Reproductive biology of *Cyrtopodium punctatum* *in situ*: implications for conservation of an endangered Florida orchid

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**Abstract**

*Cyrtopodium punctatum* (Linnaeus) Lindley is an endangered epiphytic orchid restricted in the USA to southern Florida. This species has been extensively collected from the wild since the early 1900s, and today only a few plants remain in protected areas. As part of a conservation plan, a reproductive biology study was conducted to better understand the ecology of this species in Florida. *Cyrtopodium punctatum* relies on a deceit pollination system using aromatic compounds to attract pollinators. Nine aromatic compounds were identified as components of the fragrance of *C. punctatum* inflorescences, including two compounds that are known to be Euglossine bee attractants. However, this group of bees is not native to Florida. Of the four bee species observed to visit *C. punctatum* flowers in the present study, carpenter bees (*Xylocopa* spp.) are likely to be the main pollinators. Pollination experiments demonstrated that *C. punctatum* is self-compatible, but requires a pollinator and thus does not exhibit spontaneous autogamy. In addition, the rates of fruit set were significantly higher for flowers that were outcrossed (xenogamy) than for those that were self-crossed. Thus, the species has evolved a degree of incompatibility. Examples of natural pollination and fruit set were observed during the present study (2007–2008), but the rates of reproduction were modest as a consequence of the low plant numbers and possible changes in insect densities as a result of anthropogenic influences.

**Keywords:** breeding system, *Cyrtopodium punctatum*, Orchidaceae, pollination.

Received 11 March 2009; accepted 21 May 2009

**Introduction**

Several types of plant rarity exist and different natural processes may affect species density and distribution (Rabinowitz 1981; Fiedler & Ahouse 1992). Species may be considered rare based on habitat specificity, local population size and/or the size of their geographical range. However, plants that were once common in their range may now be rare as a result of anthropogenic influences (Partel *et al.* 2005). The factors influencing rarity need to be considered for an effective conservation plan to be designed for any target species. Pollination systems are often linked to plant rarity given the dependence on insect species for cross pollination as this relationship is required for seed production and fruit set to take place. Decreased abundance or loss of pollinators often results in increased rarity of the plant. The Orchidaceae is a very diverse family, and pollination systems may range from being very specific with a plant being pollinated by only one insect, to very generalized with a plant being pollinated by many species (van der Pijl & Dodson 1966; Dressler 1981; van der Cingel 1995). A comprehensive investigation of the pollination system is a prerequisite to developing a conservation plan for any rare species.

*Cyrtopodium punctatum* (L.) Lindl. is an epiphytic orchid found in southern Florida, Cuba, Hispaniola, Puerto Rico and the north-western Caribbean coast of South America (Romero-González & Fernández-Concha 1999).
In Florida, the species is listed as endangered (Coile & Garland 2003); populations have been depleted because of cypress logging and over-collecting during the past century. Early accounts in the literature to C. punctatum as abundant throughout south and south-west Florida, particularly in the cypress swamps in the Big Cypress region (Ames 1904; Luer 1972). Photographs from the early 1900s show wagons loaded with plants being extracted from the wild. Consequently, only a few plants still remain in very small populations within inaccessible protected areas, such as Everglades National Park, Big Cypress National Preserve and the Florida Panther National Wildlife Refuge (FPNWR). Over the past 10 years, observations of plant populations in the FPNWR have indicated very low rates of capsule formation and seed production in the remaining plants (L. Richardson, pers. comm. 2005). Additional information regarding the breeding strategies and pollination mechanisms of natural populations of C. punctatum in Florida is critical to develop strategies for the recovery of the species.

The study of pollination systems, including breeding systems, is critical in rare plant conservation because it may influence the genetic diversity of populations (Hamrick & Godt 1996). Pollination aids the long-term survival of most species because population persistence depends on the recruitment of new plants from genetically diverse seeds (Dixon et al. 2003). Most of the pertinent literature on the pollination of C. punctatum refers to observations conducted in only one area of the species’ range (e.g. Puerto Rico; Ackerman 1995). In different parts of its geographical range, a species may possess different breeding strategies, which is not uncommon for tropical orchid species in Florida. For example, both Epidendrum nocturnum and Secoila lanceolata produce seeds by agamospermy in Florida, but use animal cross-pollination in the more southern part of their range (Catling 1987; Brown 2002).

Pollinator species may also vary from one location in the geographical range to another. Pollination ecotypes have been reported by Robertson and Wyatt (1990a) in Plantanthera ciliaris. In the present study, the pollination ecology of two disjunct populations was compared and the primary pollinators were found to differ between the sites. Cyrtopodium punctatum is reported to be pollinated by Euglossa bees (van der Pijl & Dodson 1966; Jeffrey et al. 1970; Luer 1972; Dressler 1993). However, as Florida lies outside the range of euglossine bees, C. punctatum’s pollinator(s) in Florida may vary (Chase & Hills 1992). In Puerto Rico, C. punctatum is reported to be pollinated by Centris or Xylocopa bees (Ackerman 1995). Bees of the genus Centris were reported by Dodson and Adams (in Luer 1972) to visit flowers of C. punctatum in Florida. However, these observations have not been fully described or verified.

Fragrances produced by plants attract pollinators and may function to select the type and number of visitors (Hills et al. 1972). Floral fragrances have been associated with deception pollination systems based on generalized food deception (Little 1983) and can be an extra tool in the determination of pollination systems. Different fragrance compounds or groups of compounds may attract different pollinators. A larger array of compounds may be beneficial in deceit pollination systems because a higher number of visitors will be attracted to the plant.

In many orchid pollination strategy studies, capsule formation is commonly observed as the final result of the breeding system study without taking into consideration seed viability (Wong & Sun 1999; Lehnebach & Robertson 2004). Other researchers have used seed number and weight as a further measurement for breeding system determination (Robertson & Wyatt 1990b; Sipes & Tepedino 1995). However, this type of measurement produces uncertain results because of differences in capsule maturity. Sipes and Tepedino (1995) reported that fruit weight and fruit size were not correlated with seed number, and instead used seed number as another measurement for breeding system determination. As capsules of C. punctatum require approximately 11 months to mature, repeated capsule measurement over the course of capsule development may minimize this limitation.

Another tool used to evaluate breeding systems is tetrazolium seed viability testing (TZ) and it has been used to assess the viability of European and North American temperate orchid species (Van Waes & Debergh 1986; Lauzer et al. 1994; Vujanovic et al. 2000; Bowles et al. 2002) and tropical epiphytic species (Singh 1981). Assessing seed viability and germinability is crucial for in vitro propagation of orchid species and their conservation and may be a helpful tool to more precisely determine effective pollination strategies for threatened and endangered taxa.

As a prerequisite to developing a comprehensive conservation plan for C. punctatum, integrated field and laboratory studies were conducted with the objectives of: (i) determining the breeding system of C. punctatum through controlled pollination experiments and the effects on both capsule formation and seed viability; (ii) determining the pollinator(s) of C. punctatum in Florida; and (iii) determining the volatile potential attractant compounds present in C. punctatum flowers.

**Materials and methods**

**Study species**

In the FPNWR (Collier County, FL, USA), C. punctatum plants are found growing epiphytically on cypress (Taxodium distichum) and less often on cabbage palm (Sabal

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**Plant Species Biology** 24, 92–103

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Palmetto) or old stumps of massive cypress trees that were logged in the 1940s. The vegetation classification for the habitat is cypress forest moderately to densely distributed cypress with an open canopy. The understory is composed of *Fraxinus caroliniana*, *Cladium jamaicense* and *Annona glabra*, among others. In other areas of Florida (e.g. Everglades National Park), *C. punctatum* can also be found growing on Buttonwood trees (*Conocarpus erecta*) in full sun. The inflorescences of *C. punctatum* are up to 1 m long and can have 30–40 flowers.

**Breeding system determination**

One population with 15 plants located in the FPNWR served as the source of plants for the determination of the breeding system. Six plants located approximately 30 m apart were used for the breeding system experiment during two consecutive years (2006–2007 and 2007–2008). Seven breeding system treatments (Table 1) were applied to five flowers of each plant. Flowers were randomly selected on the inflorescences. A total of 245 flowers were used in this study. A plant located approximately 1.61 km from the breeding system study population served as the pollen donor. Pollen was applied to flowers within 1 h.

The breeding system determination methods were adapted from Wong and Sun (1999). An extra treatment (induced xenogamy) was added based on Stewart (2007). The breeding system treatments are described in Table 1. Pollen used in the artificial xenogamy treatment was obtained from a plant originating from a different population in the FPNWR. Inflorescences were produced in February and were bagged before the flowers opened with 95 μm nylon mesh to prevent pollination events prior to initiating the experiment (Fig. 1). Plants remained bagged until signs of capsule formation were observed. The percentage of flowers forming capsules was recorded and capsule development was recorded bimonthly for 1 year by recording capsule length, width and abortion. The breeding system study was conducted during 2006–2007 and 2007–2008. Data were analyzed using general linear procedures and Waller–Duncan mean separation ($\alpha = 0.05$) with SAS v. 9.1 (SAS Institute 2003).

**Tetrazolium seed viability test**

Seed capsules produced from the breeding system study treatments (2006–2007) were collected on 23 February 2007 and dried over silica desiccant for 70 days at $23 \pm 2^\circ\text{C}$. The seeds harvested from each capsule collected were transferred to 20 mL scintillation vials. The vials were then maintained in cold storage ($-10 \pm 2^\circ\text{C}$).

Three 5 mg seed subsamples (75–100 seeds) from each capsule of each treatment were dispensed into a 1.5 mL microcentrifuge tube and homogenized with the aid of a vortex shaker. The methods of tetrazolium viability testing follow Lakon (1949). A pretreatment test conducted prior to experimentation indicated that a 15 min treatment in a seed scarification solution of 5% CaOCl$_2$ was ideal for *C. punctatum* seeds. Percentage seed viability data were collected for each of the three subsamples per capsule by placing seeds that were suspended in water droplets into Petri dishes and the seeds were then scored with the aid of a dissecting microscope. Approximately 100 seeds were counted per subsample. Embryos that contained any degree of red or pink coloration were scored as viable and white embryos were scored as non-viable (Fig. 2). Percentage viability was calculated for each replicate by dividing the number of stained embryos by the total number of embryos. Data were analyzed using general linear procedures and Waller–Duncan mean separation ($\alpha = 0.05$) using SAS v. 9.1.

**Asymbiotic seed germination test**

Seeds from the capsules produced from each specific breeding system treatment were sampled (5 mg subsamples of 75–100 seeds). Seeds from the same treatment were then pooled and dispensed into 1.5 mL microcentrifuge tubes. The seeds were surface sterilized in a solution containing 5 mL ethanol (100%), 5 mL 6.0% (v/v) sodium...
hypochlorite and 90 mL sterile distilled water for 3 min, followed by three repetitive 30 s rinses in sterile distilled water.

P723 Orchid Seed Sowing Medium was prepared from concentrated stock solutions using the formulation developed by PhytoTechnology Laboratories, LLC (Shawnee Mission, KS, USA) and adjusted to pH 5.8 with 0.1 mol/L KOH prior to the addition of 0.8% TC agar (PhytoTechnology Laboratories, LLC) and autoclaving at 117.7 kPa for 40 min at 121°C. The autoclaved medium (approximately 50 mL medium/plate) was dispersed into square 100 mm × 15 mm Petri plates (Falcon ‘Integrid’ Petri Dishes; Becton Dickinson Woburn, MA, USA). The bottom of each dish was divided into 36, 13 mm × 13 mm

Fig. 1 Breeding system experiment. (a) Flower, (b) artificial removal of pollinia, (c) capsule measurement, (d) Cyrtopodium punctatum plant with pollination bag in natural habitat at the Florida Panther National Wildlife Refuge (Collier County, FL, USA).
cells (Fig. 2). Only the 16 cells in the middle of the plate were considered for inoculation because the cells at the outer edges of the plate were more susceptible to drying. Five of the 16 interior cells were selected randomly for sowing using a computerized random number generator. Surface-disinfected seeds were sown onto the surface of the sterile germination medium using a sterile bacterial inoculating loop. Plates were sealed with NescoFilm (Karlan Research Products, Santa Rosa, CA, USA) and incubated under 12/12 h light/dark (60 μmol/m²/s) photoperiod at 25 ± 3°C. Approximately 82 seeds were sown onto each plate (mean seeds/plate = 82, mean seeds per cell = 16.4). Five replicate plates were used for each breeding system treatment. Seed germination and protocorm development stage percentages were recorded weekly for 10 weeks. Seedling development was scored on a scale of 1–5 (Table 2; modified from Stewart et al. 2003). Germination percentages were calculated by dividing the number of seeds in each germination and development stage by the total number of viable seeds in the subsample. Data were analyzed using general linear model procedures and Waller–Duncan at α = 0.05 with SAS v. 9.1. Percentage

Table 2 Seed developmental stages of Cyrtopodium punctatum (modified from Stewart et al. 2003)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Intact testa</td>
</tr>
<tr>
<td>2</td>
<td>Embryo enlarged, testa ruptured (= germination)</td>
</tr>
<tr>
<td>3</td>
<td>Appearance of protomeristem</td>
</tr>
<tr>
<td>4</td>
<td>Emergence of two first leaf primordia</td>
</tr>
<tr>
<td>5</td>
<td>Elongation of shoot and further development</td>
</tr>
</tbody>
</table>

Fig. 2 Seed viability and asymbiotic germination. (a) Germinating seeds (stage 2). (b) Protocorm at stage 4 of development showing two leaves. (c) Stained and unstained seeds from tetrazolium. (d) Falcon ‘Integrid’ Petri dishes showing cells.
Germination counts were arcsine transformed to normalize variation.

**Pollinator identification**

Pollinator observations were conducted during the 2007 and 2008 flowering seasons on a population consisting of 15 plants. Observations took place on plants in the population that had the largest number of inflorescences during both flowering seasons (12 and 14 inflorescences in 2007 and 2008, respectively). Observations took place from 07.00 to 18.00 hours for the first 2 days (13 and 14 March 2007). When peak visitation time was identified, observations took place from 10:00 AM to 4:00 PM for the remaining days (15, 23–25 March 2007). In 2008, observations took place on 18–19 March (10.00–15.00 hours) and 26 March (11.00–15.00 hours). Flower visitors were photographed, collected and identified, and their behavior in the flowers was documented. Flower visitors capable of pollinating the flowers were determined either by direct observation or by the detection of pollinaria on their bodies. The insects collected were identified at the Florida State Collection of Arthropods (Gainesville, FL, USA).

**Fragrance analysis**

Two inflorescences with 30 open flowers each from two C. punctatum plants were collected from the FPNWR during the 2007 flowering season. The inflorescences were collected while still attached to the pseudobulbs to preserve the flowers and taken to the US Department of Agriculture Chemistry Research Unit (Gainesville, FL, USA). The inflorescences were placed into a 15 cm diameter x 40 cm tall glass volatile collection chamber. Volatiles were collected over three time periods: 9:00–12:00 AM, 12:00 AM–5:00 PM and 5:00 PM–7:00 AM. The volatile traps were extracted with 150 µL methylene chloride. Gas chromatography–mass spectroscopy (GC/MS) analyses of the collected volatiles were carried out on an HP-6890 gas chromatograph coupled to an HP5973 mass spectrometer (Agilent, Santa Clara, CA, USA). The GC/MS peaks of interest were identified by comparing their mass spectral data with data from three mass spectral libraries. Only two inflorescences could be used because very limited plant material from wild populations is available owing to the endangered status of the species.

**Results**

**Breeding system determination**

Capsule formation occurred in four out of the seven treatments tested during both the 2006–2007 and 2007–2008 seasons (Figs 1c,3). There was no evidence that the agamospermy, spontaneous autogamy or open pollination (control) treatments resulted in capsule formation (Fig. 3). The induced geitonogamy (pollen from the same flower) and artificial geitonogamy (pollen from the same plant, but from different flowers) treatments resulted in the production of significantly fewer capsules (14.6 and 27.1% capsule formation, respectively) than the artificial xenogamy (pollen from a different population) and induced xenogamy (pollen from a plant in the same population).

Fig. 3 Percentage capsule set among seven breeding system treatments. Data from 2006 and 2007. Six plants and 30 flowers per treatment were used per year. Percentages with the same letter are not significantly different. Control (no treatment, open pollination); agamospermy (emasculated flower, no pollination); spontaneous autogamy (no treatment, pollen from the same flower); induced autogamy (selfing with pollen from the same flower); induced autogamy (selfing with pollen from the same plant, different flower); artificial geitonogamy (seling with pollen from the same plant, different flower); artificial xenogamy (pollen from a different population); induced xenogamy (pollen from a plant in the same population).
Viability and asymbiotic seed germination tests

We found no differences among treatments for either seed viability or asymbiotic seed germination. In general, seed germination was very low (induced autogamy, 2.9%; artificial geitonogamy, 1.9%; artificial xenogamy, 4.3%; induced xenogamy, 4.7%). This was unexpected considering the high seed viability observed across treatments that resulted in seed production, particularly induced autogamy (79.9%), artificial geitonogamy (67.6%), artificial xenogamy (87.2%) and induced xenogamy (79.1%; Table 3). However, capsules that originated from selfing (induced autogamy and artificial geitonogamy) had significantly lower seed germination (2.9 and 1.9%, respectively) than seeds that originated from outcrossing (induced xenogamy, 4.7%).

Pollinator identification

During the two flowering seasons (2007 and 2008), four bee species were observed visiting the flowers of C. punctatum (Fig. 6). Apis mellifera was the most frequent visitor in 2007 (53 visits in 46.5 h; Table 4). However, A. mellifera and Megachile xylocopoides were only observed during the 2007 flowering season (Table 4). Both Xylocopa micans and Xylocopa virginica were frequent visitors to the flowers during both flowering seasons (Table 4). Flowers were observed without pollinia; however, no visitors were observed carrying pollen to or from flowers.

Fragrance analysis

Nine fragrance compounds were identified as being produced by C. punctatum flowers: benzaldehyde, myrcene, benzyl alcohol, Z-β-ocimene, E-β-ocimene, 1,3,8-para-menthatriene, methyl salicylate, indole and E,E-α-farnesene. The relative abundance of these compounds was recorded (Fig. 7).

Discussion

Breeding system

Our results show that C. punctatum is not capable of reproducing autogamously and requires a pollinator for...
capsule set. Given that no capsules were formed from the agamospermy or spontaneous autogamy treatments implemented in the field, we demonstrated that no spontaneous self-fertilization occurs. Although a few capsules were formed from induced autogamy (selfing with pollen from the same flower; 17.2%) and artificial geitonogamy (selfing with pollen from the same plant, but from a different flower, 27.2%), significantly more capsules were produced following artificial and induced xenogamy (48.9 and 73.9%, respectively; Fig. 3). Species within the Orchidaceae are predominantly self-compatible; however, species normally require an insect vector to facilitate pollen movement (Dressler 1981). This is supported in C. punctatum; spontaneous autogamy treatments resulted in

Table 3 Seed viability, germination and development stage percentages generated from *Cyrtopodium punctatum* pollination treatments in the 2006–2007 flowering season

| Pollination treatment | Seed viability (%) | Total germination | Seedling development stages (%)† | | |
|-----------------------|-------------------|------------------|----------------------------------|---|---|---|---|---|
| Control ‡             | 0.0               | 0.0              | 0.0                              | 0.0 | 0.0 | 0.0 | 0.0 |
| Agamospermy ‡         | 0.0               | 0.0              | 0.0                              | 0.0 | 0.0 | 0.0 | 0.0 |
| Spontaneous autogamy ‡| 0.0               | 0.0              | 0.0                              | 0.0 | 0.0 | 0.0 | 0.0 |
| Induced autogamy      | 79.9 ab           | 2.9 bc           | 17.9 ab                          | 1.2 b | 1.7 a | 0.35 a |
| Artificial geitonogamy| 67.6 b            | 1.9 c            | 18.1 a                           | 1.2 b | 0.49 b | 0.44 a |
| Artificial xenogamy   | 87.2 a            | 4.3 ab           | 17.6 b                           | 2.7 a | 1.9 a | 0.55 a |
| Induced xenogamy      | 79.1 ab           | 4.7 a            | 17.5 b                           | 4.0 a | 1.1 ab | 0.29 a |

† See Table 2 for descriptions of the seedling developmental stages. ‡ Treatments produced no capsules. Six plants were used and 30 flowers were used per treatment. Values with the same letter are not significantly different (α = 0.05).

Fig. 6 Bees observed visiting flowers. (a) *Apis mellifera* (honey bee). (b) *Xylocopa virginica*. (c) *Xylocopa micans* (female). (d) *Megachile xylocopoides*. (e) *X. micans* (male).
no capsule formation, but both induced autogamy and artificial geitonogamy treatments, where pollen was manually moved to simulate the action of the pollinator, resulted in capsule formation.

Other studies with epiphytic orchid species show a degree of self-incompatibility and the need for pollinators (Ackerman 1989; Jaimes & Ramírez 1999; Borba et al. 2001; Lehnebach & Robertson 2004). Lehnebach and Robertson (2004) and Borba et al. (2001) reported that capsules were not produced following agamospermy and spontaneous autogamy treatments on the epiphytic orchid species that they examined, showing dependence on a pollinator. Some degree of self-incompatibility was also found for these species (Borba et al. 2001; Lehnebach & Robertson 2004). Significantly fewer capsules were formed from artificial selfing than from induced outcrossing. Similarly, some degree of self-incompatibility exists in C. punctatum because fewer capsules were formed from selfing than outcrossing treatments.

_Cyrtopodium punctatum_ uses deceit via aromatic compounds and visual signals to attract insect visitors. Our fragrance analysis and compound identification showed a floral bouquet strategy in which a diverse array of attractive compounds are produced. However, _C. punctatum_ flowers provide no food reward to floral visitors. There are implications of having a deceit pollination strategy. Neiland and Wilcock (1998) found that orchids that offer food rewards (e.g. nectar) had double the probability of setting capsule than non-rewarding species across all geographical areas. In North America, the rate of capsule set was 19.5% for species that offer no nectar reward compared with 49.3% for rewarding orchids (Neiland & Wilcock 1998). A closely related species in Brazil, _Cyrtopodium polyphyllum_, also uses a deceit pollination system. This plant mimics the yellow flowers of a reward-producing unrelated species that occurs in the same habitat. This species also has low capsule set as a result of low pollinator visitation (Pansarin et al. 2008). Pollinator limitation has also been observed in other deceptive orchids, such as _Serapias vomeracea_ and _Pogonia japonica_ (Matsui et al. 2001; Pellegrino et al. 2005).

Historically, populations of _C. punctatum_ in Florida were numerous and plants were abundant. In this situation, a deceit pollination system with low capsule production was viable because the probability of capsule formation was higher owing to plant abundance. However, populations are now small and fragmented; thus, the deceit pollination system may minimize future reproductive success for the species as the likelihood of capsules being produced is so low. The open pollination treatment resulted in no capsule formation during both the 2006–2007 and 2007–2008 growing seasons in the population studied. However, two capsules were formed on two different plants during the 2006–2007 season in inflorescences not used in the breeding system study, suggesting that natural pollination does occur, albeit at very low levels. The deceit pollination system alone may not be the cause of the low capsule formation at the FPNWR. A decrease in pollinator populations may also be affecting natural capsule set. A more detailed study on the insect

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**Table 4 Insect visitors to _Cyrtopodium punctatum_**

<table>
<thead>
<tr>
<th>Insect visitors</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xylocopa micans</em></td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td><em>Xylocopa virginica</em></td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td><em>Apis melifera</em></td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td><em>Megachile xylocopoides</em></td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number of observation hours for 2007 and 2008 were 46.5 h and 14.2 h, respectively.
populations of the area should be conducted, particularly because of the closeness of the populations to agricultural fields that continually use pesticide applications.

The capsule size measurements (length and width) taken during the 11-month maturation period in both years indicated that the induced autogamy and artificial geitogamy treatments resulted in capsules that were relatively smaller than capsules produced from other treatments (except for capsule length in 2006–2007). However, capsule size may vary from year to year owing to significant variation in environmental factors, such as rainfall and temperature, which can influence capsule formation and maturation over time. Our research suggests that breeding system studies should be conducted across multiple growing seasons.

**Seed viability and asymbiotic germination**

Tetrazolium seed viability testing showed high seed viability in all breeding system treatments that formed capsules in *C. punctatum*. However, germination was very low across all treatments. This may be because of suboptimal in vitro cultural conditions for asymbiotic germination. Light may inhibit germination of *C. punctatum* seeds (Dutra et al. 2009). By sowing seeds and initially incubating them in the dark, germination could be significantly improved in this species.

**Pollinator identification**

During the 2007 flowering season, honey bees (*Apis mellifera*) were the most common visitors to *C. punctatum* flowers. However, these bees were too small to remove pollinia and efficiently act as pollinators. In the 2008 flowering season, honey bees were not seen visiting *C. punctatum* flowers. *Xylocopa* bees (*X. micans* and *X. virginica*) were observed visiting the flowers, but removal or deposition of pollinia was not observed during the field observation period, despite being large enough to fit inside the flowers. In Puerto Rico, *C. punctatum* is reported to be pollinated by *Xylocopa* bees (Ackerman 1995). Pemberton and Liu (2008) observed two species of *Centris* bees, visiting the flowers of *C. punctatum* in southeastern Florida. However, these pollinator observations were conducted at Fairchild Tropical Botanical Garden and in Fort Lauderdale, Florida, in an artificial setting with plants of unknown origins. Observations conducted in a garden setting do not accurately reflect the real ecological links because a mixture of exotic and native plants are planted in unnatural arrangements causing insect pollinator densities to change. Pollination observations conducted in situ are more representative because it is where natural conditions prevail and where future plant reintroductions will take place.

**Fragrance analysis**

The compounds identified in the fragrance analysis of *C. punctatum* are considered relatively common pollinator attractants produced by many other orchid species (Kaiser 1993). However, two compounds identified, indole and methyl salicylate, can be associated with the Euglossini pollination system (Williams & Whitten 1983). Although Florida is located outside the range of Euglossini bees, *C. punctatum* is reported to be pollinated by *Euglossa* bees in part of its range (van der Pijl & Dodson 1966; Jeffrey et al. 1970; Luer 1972; Dressler 1993). An introduced species of *Euglossa* (*E. viridissima*) has recently become naturalized in southern Florida (Skov & Wiley 2005). Pemberton and Wheeler (2006) extracted and identified compounds collected by *E. viridissima* in southern Florida. Although methyl salicylate and indole have been detected in many other Euglossini species in the tropics (Ramírez et al. 2002), these compounds were not found in the collection storage organs of *E. viridissima* in southern Florida (see Pemberton & Wheeler 2006). It is possible that even if *E. viridissima* was found in the same location as *C. punctatum*, it would not be attracted by the types of compounds that the plant produces and would not affect pollination.

**Conservation implications**

Understanding a plant’s breeding system and pollinator diversity can help conservation and management strategies because plant populations may be greatly impacted by a reduction in pollinator visitations ( Kearns et al. 1998; Havens 1999). For example, Sahli and Conner (2006) found that pollinator visitation and not pollinator effectiveness is the main driving factor in determining pollination importance. In a generalized pollination system, such as that in *C. punctatum*, the pollinators that visit the plants the most are most likely to be the ones responsible for capsule formation and not simply infrequent visitors. *Xylocopa* bees visited the plants during both seasons and were responsible for most of the visits during 2008. Although *A. mellifera* was the most frequent visitor in 2007, it did not visit the plants during the 2008 season.

Observations made during the present study and by biologists over the past 10 years at the FPNWR indicate that very few capsules were formed by *C. punctatum*. Pesticide use in nearby agricultural areas is another possible direct cause of low capsule formation as pesticide use may decrease pollinator populations at the study site. Similarly, habitat degradation may affect insect populations. The area has been impacted from hydrological changes that have shortened the hydroperiod. Local insect population dynamics should be studied for conservation purposes and land management in areas were *C. punctatum* occurs.

Our study indicates that sexual reproduction in *C. punctatum* is severely depressed, suggesting that recruitment is...
highly unlikely. Consequently, the long-term viability/persistence of the existing population is at risk. In response to a likely need for a reintroduction program, additional studies aimed at determining the population genetic diversity and structure of the existing populations and developing efficient asymbiotic germination procedures for reintroduction are underway. Although genetic diversity studies are being conducted, manual pollination should be conducted within populations to ensure that seeds are being produced for in situ recruitment and ex situ propagation for future reintroductions.

Pemberton and Liu (2008) suggested that restoration of *C. punctatum* could be aided by planting *Brysonima lucida*, a rare species in the Malpighiaceae that is restricted to southern Florida, which attracts *Centris* bees. However, *B. lucida* is not naturally found growing in the big cypress region where natural populations of *C. punctatum* are found (Wunderlin & Hansen 2004). We suggest that local recovery plans for the species be developed given the high habitat variation (e.g. between Everglades National Park vs FPNWR) and fragmentation found in Florida. Recommendations should not be made solely from the relatively few plants in cultivation and should be based on in-depth studies that will likely affect the survival of the species in the wild.

**Acknowledgments**

We thank Hans Alborn (USDA) for his help with the fragrance analysis, Jim Wiley (Florida Department of Agriculture and Consumer Services) for his help with insect identification and the Florida Panther National Wildlife Refuge staff for logistical support in the field. Special thanks to Nancy Philman, Timothy Johnson, Scott Stewart and Philip Kauth. This project was supported by both the US Fish and Wildlife Service and the American Orchid Society.

**References**


