	6	41
NRJP	2	1	3	6	48.5
NRJP	2	2	3	7	46.5
NRJP	3	1	3	5	36
NRJP	3	2	3	6	.
NRJP	4	1	3	.	.
NRJP	5	1	3	2	48.5
NRJP	9	1	3	2	48
NRJP	10	1	3	2	45
NRJP	11	1	3	1	35.5
NRJP	1	1	1	9	31
NRJP	1	2	1	8	24
NRJP	2	1	1	.	.
NRJP	2	2	1	12	25.5
NRJP	3	1	1	4	24.5
NRJP	3	2	1	.	.
NRJP	4	1	1	7	33
NRJP	5	1	1	6	37
NRJP	9	1	1	0	.
NRJP	10	1	1	5	30.5
NRJP	11	1	1	4	27
NRJP	1	1	2	6	40.5
NRJP	1	2	2	6	37
NRJP	2	1	2	6	40
NRJP	2	2	2	8	37.5
NRJP	3	1	2	9	29.5
NRJP	3	2	2	6	36.5
NRJP	4	1	2	8	36
NRJP	5	1	2	8	38.5
NRJP	9	1	2	.	.

The vita has been removed from
the scanned document