Pollination Plan B:
The Floral Phenology and Mechanism of Delayed Selfing in *Jaltomata repandidentata* (Solanaceae)

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A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Arts in Ecology and Evolutionary Biology

Department of Biology

Central Connecticut State University

New Britain, Connecticut

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Thesis Advisor

Dr. Thomas Mione

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Jaltomata repandidentata, similar to other members of its genus, is known to be self-compatible. The following study identifies J *repandidentata* as a species capable of delayed self-pollination and describes the mechanism of delayed selfing through changes in floral morphology during anthesis. This work was accomplished through monitoring changes in flower phenology and quantifying delayed selfing success through a series of pollination experiments. On the first day of flowering, flowers are pistillate and anthers remain undehisced. The stigmatic surface is functionally receptive throughout anthesis, indicating that J *repandidentata* is able to import pollen during its pistillate phase. Elongation of stamen filaments and the pistil occur over a two-day flowering period. Flowers become hermaphroditic on the second day, and anthers on long filaments dehisce earlier than anthers on short filaments at this time. Prior to corolla abscission, anthers collapse onto the stigma, and self-pollination is achieved. Therefore, temporary herkagomy and dichogamy are demonstrated in the species, which prevents premature self-pollination while promoting outcrossing prior to selfing. No significant difference was found between the lengths of short stamens and pistils, and this proximity allows for self pollination by short stamens rather than long stamens, whose pollen is more likely to be exported than used in self-pollination. Delayed selfing may be further promoted through diurnal changes in flower opening size during anthesis, extensive filament bending that occurs during the final hours of flowering, and pollinator visitation. A high success of self pollination was demonstrated in bagged flowers of various accessions of J *repandidentata,* and a significant difference in self-pollination success was seen when compared to *Jaltomata procumbens,* another species with similar floral phenology. Evidence indicates that J *procumbens* as well as other *Jaltomata* species are also likely to carry out delayed selfing. Ecological and evolutionary ramifications of delayed selfing in *J repandidentata* are discussed, as well as recommendations for further research.

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Introduction

Lloyd and Schoen (1992) categorized self-pollination for self-compatible, outcrossing species (intermediate selfers) by temporal "windows" in self-pollination events during anthesis. Prior self-pollination ensures reproductive success by providing autogamous pollen to the stigma before an opportunity for cross-pollination arises. Competing (simultaneous) self-pollination allows autogamy to occur when outcrossing is possible. This scenario has been witnessed in some species that are normally considered delayed selfers (Lloyd's third category), as there is intraspecific variation in the timing of the delayed selfing mechanisms (Leclerc-Potvin and Ritland, 1994; Kalisz et al. 2004). There are obvious detriments to the first two systems, as the selective benefits of genetic diversity and inbreeding reduction that accompanies outcrossing is usually favored over premature selfing (but see Eckert et al., 2006). In delayed self-pollination, sometimes considered the "best of both worlds" situation (Kalisz and Vogler, 2003), cross-pollination is first attempted with the primary goal outcrossed offspring, and if that fails, self-pollination ensues, assuring fertilization.

Delayed selfing requires a change in the spatial and/or temporal arrangement of the flower's structures. Temporary herkogamy (distance between anthers and the stigmatic surface) and/or temporary dichogamy (a difference in the timing of stigma or pollen maturation) is required to promote outcrossing and inhibit accidental contamination of the stigma with autogamous pollen. Then, some modification in flower morphology during anthesis allows for mature pollen to contact the receptive stigmatic surface often just prior to corolla abscission. The mechanisms by which delayed self-pollination takes place is varied and reflect changes made to preexisting flower morphology. The most common methods resulting in self-pollination include shape changes in stylar branches, elongation of anther filaments causing pollen to be deposited on the stigma, and corolla dragging during abscission (Klips and Snow, 1997).

In *Hibiscus laevis* AlL, a perennial marsh plant of the Eastern United States, it is the gynoecia that alter during anthesis (Klips and Snow, 1997). *H. laevis* is a monadelphous species, with a branched style terminating in multiple stigmas above the stamens. As flowers age, the downward curvatures of the stylar branches increase and orient the stigmatic surface towards the anthers. In some populations of *H. laevis,* the style branches curl far enough down the style that the stigmatic surface contacts the dehisced anthers and self-pollination is achieved (Klips and Snow, 1997). An identical process was found in *H trionum* var. *vesicarius* Hochr. (Seed et aI., 2006), and for both species an increase in pollen number attached to the stigmatic surface decreased style curvature, thus delayed selfing only proceeds with unsuccessful outcrossing (Klips and Snow, 1997; Seed et aI., 2006). It is thought that delayed selfing present in the northern populations of *H laevis* assists both in colonization and reproductive assurance if pollinator populations are decreased (Klips and Snow, 1997). Style curvature has also been known as a mechanism for delayed selfing in some species of *Campanula* (Faegri and van der Pilj, 1979). In these examples, premature self-pollination is prevented primarily by herkogamy.

For many delayed selfing species it is change in the androecia that permits self-pollination. The protogynous *Sanguinaria canadensis* L. self-pollinates in the absence of insect pollen vectors when specific anthers come in contact with the stigma, usually 3 days after anthesis begins (Lyon, 1992). Temporary protogyny is also the case for *Aquilegia canadensis* L., in which stamens elongate and anthers dehisce close to a receptive stigma days after the flower opens (Eckert and Schaefer, 1998). The stigmas, however, are receptive upon the start of anthesis, allowing for outcrossing to occur prior to the selfing event (Eckert and Schaefer, 1998). *Kalmia latifolia* has spring-like stamen filaments that dust pollen when triggered by visiting pollinators. Filaments still release pollen prior to senescence if pollinators do not visit, and self-pollination was achieved in Virginia populations of the species (Rathcke and Real, 1993). The spring mechanism for delayed selfing for *K. latifolia,* however, was found to be inefficient (Nagy et aI., 1999), and autonomous selfing also takes place during corolla abscission, in a process termed "corolla dragging" (Dole, 1990). For *Collinsia verna* Nutt., an ephemeral spring flower of eastern North America, stigmatic receptivity does not occur until late in anthesis (Kalisz et aI., 1999). After the flower opens, four stamens elongate and their anthers dehisce sequentially. Self-pollination is achieved through two methods: the late-dehiscing anthers contacting the stigma and from viable pollen deposited in the corolla keel from previously dehisced anthers dusting the stigmatic surface upon corolla abscission (Kalisz et aI., 1999). Synchrony between dehiscing anthers and stigma receptivity is also the mechanism of delayed selfing for *Crotalaria micans* Link. (Etcheverry et aI., 2003). In *Caulokaempferia coenobialis* (Hance) K. Larsen, a woody plant from South China, pollen is dropped onto the horizontal style in an oily film, which slowly travels towards the stigmatic surface, providing time for outcrossing prior to selfing (Wang et aI., 2005).

Other species rely on structural changes outside of the androecia and gynoecia for delayed selfing. *Mimulus guttatus* Fisch. ex DC. self-pollinates during corolla abscission, when the anthers are brushed against the stigmatic surface as the corolla separates from the calyx (Dole, 1990), although in some populations anther/stigma contact occurred earlier in anthesis, suggesting competing selfing (Leclerc-Potvin and Ritland, 1994). A similar mechanism was found in *Pedicularis dunniana* Bonati., in which corolla wilting brought the anthers in contact with the stigma (Sun et aI., 2005). A combination of stylar branch curvature with pollen inhibition similar to *H laevis* and *H trionum* var. *vesicarius* and corolla dragging as in *M guttatus* is involved in the delayed selfing of *Kosteletzkya virginica* (L.) Presl (Ruan et aI., 2004). Self-incompatibility breakdown during anthesis allows selfing pollen to be used in fertilization at the later stages of some species such as *Lilium longifolium* Thunb. and *Campanula rapunculoides* L. (reviewed in Kalisz et aI., 1999). For *Leptosiphon jepsonii* (D.W. Schemske & C. Goodwillie) Porter & Johnson, a California annual, individuals differ in the timing of self-incompatibility breakdown, thus reproductive systems range from obligate outcrossing through competitive selfing to delayed selfing for different individuals, even within the same populations (Goodwillie et aL,2004).

Therefore, there are a diversity of mechanisms that have evolved to allow delayed selfing in a variety of plant species and families. The evolution and maintenance of autogamy in outcrossing organisms as an evolutionary stable strategy is still highly debated and many models have been proposed to explain its paradoxically ubiquitous nature (reviewed in Goodwillie et aI., 2005). Two of the more encompassing theories are automatic selection and reproductive assurance (reviewed in Schoen et aI., 1996). In automatic selection, a mutation for selfing in a population appears and has a greater chance of being passed onto the next generation than its non-mutant equivalent. The mutant gene can be passed either through a selfing event or through regular crossing events, while the regular gene can only rely on cross-pollination. However, inbreeding depression and other negative factors associated with autogamy should result in few founding events (Schoen et al., 1996). Contrary to this model, autogamous species predominate isolated habitats (Pannell and Barrett, 1998; Etcheverry et al., 2003; Eckert et aL 2006). In the reproductive assurance hypothesis, lack of pollinating vectors and/or mating partners provides a selective advantage for autogamy. However, reproductive assurance through delayed selfing has not been observed in some species (Eckert and Schaefer, 1998; Nagy et al., 1999; see also Goodwillie et al., 2005). There have also been many examples that support automatic selection and reproductive assurance hypotheses. For example, many of the species that practice delayed selfing, such as *K. latifolia,* C. *verna,* and S. *canadensis* are spring blooming and may have decreased pollinators during some years. The effects of delayed selfing at a population level are also not well understood, and this type of autogamy most likely remains undiscovered in many plant species (Kalisz et al., 1999). Detailed studies of delayed selfing species are necessary for further understanding of this reproductive system (Goodwillie et al., 2005).

Populations resulting from mixed mating systems should include individuals from selfing and outcrossing events with proportions determined by factors such as distance of seed dispersal, cross-pollination, and self-pollination success. It was originally thought that over time inbreeding would rid the species genome of deleterious alleles, which would result in a species either favoring strong outcrossing or strong inbreeding (reviewed in Goodwillie et al., 2005). However, it is clear that intermediate selfing species exist, providing evidence that varied proportions of outcrossing and selfing are possible (Goodwillie et al., 2005). Another theory is that the presence of partial selfing in a species was transient and would eventually drift through selective force to either strict outcrossing or selfing (Schemske and Lande, 1985). Inbreeding depression through heterozygote advantage or multilocus genotypes are examples of theories that may provide a stabilizing effect and allow mixed mating systems to be sustained (Charlesworth and Charlesworth, 1987; Holsinger, 1988), but these and other theories on the evolutionary stable systems of intermediate selfing is still widely debated, mostly due to lack of research (reviewed in Goodwillie et al., 2005).

It would seem that the reproductive assurance provided by delayed selfing in particular would be a selective advantage in species with the ability, since pollen limitation is a strong factor in outcrossing species (Holsinger, 1991). However, the results of mixed mating in populations have more complex implications than simply reproductive assurance (Goodwillie et al., 2005, Eckert et al., 2006). In *Kalmia latifolia,* for instance, delayed selfing did not contribute to fruit production in the wild in 1996, but pollinator abundance was noted to be high during the study, which is not the case in all years (Nagy et al., 1999, but see also Rathcke and Real, 1993). Studies on *Aquilegia canadensis* indicate that its delayed self-pollinating ability does not provide reproductive assurance, since emasculated flowers prior to dehiscence and intact flowers did not show significant differences in fruit set (Eckert and Schaefer, 1998). However, subsequent studies of *Collinsia verna* show a strong correlation between delayed selfing and reproductive assurance in a plant whose pollinator abundance is unpredictable year to year (Kalisz and Vogler, 2003). Preliminary studies find this also to be true of *Caulokaempferia coenobialis* (Wang et aI., 2005). Although delayed selfing has the further advantage of only using seed and pollen resources after outcrossing has failed (i.e. no pollen or seed discounting) (Holsinger, 1991; Kalisz et aL 2004), strong inbreeding depression should theoretically negate the advantages of both reproductive assurance and discounting (Kalisz et aL 2004 Eckert, et aI., 2006). Furthermore, lower quality seed can rob maternal resources from concurrent seeds that are genetically more successful (Harder and Routley, 2006). The extent of inbreeding depression when studied in species that practice delayed selfing is variable (Rathcke and Real, 1993; Klips and Snow, 1997; Kalisz and Vogler, 2003; Eckert et aI., 2006). However, it is generally assumed that species practicing autogamy as only part of their reproductive system should have low levels of inbreeding depression than strict autogamous species (Charlesworth and Charlesworth, 1987). It may also be that selfing is a temporary system on a macroevolutionary level and not sustainable in the long-term (Takebayashi and Morrell, 2001). The role that inbreeding depression has on the evolution of self-pollination is still widely debated (Charlesworth and Charlesworth, 1987; Holsinger, 1988; Eckert et aI., 2006).

Despite the predictions of automatic selection, the ability to produce offspring when another individual is not present is another observed advantage to autogamy, especially for colonizing species. Baker's Law predicts that for both animal and plant species, self-compatibility would be prevalent in isolated habitats such as islands, where the chances of mating partner availability for pioneer species are initially greatly reduced (Baker, 1955). Therefore, the evolution of self-compatibility would increase a species ability to disperse and pioneer colonies, especially in isolated environments. In a reassessment of Baker's Law, Pannell and Barrett (1998) concluded that delayed selfing would be the superior reproductive strategy for colony establishment in these types of habitats over obligate outcrossing or selfing, particularly if inbreeding depression is a factor for outcrossed offspring. Increased extinction rates in colonies promote an increase in reproductive assurance through self-compatibility, as do decreases in immigration rates and/or seed productivity (Pannell and Barrett, 1998). Therefore, the ratio of delayed selfing plant species to species of other reproduction systems should increase in isolated environments because of their ability to create progeny without a mating partner without sacrificing the priority to outcross if another individual is in proximity. This has been shown to be true for *Crotalaria micans* as well as in peripheral populations of the self-compatible *Leavenworthia alabamica* Rollins (Etcheverry et aI., 2003; Busch, 2005). However, over generations this could result in biparental inbreeding (inbreeding caused by mating between related individuals) in a population started by a single colonizer (Goodwillie et aI., 2005).

Preliminary observations of *Jaltomata repandidentata,* a self-compatible herbaceous perennial of the neotropics, suggested that delayed self-pollination may be a reproductive strategy of this species. A high rate of autogamy combined with a distinct initial pistillate phase that is considered protogynous reinforces this hypothesis. Due to these speculations and the exceptional flower morphology of *J repandidentata,* this study was undertaken to confirm the presence of delayed selfing in this species. Specifically, the objectives were to (1) monitor the changes in the corolla, androecium and gynoecium during anthesis, (2) record the specific mechanism of delayed selfing if present, (3) confirm self-compatibility and its success rate in *J repandidentata* while excluding the possibility of agamospermy, (4) confirm receptivity in the stigmatic surface prior to anther dehiscence, allowing for pollen import to occur prior to selfing, and (5) compare some of these findings to results for *Jaltomata procumbens,* a related species also with the potential for delayed selfing. Although the study was done outside of *J repandidentata*'s natural habitat, pollinator observations and cross-pollination experiments were carried out to provide insight on possible future field research.

Materials and Methods

Study species-

Jaltomata repandidentata (Dunal) Hunz. (Solanaceae, 2n = 24) is a shrubby perennial which grows from Mexico to Bolivia. It was at one time considered a variation of *Jaltomata procumbens* (Mione, 2005). Within its range, populations are common but patchy, such as under coffee plants in plantations not well weeded (Mione, pers. com.) The flowers of the species are characterized by sigmoid filaments and a style curved towards the stigmatic end (enantiostyly). Inflorescences usually consist of five flowers that open asynchronously. It is a heterantheric species, with two larger anthers on longer filaments producing greater quantities of pollen than three smaller anthers on short filaments (Polsgrove et al., 1993). It is also considered to be protogynous. Nectar is offered from a nectary disc at the base of each stamen filament surrounding a superior ovary, characteristics found in all species of *Jaltomata* (Mione et al., 1993). Also thought to be shared with other members of its genus is *J repandidentata's* self-compatibility (Polsgrove et al., 1993; Mione, unpublished data). Flowers are pistillate upon opening, with short filaments, but flowers become hermaphroditic as the filaments elongate prior to anther dehiscence. It has been hypothesized that the anthers with longer filaments function in cross pollination while the shorter stamens are used for selfing (Polsgrove et al., 1993). The fruit of *J repandidentata* consists of a dark blue, edible berry. Photographs of *J repandidentata* and various reproductive stages can be found in Appendix A (fig. 1a- 11a).

Jaltomata procumbens (Cav.) 1. L. Gentry (Solanaceae, 2n = 24) is similar to *J repandidentata,* yet has distinctions crucial to this study. Both *J procumbens* and *J repandidentata* are members of the 'Mesoamerican' clade of *Jaltomata,* with comparable fruits and corollas (Mione et al., 1994). The range of *J procumbens* is Arizona through Central America to Ecuador, and it lives in patchy populations, particularly in agricultural fields and disturbed areas (Davis, 1986). *J procumbens* flowers also exhibit dichogamy and herkogamy through delayed filament elongation and anther dehiscence. Unlike *J repandidentata, J procumbens* has straight filaments of equal length set at approximately a 45° angle away from the straight style during the hermaphroditic phase of anthesis. The stamens have been known to collapse onto the style prior to corolla abscission, perhaps as a method of delayed self-pollination (Davis, 1986). The fruit of *J procumbens* is similar to that of *J repandidentata. J procumbens* was grown for this study to see if filament shape and/or style shape was important to the mechanism of delayed selfing in *J repandidentata* as well as to verify the presence and mechanism of delayed selfing in *J procumbens.* Photographic representatives of *J procumbens* flowers can be found in Appendix A (fig. 12a and 13a).

Individuals of *J repandidentata* grown for this study were from accessions representing areas of the species' distribution (table 1). Three individuals from each accession were chosen for the study (a total of 21 for *J repandidentata* and 9 for *J procumbens).* Plants were germinated indoors on windowsills by Dr. Thomas Mione at Central Connecticut State University, then individually transplanted into at least 1 gallon pots on 30 June, 2006 containing soil with slow release fertilizer (Miracle Gro® Potting Mix with Miracle Gro® Continuous Release Plant Food) and grown outside in Tolland, Connecticut (fig. 1). Plants were watered regularly and pots were moved and rotated occasionally for optimal sunlight. Experimentation on both species was conducted from 25 July, 2006 to 12 October, 2006. Due to excessive heat and its negative effect of pollen viability (Mione, pers. com.), all first and second day flowers were removed from all plants between 1 August, 2006 and 3 August, 2006.

fig. 1. J. *repandidentata* and J. *procumbens* individuals at experimental site.

Species	Accession Number (T. Mione)	Origin	Years of Seed Storage	Days for Seed Germination
J. repandidentata	362	Mexico	17	18
J. repandidentata	555	Nicaragua	6	10
J. repandidentata	565	Bolivia	14	25-26
J. repandidentata	566	Bolivia	13	11
J. repandidentata	571	Mexico	No data	10
J. repandidentata	605	Cost Rica	11	10
J. repandidentata	701	Venezuela	2	9
J. procumhens	392	Mexico	No data	8
J. procumhens	548	Mexico	2	8
J. procumhens	599	Mexico	10	9-10

Table 1. Data courtesy of Dr. Thomas Mione, CCSU.

Flower phenology-

Data collection for *Jaltomata repandidentata* flower phenology occurred between 8 October and 12 October, 2006. To document changes in flower phenology and the morphological changes that may allow for delayed selfing in *J repandidentata, 25* inflorescences were haphazardly chosen from the three individuals of accession 565. As each bud was chosen, all other flowers, buds and fruit were removed from the inflorescence by severing their pedicels. Buds were labeled using 1 3/4" x 1 3/32" white marking tags hung from the peduncle by floss. Each flower was monitored from initial opening to corolla abscission, and measurements were taken at various times throughout each day to monitor growth and morphological changes. Because *J repandidentata* flowers are actinomorphic and sympetalous, corolla opening size was measured adaxially from the lobe tip of one petal to the edge of the opposite sinus (fig. 2). Corolla shape was classified as concave, explanate, or reflexed (fig. 8) when open. Petal length, from the base to the tip of the lobe along the midline, was measured abaxially by flattening the corolla against the ruler (fig. 3). Stamen lengths of both short and long petals were measured from base to anther tip. One haphazardly chosen short stamen and one long stamen were measured on each flower for each recording session. The sigmoid filaments were not straightened for measuring after elongation. The position of the anthers relative to the stigmatic surface was observed and the point of anther dehiscence was recorded for all long and short stamens for all flowers. Enantiostylic pistils were not straightened for recording measurements. Pistil length was measured from the base of the corolla to the stigmatic tip, and included the length of the exposed ovary, as ovaries in *J repandidentata* flowers are superior. Changes during anthesis as well as other events in the reproductive system of *J repandidentata* were also recorded using representative photographs (Appendix A).

The production of nectar was monitored throughout the study. Pollinator visits were not quantified, but pollinator type, flower type (day 1 or day 2), and basic behavior were noted while flower data was being recorded. Although pollinator observations were informal, they are presented in the results to provide insight in possible pollinator visitation and behavior in *J repandidentata's* native habitat.

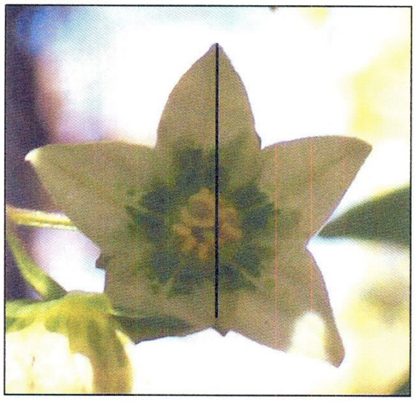


fig. 2. Black line indicates measurement of corolla opening size.

Fig. 3. Black line indicates measurement of petal length prior to flattening.

Stigma receptivity during flower's pistillate phase-

To determine if cross-pollination is possible during the pistillate phase of *J repandidentata,* day 1 (pistillate) flowers were haphazardly selected and other members of its inflorescence were removed at the pediceL Flowers were emasculated during the pistillate phase (day 1) to prevent self pollination during the flower's hermaphroditic phase by severing all filaments and removing the anthers, all of which were confirmed undehisced. These flowers were then hand-pollinated with dehisced anthers either taken from flowers of either the same accession or different accessions of *J repandidentata,* depending on availability. Flowers were bagged using nylon tulle tied with floss to prevent cross pollination and tagged using the labels described above. Bags remained on the plants until either fruit set (fig. 4) or peduncle drop presumably from unsuccessful fertilization. Twenty-two flowers were used for this experiment, 18 of which were crossed with their own accession. Flowers were crossed between 27 July and 24 August, and all accessions except 565 were tested. Fruit set and seed presence were used to determine the amount of successful pollination.

Ability to self-pollinate-

Both *J repandidentata* and *J procumbens* were tested for their ability to self-pollinate by bagging and tagging buds prior to opening using the tulle and label mechanisms described above. As in the previous experiments, other members of the bud's inflorescence were removed to prevent any incidence of accidental geitonogamy, and bags remained on the plant until fruit set (fig. 4). Fruit set and seed presence were subsequently recorded. This experiment occurred between 30 July and 24 August 2006, and all accessions were tested (n = 24 for *J repandidentata* and n = 19 for *J procumbens).*

Incidence of cross-pollination and/or agamospermy-

To determine the incidence of cross-pollination in *J repandidentata,* pistillate flowers were emasculated to prevent self-pollination and left unbagged for pollinators to visit. Low incidence of fruit set under these conditions would indicate low amounts of cross-pollination and eliminate the possibility of agamospermy (a form of apomixis in which seed is set without fertilization or pollen transference). Again, all flowers, fruit and buds but the one for experimentation were removed from the inflorescence and the peduncle was tagged, and fruit and seed set were recorded for each experimental flower (fig. 4). Although quantitative information concerning the incidence of pollinator visit was not recorded, pollinator type was recorded when noticed. All *J repandidentata* accessions but 565 were used in this experiment (n = 32 flowers), which occurred from 15 August to 24 August, 2006.

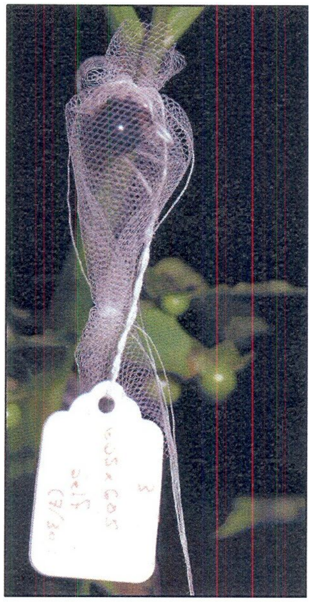


Fig. 4. Bagged and tagged fruit prior to harvesting on J. *repandidentala*.

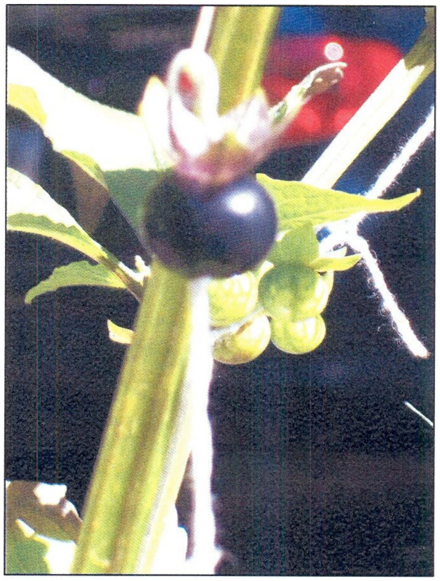


Fig. 5. J. *repandidentala* fruit tagged but not bagged.

Tagged, and left for open pollination-

For both *J repandidentata* and *J procumbens,* fruit production without bagging was monitored on individual flowers (fig. 5). Buds were haphazardly chosen, other members of the inflorescence removed, and peduncles tagged with labels until fruit set. These data was used to analyze the effects of bagging flowers, compare the experiments above to flowers which have not been manipulated, and to see pollination success under more ordinary conditions. All accessions of *J procumbens* (n = 26) and *J repandidentata* (n = 23) were used in this experiment except for accession 565, and the experiment was conducted between 4 August and 4 September, 2006.

Results

Flower phenology-

Although *J repandidentata* has been observed to flower for two days (Mione, pers. com), 13 of 25 flowers in this study lasted for three days. Ten flowers were pistillate for two days followed by one day hermaphroditic, and 3 flowers were pistillate one day and hermaphroditic two days. In the flowers that were pistillate two days, little growth of the corolla, androecia, and gynoecia was noticed during the first day. These results were not consistent with other observations of *J repandidentata's* flowering time (Mione, pers. com.). Following this study, a smaller investigation was conducted at the Central Connecticut State University greenhouse on five flowers of accession 605 and none of the individuals were pistillate for two days (Mione, pers. com.). These results are in agreement with past studies on *J repandidentata* floral longevity. This study's aberration is most likely due to the cold temperatures and non nonnative habitat in which the floral phenology data collection took place. Accession 565 (the same accession used in this flower phenology study) has been previously recorded to produce flowers that are hermaphroditic for two days (Mione, unpublished data), so these results were not unusual.

Representative photographs of the flower phenology were taken for *J repandidentata* (Appendix A). All figure references with an 'a' following the figure's number herein refer to this appendix. Anthesis for *J repandidentata* will be referred to by the following terms herein: day 1 (the pistillate phase) is the day prior to day 2 (the hermaphroditic phase). Please refer to figures 4a and 5a for examples of pistillate flowers and figures 8a-10a for hermaphroditic flowers. A summary of paired *t* test results used to investigate significant differences between the petals, stamens and pistils in day 1 and day 2 flowers can be found in table 2.

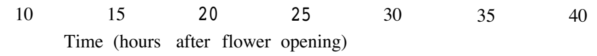


Fig. 6. Change in flower opening size during anthesis. Points are means for each data recording session. Measurements were taken at hours 1,6,10, 15,24, 30,35 and 39. Hour 1 data recorded at 7am. Hour 1-10=day 1, hour 10-23=night 1, hour 23-35=day 2, hour 36-40=night 2. 95% CI for the mean.

Corolla opening length and shape- All flowers were diurnal, with buds and day 2 flowers opening in the morning between 0700-1000 hr and closing at night between day 1 and day 2 (fig. 6 and 6a). Flowers within an inflorescence bloomed asynchronously (fig.3a), although this was observed on flowers not included in the following experiments, since all but one flower were removed from the inflorescence. Increases in the corolla size between day one and day two flowers resulted from petal elongation and change in the corolla's shape (concave, explanate, or reflexed). Measurements taken on petal length indicate that there is growth of the corolla during the anthesis (fig. 7). Petals averaged 9.5 mm (SD ± 0.87 mm) upon opening during the pistillate phase and 11.5 mm (SD ± 0.56 mm) as the anthers dehisced. A paired *t* test showed a significant difference between the petal length of day 1 and day 2 flowers (t = 13.11, df= 24, $P < 0.001$, see table 2). Fourteen of the 25 flowers (56%) monitored on day 1 were explanate during the pistillate phase of flowering by midday of the first day (recorded at hour 6), while the rest (11 of25, 44%) were concave. Twenty (80%) were explanate and the rest of the flowers were reflexed on day 2 while the anthers were in the process of dehiscing asynchronously (hour 30). Refer to fig. 8 for representatives of concave, explanate, and reflexed flowers. Corolla abscission occurred either during the second night of anthesis or early in the morning of day 3 for 22 of the 25 flowers (88%). The remaining three flowers spent a third full day hermaphroditic and abscised the night after day 3.

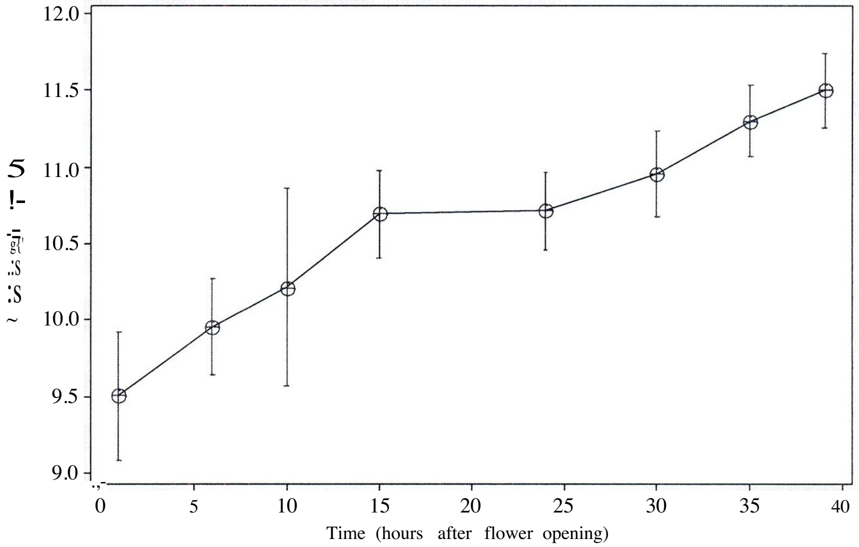


fig. 7. Change in petal length during anthesis. Points are means for each data recording session. Measurements were taken at hours 1,6, 10, 15,24,30,35 and 39. Hour 1 data recorded at 7am. Hour 1-10=day 1, hour 10-23=night 1, hour 23-35=day 2, hour 36-40=night 2. 95% CI for the mean.

a. b. c.

Fig. 8. Corolla opening shape. a. concave day 1 flower, b. explanate day 2 flower, c. reflexed day 2 flower.

Pistils- Day 1 flowers had an average pistil length (for this study, considered the length of stigma, style, and ovary) of 5.4 mm (SD ± 0.46 mm) and styles were already slightly curved near the stigmatic end upon the start of anthesis. Day 1 pistils were longer than all stamens when the flower opened (see stamen data below and fig 4a, 5a). Day 2 pistils had an average length of 6.3 mm (SD ± 0.56 mm) (fig. 9) and styles remained slightly curved, with the stigmatic end oriented to the side rather than straight from the flower (fig. 9a). A paired *t* test indicated that day 1 and day 2 pistil lengths were significantly different ($t = 6.19$, df = 24, $P < 0.001$, see table 2). A comparison between lengths of long stamens, short stamens and pistils is provided below.

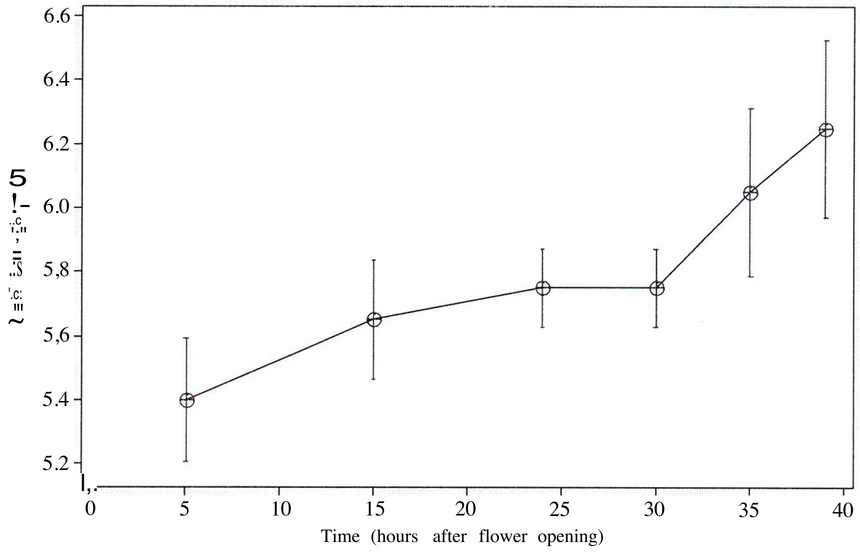


fig. 9. Change in pistil length during anthesis. Points are means for each data recording session. Measurements were taken at hours 6, 15,24,30, 35 and 39. Hour 1 data recorded at 7am. Hour 1-10=day 1, hour 10-23=night 1, hour 23-35=day 2, hour 36-40=night 2. 95% C1 for the mean.

	n	$\bar{x}$ **Day 1**	$\bar{x}$ Day 2	**of Difference**	**St. dey. of Difference**	**SE:r of Difference**	**t-value**	**P-value**
Petal Length	25	9.5mm	11.5 mm	2.0mm	0,77	0,15	**13,11**	<0.001
Pistil Length	25	5.4mm	6.3 mm	0,9mm	0.71	0,142	6,19	<0.001
Long Stamen Length	25	4,7mm	8.0mm	3.3mm	0.91	0.18	18.68	<0.001
Short Stamen Length	25	3.1 mm	6.0mm	2.9mm	0.72	0,14	20,54	<0,001

Table 2. Results of paired *t* tests on measured flower parts. Day 1 is the mean of the first measurement taken after the flower opened. Day 2 is the mean of the measurement taken after all anthers had dehisced.

Stamens- Long stamens and short stamens were visually different as the flower opened, with 2 stamens with longer filaments and larger anthers and 3 stamens with shorter filaments and smaller anthers in every flower (fig. 4a and 5a), The two longer stamens were separated by one short stamen on one side and two short stamens on the other, and all were positioned radially around the style in every flower. Longer stamens were even more distinct from shorter stamens by the second day (fig. 8a and 9a). After dehiscing, all anthers were attached to the ventral face and extrorse, which was promoted by their excurved, sigmoid shape. At 2200 hr of day 2 (hour 39 of anthesis), 32 of 50 (64%) of the long anthers became heavily curled and had lost their characteristic shape (fig, lOa). Between the late afternoon of day 2 and the morning of day 3, the anthers on short stamens had collapsed onto the style (fig. 9a). This was documented hours prior to corolla abscission.

Longer stamens averaged 4,7 mm (SD ± 0,55 mm) upon opening on day 1 and 8.0 mm (SD ± 0,98 mm) once the anthers had dehisced on day 2 (fig, 10), There was a significant difference between day 1 long stamen length (at the beginning of anthesis) and day 2 long stamen length (at dehiscence) in a paired t test (t = 18,68, df= 24, $P <$ 0.001, see table 2). Although slight curvature was noticed in day 1 filaments, filaments exhibited the sigmoid form indicative of the species on the morning of day 2 (fig 7a). All anthers on long filaments remained undehisced though day 1 and the early morning of day 2, By 1200 hr of the second day (30 hours since flower opening), 41 of 50 (82%) of anthers on long filaments had dehisced. By the next data recording (1700 hr, or 35 hours since flower opening), 100% of the anthers on long filaments had dehisced.

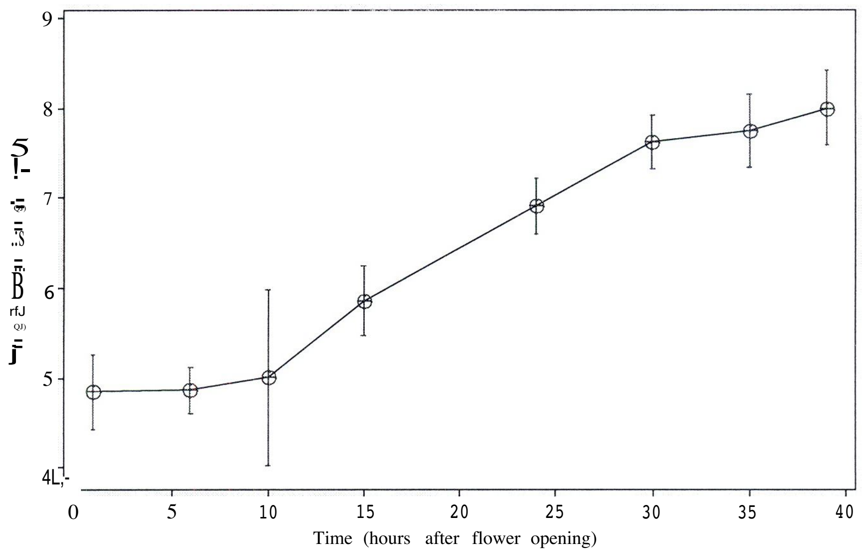


fig. 10. Change in long stamen length during anthesis. Points are means for each data recording session. Measurements were taken at hours 1,6, 10, 15,24, 30,35 and 39. Hour I data recorded at 7am. Hour 1-10=day 1, hour 10-23=night I, hour 23-35=day 2, hour 36-40=night 2. 95% CI for the mean.

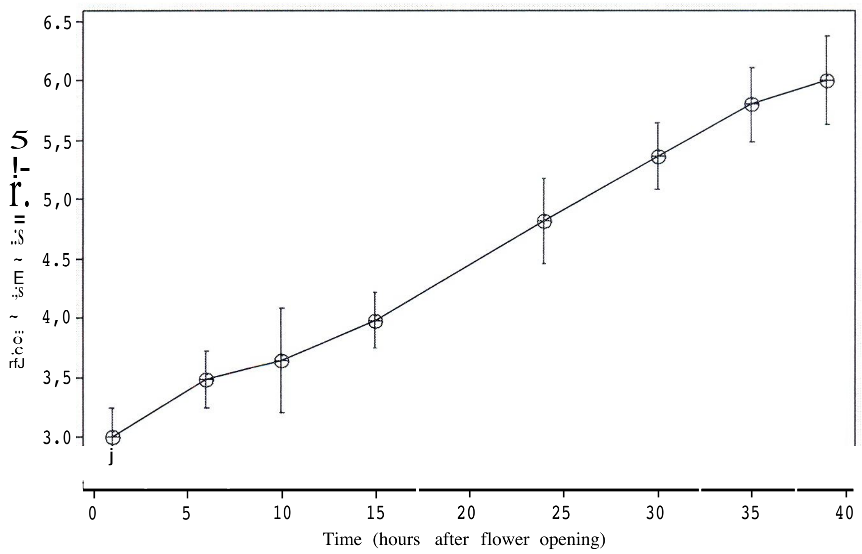


fig. 11. Change in short stamen length during anthesis. Points are means for each data recording session. Measurements were taken at hours 1,6,10,15,24,30,35 and 39. Hour 1 data recorded at 7am. Hour 1-10=day I, hour 10-23=night 1, hour 23-35=day 2, hour 36-40=night 2.95% CI for the mean.

Short stamens averaged 3.1 mm upon opening (SD ± 0.43 mm) and had lengthened to 6.0 mm at dehiscence (SD ± 0.82 mm) (see fig, 11). A paired t test revealed a significant difference between short stamens upon flower opening and short stamens once anthers had dehisced ($t = 20,54$, df= 24, $P < 0,001$, see table 2), The filaments of short stamens also fully displayed their sigmoid shape after some elongation, beginning on day 2, Similar to long stamens, the anthers of short stamens remained undehisced throughout day 1 and through the morning of day 2, However, on 1200 hr of day 2 (30 hours since flower opening), only 10 of75 (13%) of anthers on short stamens had dehisced, A G test was used to compare dehisced and undehisced anthers on long and short filaments at 1200 hr of day 2. This was used in lieu of a Category I (no fixed margins) **/** test (Zar, 1999), The G test indicated that there was a significant difference between the number of dehisced anthers on long filaments and short filaments (G = 62.988, df= 1, $P < 0.001$), Fig. 12 illustrates the variation in dehiscence between short and long anthers at hour 30. Note the large number of dehisced anthers on long filaments and large number of undehisced anthers on short filaments, All anthers had dehisced by 1700 hr of day 2, or 35 hours after the anthesis had begun.

A fixed-effects, one-way ANOVA comparing pistils, short stamens, and long stamens at dehiscence showed that length varied significantly ($F = 46.58$, $P < 0,001$), Furthermore, a Tukey multiple comparison test indicated that while long stamens were significantly different from both short stamens and pistils, pistils and short stamens were not significantly different when compared to each other. The Tukey test was chosen over the Newman-Keuls test due to the conservative nature and the decreased chance of a Type I error in the Tukey test (Zar, 1999). Table 3 summarizes the results of this test. A

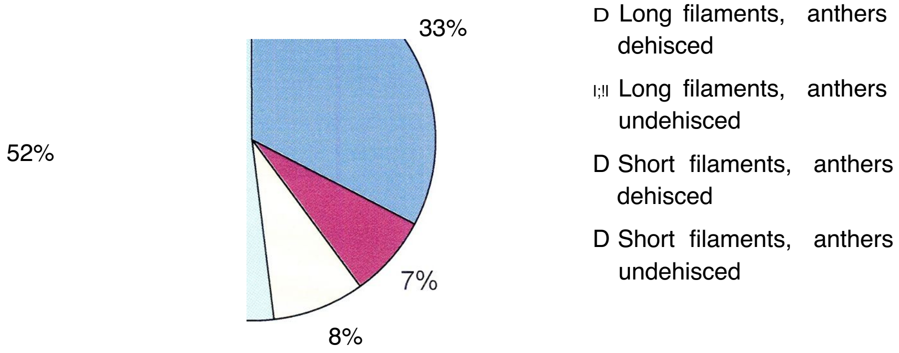


Fig 12. Percent of short and tall stamens and their state of dehiscence at 1200 hr of day 2 (hour 30 after flower opening).

paired t-test comparing day 2 pistil and short stamen lengths once anthers on short stamens dehisced confirmed that there is no significant difference between the lengths of the two structures in each flower ($t = 1.40$, df = 24, $P = 0.001$), Elongated filaments of short stamens (day 2 lengths) were also compared to pistil lengths upon the beginning of anthesis (day 1 pistil lengths) with a paired *t-test* to understand the role of pistil elongation in self-pollination, In this case, short pistils and elongated stamens were significantly different ($t = 4.13$, df= 24, $P < 0.001$).

Comparison	**Difference** ;X_{B}- ;fA	**SE**	q	$q_{,05,72,3}$	**Conclusion**
Length of long stamens vs. short stamens	2,0	0,1607	12.45	3.384	long stamens /- short stamens
Length of long stamens vs. pistils	1.78	0.1607	11.07	3,384	long stamens /- pistils
Length of pistils vs. short stamens	.22	0.1607	1.37	3.384	pistils = short stamens

Table 3. Tukey multiple comparison test on J. *repandidentata* long stamens, short stamens, and pistils at dehiscence.

Nectary disc and pollinators- All nectary discs offered clear, glistening nectar during both the pistillate and hermaphroditic phases. No odor was detected coming from the nectar on either day 1 or day 2 flowers. *Bombus* (Hymenoptera) individuals were the most frequently observed pollinator recorded flying among the experimental plants during data collection, followed by species of Syrphidae (Diptera) and *Apis* (Hymenoptera). *Bombus* were observed actively visiting day 1 and day 2 flowers, although more often visiting day 2 flowers, and were seen using the elongated stamens and style for grasping while attempting to gather nectar at the corolla's base. Visits were shorter on day 1 flowers and seemed more difficult for *Bombus,* as the bumble bees made repeated landing attempts, some of which were unsuccessfuL Successful visitation on day 1 flowers usually resulted from the insect grasping the undehisced anthers and hanging upside-down, Syrphid individuals were seen gathering nectar while hovering, and they were not observed **in** contact with either the androecia or gynoecia, The Syrphids observed did not seem to have a preference between day 1 and day 2 flowers, *Apis* individuals were seen more often moving through the plants rather than visiting flowers, but similar to the hoverflies *Apis* did not have a preference for pistillate or hermaphroditic flowers. Overall, both *J repandidentata* and *J procumbens* seemed to be visited by pollinators less frequently than the garden plants that were in proximity to the *Jaltomata* plants in the experiment (fig, 1).

Pollination studies-

Successful hybridization between *J repandidentata* and *J procumbens* is rare (0-10%), with slightly higher amounts of crossing success when *J repandidentata* is the pollen donor for a particular race of *J procumbens* (Mione, 1992). To reaffirm that cross pollination between the two research species was not possible, a smaller study was performed in which pistillate flowers were emasculated and hand pollinated with an the alternate species. After pollination, flowers were bagged and tagged using the techniques and materials described in the Materials and Methods section. This was done for 6 flowers, three of each species. For all flowers in this experiment, the peduncle abscised a few days after the attempted cross, indicating failure in fertilization. Although the sampling size in the study was extremely small, it was used to reiterate cross pollination not being a strong factor in the following pollination experiments. Table 4 summarizes the pollination tests, and table 5 summarizes the X2 tests on pollination data, For each experiment, fruit was collected and opened to confirm seed set. It was observed that fruit matures asynchronously on the infructescence, although this was not seen on the experimental flowers, since other members of their inflorescence were removed.

Species	**Treatment**	Number of **Flowers**	Percent **Fruit** Set
J *repandidentata*	Hand-pollinated during pistillate phase and emasculated (for stigma receptivity)	22	100%
J repandidentata	Bagged to exclude pollinators prior to anthesis and left to self-pollinate	24	95.8%
J repandidentata	Emasculated during pistillate phase and left unbagged	32	9.4%
J repandidentata	Tagged and left for open pollination	23	91.3%
J procumbens	Bagged to exclude pollinators prior to anthesis and left to self-pollinate	19	68.4%
J *procumbens*	Tagged and left for open pollination	26	76,9%

Table 4. The percent of fruit set success under pollination treatments.

Stigma receptivity during flower's pistillate phase- There was a high rate of functional receptivity in the stigma during the pistillate phase of the flower. Pistillate flowers on *J repandidentata* that were hand pollinated after emasculation and then bagged to prevent visitation by pollinators set fruit and seeds in 100% of the trials (n = 22).

Ability to self-pollinate- In *J repandidentata,* 23 of24 flowers (95.8%) were able to self pollinate when buds were bagged and left to set fruit and seeds. Thirteen of 19 flowers (68.4%) succeeded in fruit and seed set through self pollination for *J procumbens* under the same experimental conditions, In a category I (no fixed margins) X2 test using the Haber correction for continuity, there was a significant difference between the success of fruit set in *J repandidentata* and *J procumbens* (l = 4,324, df = 1, ,025 < *P* < .05). The Haber correction of continuity, similar to Yates correction, is used to correct overestimation of significance for contingency tables with small expected values (Zar, 1999).

Incidence of cross-pollination and/or agamospermy- *J repandidentata* flowers that were emasculated in the pistillate phase and left unbagged to be cross-pollinated resulted in a low amount of fruit and seed set. Three of 32 (9.4%) flowers were able to produce fruit under these experimental conditions.

Unbaggedflowersfor open pollination- Twenty-one of23 flowers (91.3%) *onJ repandidentata* resulted in successful fruit and seed set when left alone and unbagged in the experimental area, while 20 of26 (76.9%) of *J procumbens* flowers had successful fruit and seed set. A category I X2 test using the Haber correction for continuity indicated that there was no significant difference between *J repandidentata* and *J procumbens* in the success of fruit and seed set under these conditions (*l*= 1.349, df= 1, $.10 < P < .25$). Furthermore, it was found that bagging flowers did not affect the flowers' reproductive capability, When bagged flowers of *J repandidentata* (data taken from "ability to self-pollinate" experiment) were compared to unbagged flowers of *J repandidentata* using a category I X2 test with the Haber correction for continuity, there was no significant difference found (X^2= .3562, df= 1, $.50 < P < .75$). This was found also to be true of *J procumbens* in a category I X2 test in which no Haber correction was necessary (X^2= .087, df= 1, $.75 < P < .90$),

Treatment 1	**Treatment 2**	**Pvalue**	**Comments**
J repandidentata, flowers bagged	*J procumbens,* flowers bagged	*P<,05*	*J repandidentata* set a significantly higher percentage under these conditions than *J procumbens.*
J repandidentata, flowers unbagged	*J procumbens,* flowers unbagged	*P>.05*	No difference of successful fruit set
J repandidentata, flowers bagged	*J repandidentata,* flowers unbagged	*P>.05*	Bagging flowers did not alter fruit set success
J procumbens, flowers bagged	*J procumbens,* flowers unbagged	*P>,05*	Bagging flowers did not alter fruit set success

Table 5. Results OfX2 tests on fruit set success, See text for specific X2 and *P* values.

Discussion

Changes that occur during anthesis of *Jaltomata repandidentata* allow for self-pollination after a period of promoting outcrossing. Day 1 flowers are distinctly pistillate, with undehisced anthers on short stamens, a long style with a functionally receptive stigma, and nectar available from the nectary disc as a reward to pollinators, During this phase of anthesis, *J repandidentata* exhibits herkogamy through little elongation of anther filaments and dichogamy through undehisced anthers (fig, 4a and 5a). Early day 2 flowers also display characteristics that promote outcrossing over self-pollination, At this point in anthesis, the stamens with long filaments extend their anthers beyond the stigma prior to dehiscing to avoid premature autogamy through stigmatic contamination. Short stamens, which dehisce after the long stamens, orient their anthers away from the stigma. Therefore, mechanisms to promote cross pollination are prevalent throughout most of anthesis.

Both day 1 and day 2 flowers opened in the early morning for all the flowers studied, suggesting that light availability and/or temperature change plays a role in initiating the corolla to open rather than endogenous rhythms. It was also observed during the study that flowers did not open as wide during overcast days, which provides further evidence to this hypothesis (van Doorn and van Meeteren, 2003). The change from pistillate to hermaphroditic flowers is punctuated by nocturnal corolla closing (fig.6 and fig, 6a). The specific purposes of plants to close their corollas at night is varied and not widely understood for most species with similar behavior, although it has been hypothesized that it may reduce the damage of reproductive organs from precipitation, reduce pollen degradation from humidity and pathogens, or limit water loss (van Doorn and van Meeteren, 2003). Many of these factors may playa role in flower closing for *J repandidentata,* and similar species in the genus *Jaltomata* share this behavior with *J repandidentata* (Mione et aI., 1994). Interestingly, flowers close again the night before corolla abscission, although not as tightly (fig. 6), Flower closing prior to senescence is known in other species (van Doorn and van Meeteren, 2003), but in the case of *J repandidentata* closing prior to corolla abscission may play an additional role, pressing the anthers of short stamens against the stigmatic surface and assisting self-pollination. Further observations are needed on *J repandidentata* to confirm corolla closing assists delayed selfing,

Asynchronous flowering is essential to the outcrossing success prior to delayed selfing in *J repandidentata* (fig.3a). Flowers with dehisced anthers available in proximity to pistillate flowers on other individuals allow for export of pollen from hermaphroditic flowers onto pistillate flowers by visiting pollinators. Unlike the steady growth of the stamens or pistils, petal elongation occurred mostly during the day hours, and there seemed to be little petal elongation on the night between pistillate and hermaphroditic phases (fig. 7). Due to both a change in the corolla shape (concave to explanate or reflexed) and petal elongation during anthesis, hermaphroditic flowers appear more visually pronounced than their pistillate counterparts (fig. 8).

The visual difference between day 1 and day 2 flowers could playa critical role in the sequence of pollinator visits among flowers. By presenting flowers with exportable pollen more conspicuously than flowers with undehisced anthers, pollinators may be visiting hermaphroditic flowers first, then proceed to move among nearby flowers, subsequently visiting pistillate phased flowers. Since a nectar reward is available from both flower phases, pollinators would have an incentive to visit either type. For example, if it is imagined that an optimal foraging pollinator visits an average of five flowers in a population before leaving a patch, it would be more profitable to *J repandidentata* if pollen is deposited on a pollinator in the first one or two flower visits rather than the last few. Similarly, incoming pollinators would be more likely to import pollen from a different species, which does not assist the first flowers visited. This scenario would be true for isolated or patchy populations, where there is an increased chance that the pollinator's next flower population visit will not be of the same species, This type of population demographic is characteristic of both *J repandidentata* (Mione, per.com.) and *J procumbens* (Davis, 1986). Adaxial corolla coloration patterns, which are more revealed in explanate or reflexed corollas (mostly hermaphroditic flowers), may also play a role in attracting pollinators initially to hermaphroditic flowers (fig 8). Furthermore, Sakai *(199S)* predicted that delayed selfing could become evolutionarily stable if there are nonlinear constraints on flower size and number, this way conserving maternal resources. *J repandidentata* and *J procumbens* may exemplify this model, with smaller flowers and multiple seeds per fruit.

Pistils lengthened during anthesis, most likely through cell elongation in the style, increasing their length by almost 17% from the beginning of flowering to anther dehiscence. This increase, although seemingly small (S.4mm to 6.3mm), is essential for self-pollination during the hermaphroditic phase, since the stigmatic surface would not be able to contact the anthers on the short stamens during the selfing event. Although a Tukey multiple comparison test indicated that the short stamens and the stigmatic surface were not significantly different at dehiscence, a paired *t* test between short stamens at dehiscence and pistils when the flower opened (as if they had not elongated during anthesis) showed significant difference. The stigmatic surface of a style before elongation was almost 0.7mm below the average elongated filament at the tip of the anther, compared to elongated pistils with elongated styles, which were 0.3mm above the anthers. Therefore, *J repandidentata* demonstrates growth of both the pistil and the stamens for optimal contact during the self-pollination event.

Style curvature, or enantiostyly, near the stigmatic end (best illustrated in fig. 7a) plays an indispensable role in successful self-pollination for *J repandidentata*. Although for most of day 2 anthers are positioned extrorse preventing premature self pollination, anthers from short stamens collapse onto the stigma prior to corolla abscission. The chance of pollen transfer onto the stigmatic surface is increased by the repositioning of the stigmatic surface through curvature. In an analysis of 80 anther lengths on short stamens by Mione (unpublished data) for the same accessions of *J repandidentata* used in this study, the average length was 2.32 mm, Although the lengths between the short stamens and the pistils were not found to be significant, small variations in pistil length, particularly shorter pistil lengths, will still allow for delayed selfing. Style curvature used as a method of delayed selfing has been documented in other species, such as *Hibiscus laevis* and *H trionum* var. *vesicarius* (Klips and Snow, 1997; Seed et aI., 2006), but in these cases the multiple styles curl to connect to dehisced anthers rather than positioning the stigmatic surface for optimal contact during stamen collapse, as in *J repandidentata*. Although the style in *J procumbens* is not curled, filaments are not sigmoid and anthers are not extrorse, so modification to the stigmatic surface position is not advantageous (fig. 12a),

Interestingly, the presence of enantiostyly, thought to have evolved multiple times, has been correlated with other flower characteristics, including heteranthery evolved for specialized pollen deposition on specific areas of the pollinator's body, poricidal anthers, and lack of nectar (Jesson and Barrett, 2003). Heteranthery in this case refers to "reciprocal enantiostyly," in which a specialized stamen unique to others in the flower elongates and bends opposite the style (see Jesson and Barrett, 2003). The purpose of this morphology is hypothesized to not only increase the chance of pollen deposition on the stigmatic surface and increase the chances of successful exportation of pollen on a pollinator, but also to decrease the chances of interference between the gynoecia and androecia during pollination events. *J repandidentata,* which produces nectar and has anthers that dehisce longitudinally without pores (Mione et aI., 1993), is therefore a rather unique in enantiostylic species. Jesson and Barrett (2003) refer to the more uncommon form of enantiostyly without the opposing stamen (similar to *J repandidentata)* as "nonreciprocal enantiostyly." Furthermore, it seems that the evolution of enantiostyly and heteranthery in *J repandidentata* is to delay self pollination until a morphological change occurs in the androecia (i,e. stigmas collapsing onto the style) at which point the bent style with an extrorse stigmatic surface promotes self-pollination. Enantiostyly in the case of *J repandidentata* seems to improve chances for self-pollination due to sigmoid stamens with extrorse anthers.

Although this study does not directly provide confirmation that pollen of longer stamens are exported more often than pollen of shorter stamens as hypothesized

(Polsgrove et aI., 1993), it is evident that only short stamens are used in self-pollination since anthers on long stamens are significantly longer than the pistiL Furthermore, anthers on long stamens dehisce significantly earlier than anthers on shorter stamens, allowing a longer period of time for export. Short stamens elongate to a length similar to the pistil, allowing dehisced anthers to collapse onto the stigmatic surface on the second night after opening or early on the third day's morning just prior to corolla abscission. Anthers on long stamens carry a larger pollen load than the smaller anthers on short filaments (Polsgrove et aI., 1993), but overall more pollen seems to be allocated to selfing, since three stamens are short and two stamens are long. This disparity may allow for an increased chance in self-pollination, as the pollen collapse mechanism for delayed selfing could be haphazard. Heteranthery is present in other genera of the family Solanaceae, but is considered an unusual characteristic, and its purpose is not well understood (Bohs and Olmstead, 2001; Garcia, 2003).

In other species, heteranthery has been known to serve a variety of functions, usually focused on the plant's pollinating vector and successful cross-pollination. In *Sternbergia clusiana,* each stamen length advertises to different pollinating species, optimizing the chance to pollen export and reducing interspecific competition between visiting pollinators (Dafni and Werker, 1982). *Lagerstroemia indica* flowers as well as the genus *Melastoma* are examples of species that provide specialized stamens with "feeder" pollen which is usually more digestible than the reproductive pollen, of which the latter is used for adherence to the pollinator (Nepi et aI., 2003; Gross, 1993). Feeder and pollinating pollen from different anthers, differentiated by their location in the corolla, was also found in the heterantheric and enantiostylous *Solanum rostratum,* though both types of pollen were equally viable (Bowers, 1975). Some plant species may also be using certain anthers as visual cues for pollinator attraction since pollen is the food source for many pollinating species (Lunau, 2000). Similar to S. *rostratum,* it was found that *J repandidentata* pollen quality and size did not differ between stamen types (Polsgrove et aI., 1993), unlike plants such as *L. indica,* in which feeder pollen shows a reduction in viability (Nepi et aI., 2003). It may be that heteranthery in *J repandidentata* has multiple functions besides optimizing self-pollination while providing pollen for exportation, but this requires further study on *J repandidentata's* pollinating vector.

Because this study was outside of *J repandidentata's* native habitat and the pollinator observations were fleeting, not much can be concluded about the mechanisms of cross-pollination in this species. Although successful cross-pollination did occur during the experiment (9.4% in emasculated flowers), it is unknown if this was due to anemophilic or entomophilic pollination. However, the recorded pollinator behaviors may provide some insight into future research. *Bombus,* for example, clasped the entire androecia and style when landing on the flowers and held them while collecting nectar. While clasping, all of the short stamens were seen to be pressed against the style, which is positioned at the center of the stamens. If the native pollinator of *J repandidentata* alights onto the flowers in a similar fashion when anthers on short stamens have dehisced, *J repandidentata* may also be self-pollinated through facilitated self-pollination during the late stages of the hermaphroditic phase (Lloyd and Schoen, 1992). This would also mean that pollen from both short and long stamens could be exported and heteranthery serve as a 'landing pad' for pollinators. Due to the position of the nectary at the corolla's base, avoidance of facilitated self-pollination would be difficult once anthers on short stamens have dehisced. Premature self-pollination through facilitated selfing would be avoided by asynchronous dehiscence, which most likely explains the late day dehiscence of anthers on short stamens during day 2. Syrphids were seen robbing nectar, and were not seen in contact with the anthers, Again, observations of *J repandidentata* and its native pollinators in the field would provide more insight into pollination of this species.

The pollination experiments indicate that *J repandidentata* is at least functionally protogynous in the pistillate phase of anthesis, Although actual stigma receptivity was not tested, all day 1 flowers that were hand-pollinated successfully set fruit and seeds. This capability is essential for delayed selfing in *J repandidentata,* allowing for outcrossing prior to selfing. Self-pollination success was slightly lower in flowers that were not manipulated (95.8%), and the discrepancy can most likely be attributed to a more haphazard mechanism of stamen collapse and the more dense pollen loading of hand-pollination. Pollinators were not necessary for self-pollination to be successful, and geitonogamy from members of the same inflorescence was not possible in this experiment, since all other flower buds were removed from the inflorescence except for the one under investigation, There was no significant difference between bagged and unbagged fruit setting success in flowers, indicating that the experimental procedure did not significantly affect the process of self-pollination, The lower incidence of fruit set in emasculated flowers indicates that pollinators were not cross-pollinating at high rates in the experimental site, Lower fruit set in emasculated flowers also eliminated agamospermy as a potential mode of reproduction in *J repandidentata.* Pseudogamy (for plants, the case in which pollination is required for endosperm production but the egg nucleus remains unfertilized) cannot be ruled out through the experiments here, since lack of fruit set occurred because of pollen absence. However, most pseudogamous species are polyploids or hybrids (Stenseth et aI., 1985), neither of which is recognized in *J repandidentata.*

Summarizing the findings of this study provides a synopsis of *J repandidentata's* reproductive mechanisms, Day 1 flowers are pistillate and less conspicuous than their hermaphroditic counterparts, attracting pollinators more likely laden with *J repandidentata* pollen. The nectary disc offers nectar and the stigmatic surface can receive imported pollen. Filaments remain shorter than the pistil at this time, As the first night after anthesis begins, the flowers close and filaments of the stamens continue to elongate, preparing for the hermaphroditic phase. Flowers open again in the morning, now with two longer stamens beyond the stigma and three shorter filaments at the same lengths as the pistiL Flowers continue to inhibit premature self-fertilization by extrorse anthers and sigmoid filaments. Anthers on filaments beyond the stigma dehisce safely away from the stigmatic surface, while the anthers on short filaments remain undehisced most of the second day, In the afternoon, all anthers are dehisced and flowers have yet to be selfed, although pollinator visitation at this time may promote contact between antheridia and stigma. Self fertilization most likely occurs either during the night between the second and third day, when the corolla closes and filaments bend exceedingly or the next morning as stamens collapse onto the gynoecia prior to senescence. In the absence of cross-pollination, delayed selfing in *J repandidentata* is highly successful.

J procumbens also exhibits delayed self-pollination as predicted by Davis (1986), although with a significantly lower success rate than *J repandidentata*. Its flowering phenology is similar, with diurnal flowers and a pistillate phase preceding hermaphroditic flowers on the second day (fig 12a). Stamen filaments are short and close to the base of the corolla, then elongate to a length similar to the pistiL Just prior to corolla abscission, stamens close onto the gynoecia (fig 13a), Although the stigma was not tested for protogyny, anthers are undehisced during the early phases of anthesis and the species readily self-pollinates. The mechanisms for delayed selfing between the two species are similar, but the mechanisms of preventing premature self-pollination during the hermaphroditic phase differ. Straight stamens are angled away from the stigmatic surface rather than extrorse anthers and sigmoid filaments as in *J repandidentata*. Most observations of *J procumbens* do not exhibit asynchronous anther dehiscence, since this is unnecessary with radially symmetrical anthers. Delayed selfing may be present in many other *Jaltomata* species, as all species tested have demonstrated self-compatibility and are usually protogynous (Mione, pers. com,).

Preliminary evidence suggests that delayed selfing in *J repandidentata* is not compensating for infrequent pollinator visitation, as has been noted in other delayed selfing species (Dole, 1990; Lyon, 1992; Rathcke and Real, 1993; Klips and Snow, 1997; Kalisz et aI., 1999). However other species, such as *Aquilegia canadensis,* have evolved delayed selfing without providing reproductive assurance (Eckert and Schaefer, 1998). Generalist pollinators were observed visiting *J repandidentata,* and this is also true of *J procumbens* within its native habitat (Davis, 1986). At least in *Jaltomata,* pollinator scarcity is not a prerequisite for the evolution of delayed selfing. It is clear that delayed selfing has evolved multiple times for various reasons (Goodwillie et al., 2005).

Both *J repandidentata* and *J procumbens* demonstrate the advantages of delayed selfing in the ability to colonize isolated habitats, creating patchy, separated populations. This would be predicted by Baker's Law (Baker, 1955; Pannell and Barrett, 1998). Both species have been found in isolated habitats, such as agricultural fields, coffee plantations, disturbed areas and river banks (Davis, 1986; Mione, pers. com.). It has been proposed that the distribution of *J procumbens* in particular may be linked to human influenced dispersal through progressive domestication, as the edible fruits are consumed by local inhabitants (Davis and Bye, 1982). However, the ability to disperse and establish colonies through delayed self-pollination may also playa role, particularly in areas outside of human habitation, This propensity for colonization may be influenced by its high seed number per fruit (:13= 56 in field individuals of *J procumbens)* and bird dispersal, which has been documented (Davis, 1986). Although both *Jaltomata* species are perennial, which has been predicted to reduce the selection for delayed selfing, this may be compensated by the transitory nature of the disturbed habitats in which they inhabit, increasing the colony extinction rate, and thereby increasing the selection for species with the delayed selfing ability (Pannell and Barrett, 1998). Research on another delayed selfing colonizer of disturbed habitats, *Crotalaria micans,* revealed similar results (Etcheverry et al., 2003), More research is necessary regarding the life histories of *J repandidentata* and *J procumbens* to gain insight on this hypothesis, particularly in relation to seed dispersal mechanisms and rates, pollinator abundance, and population dynamics.

Inbreeding depression resulting from self-pollination in *J repandidentata* was not observed or analyzed during this study. Seeds for experimental plants were collected in different years, and any variation resulting from seeds storage could be perceived as inbreeding depression. Furthermore, plants in this study were not observed over generations. By collecting seeds and observing seed counts in set fruits of known pollen origin as well as observing the differences in success of progeny resulting from selfing and crossing events, inbreeding depression, if present, could be observed. Even so, the advantages of delayed selfing from reproductive assurance and/or optimal colonization ability being greater than the repercussions of inbreeding depression could be a possibility for *J repandidentata*. Because inbreeding depression varies with the environment, it should be studied in all life history aspects of the individuals in question under field conditions (Goodwillie et aI., 2005). Despite the lack of this type of data from the field, results of this study concluded that *J repandidentata* is a prolific seIfer, and effects of early inbreeding depression (such as fruit set success) were not observed. This is not true for all delayed selfing plants, in which self-pollination success varies with mechanism and pollinator availability (Lyon, 1992; Rathcke and Real, 1993; Kalisz et aI., 1999). There are conflicting theories as to the influence inbreeding depression has on autogamous and partially autogamous species, although it is assumed to have a greater effect on outcrossing species than on those that partially self (Charlesworth and Charlesworth, 1987; Holsinger, 1988; Eckert et aI., 2006).

Another possible consequence of delayed selfing in *J repandidentata* and *J procumbens* is a combination of seeds coming from both self-pollination and cross-pollination events. Since the fruit of *Jaltomata* species are berries containing many seeds (fig. 11a), ovules within the ovary can be fertilized from multiple pollen sources, in intermediate selfing species, termed part-flower self-pollination (Schoen and Brown, 1991). The proportion of seeds originating from selfing or outcrossing events would be determined by pollinator abundance and delayed selfing success. If pollinators are frequent in the native habitat of *J repandidentata,* delayed selfing may be used to ensure the fertilization of all ovules before fruit production. Autogamy would occur at smaller rates, and dispersal (through bird consumptions, for example) would create a founding population of siblings with potentially different paternal genomes, resulting in more genetic diversity in the founding group, Part-flower self-pollination in populations of *Glycine argyrea* and G. *clandestine* was theorized to be important to their reproductive assurance (Schoen and Brown, 1991). Selfed seeds housed within the same fruit as outcrossed seeds may not always be advantageous, however. If, for example, the resulting selfed embryos are of lower quality than outcrossed embryos, perhaps due to inbreeding depression, allocated maternal resources are not being optimized by the seeds having the greatest fitness (Harder and Routley, 2006). Within these models, however, if delayed selfing provides reproductive assurance when pollen is limited or if selfed seeds have a significant survival rate, mechanisms of delayed selfing should still prove to be beneficial (Harder and Routley, 2006),

It is hypothesized that most if not all species within the genus *Jaltomata* are self-compatible (Mione, pers.com.), and therefore it is extremely likely that other members of the genus are practicing delayed self-pollination at various levels of success and frequency depending on the environmental and morphological factors described above, Delayed selfing would be especially probable for those species within the

"Mesoamerican" clade, which are most closely related to *J repandidentata* and *J procumbens* (Mione et aI., 1994). Presence of a pistillate phase followed by a hermaphroditic phase, functional protogyny, and elongation of filaments bringing dehisced anthers towards the stigma would be indications that delayed self-pollination is possible. Facilitated self-pollination could also be a factor in the reproductive system of certain *Jaltomata* species, particularly in the absence of a stigma/anther contact mechanism. Preliminary evidence through representative photographs of various *Jaltomata* species points to some type of autogamy possible in most species. The prevalence of multiple delayed selfing species within the same genus combined with an environment in which pollinator scarcity is not a likely factor in favoring the selection of delayed selfing provides a number of opportunities to better understand this reproductive system. Not only can greater insight be gained through study of individual species, but evolutionary stable strategies can also be tested within the genus. For example, *Jaltomata* would be an excellent subject to study the correlations between the presence/absence of delayed selfing with flower number and size predicted by Sakai (1995) by comparing corolla morphology and delayed selfing success in species with corolla differences. In fact, through further study, *Jaltomata* species have the potential to solve many of the unanswered questions concerning intermediate autogamy and mixed mating, considered one of the most perplexing problems of modern evolutionary biology (Goodwillie et aI., 2005),

Appendix A

Photographic Representatives of *Jaltomata repandidentata* and *Jaltomata procumbens*.

Photographs 1a-6a, 9a, 11a, 13a courtesy of Deborah MacDonald.
Photographs 7a and 12a courtesy of Dr. Thomas Mione.
Photographs 8a, 10a and 13a by the author.

Fig. 1a. *J. repandidentata.*

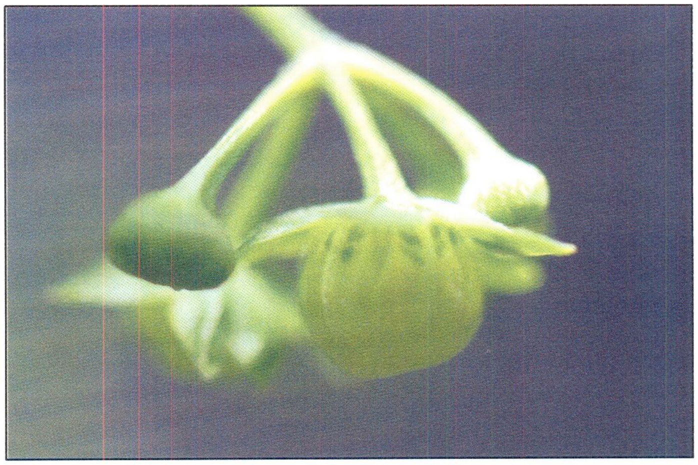


Fig. 2a. *J. repandidentata* flower buds.

fig. 3a. J. *repandidenlata* inflorescence.
Note asynchronous flowering,

fig, **4a** (left) and 5a (right). Day 1 (pistillate) flowers of J. *repandidentata,* Note undehisced anthers, concave corolla and long stamens already distinct from short stamens.

Fig, 6a, J. *repandidentata* flower closing for the night between day 1 and day 2 (flower on the right). Note anthers still undehisced.

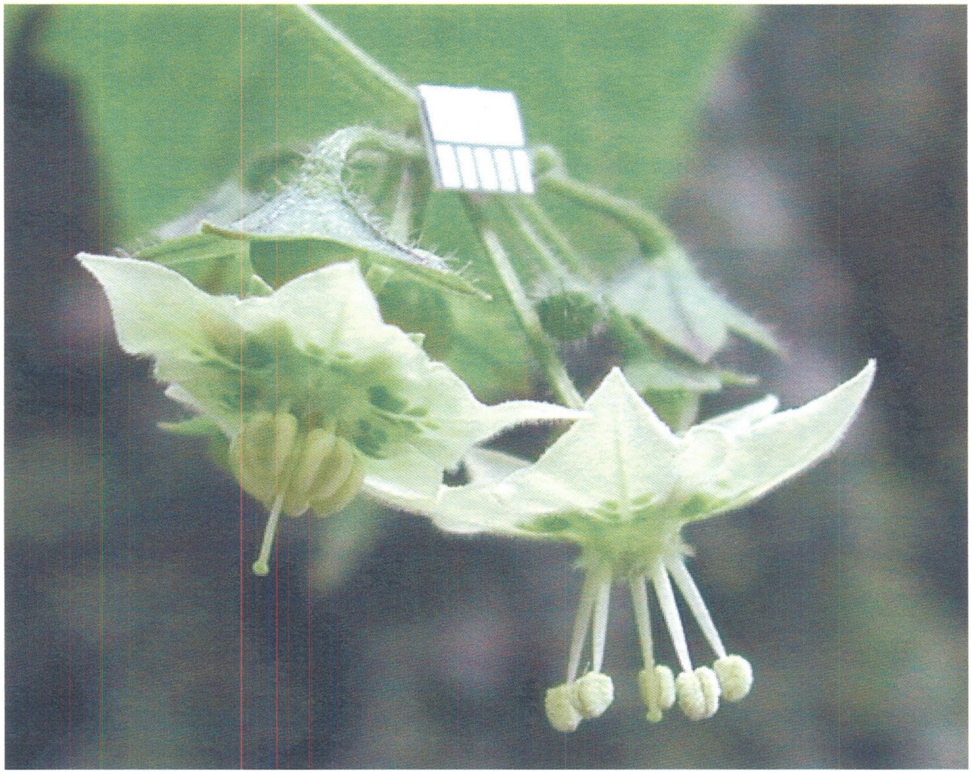
fig, 7a, Day 1 (right) and day 2 (left) J. *repandidentata* flowers, Note differences between pistillate and hermaphroditic phases. Compare with J. *procumbens* (fig 12a).

Fig. 8a. Early day 2 flower of J. *repandidentata*. Note explanate corolla shape and elongated, sigmoid filaments, Prior to dehiscing.

Fig. 9a. Stamen collapse of night 2 in J. *repandidentata*, Note fully dehisced anthers and stigmatic surface in contact with short anther with pollen on stigmatic surface (arrow).

Fig. lOa. Night 2 flower of J. *repandidentata,* just prior to flower abscission. Note heavily curved long anthers (compare to fig. 9a)

fig, 11 a. Asynchronous fruiting maturation of J. *repandidenlala*.



fig, 12a. Day 1 (left) and day 2 (right) J. *procumbens* flowers. Note differences between pistillate and hermaphroditic phases. Compare with J. *repandidentata* (fig 7a). Units on ruler are mm.

fig. 13a, Late afternoon of day 2 J. *procumbens* flower. Note stamen collapse onto style and pollen on stigmatic surface.

Appendix B

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