Reproductive Ecology of *Pinckneya bracteata* (Bartram) Rafinesque (Rubiaceae)

George H. Rountree

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REPRODUCTIVE ECOLOGY OF
Pinckneya bracteata (BARTRAM) RAFINESQUE
(RUBIACEAE)

George H. Rountree
165. BACHMAN'S SPARROW
AIMOPHILA AESTIVALIS

Range: Breeds from Iowa and Pennsylvania to Texas and Florida. Winters from North Carolina south. The Pine-woods Sparrow of Florida is a race. Habitat: Open woods, deciduous in Midwest, pineries in Florida. Identification: Length, about 6 inches. "... a brown-backed bird with a clear dingy-buff breast" (Peterson). This bird was named in honor of Audubon's friend, John Bachman.
Reproductive Ecology of

*Pinckneya bracteata* (Bartram) Rafinesque

(Rubiaceae)

by:

George H. Rountree

A Thesis Submitted to the Faculty
of the College of Graduate Studies
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Requirements for the Degree of
Master of Science
in Biology

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Reproductive Ecology of

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Abstract

*Pinckneya bracteata* is a small tree or large shrub that is indigenous to the coastal plain of extreme southern South Carolina, Georgia, and northern Florida. It occurs in sunny, wet habitats and in small populations that are usually separated by distances of kilometers. This study examined the mating, dispersal, and germination systems of the species. *Pinckneya bracteata* was found to be protandrous and self-incompatible. Ruby-throated Hummingbirds and bumblebees (*Bombus* spp.) appear to be the primary pollinators. Seed fall velocity is important to the wind dispersal of seeds and decreases as seed size increases. Fall velocity is also slower in seeds with more centrally located embryos. The seeds of this plant were found to be positively photoblastic, but this photoblastism can be overcome by temperature fluctuation. The mating system, dispersal system, and germination requirements of *P. bracteata* are compatible with and adapted for the habitats in which it is found.
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INTRODUCTION

This thesis documents a study of the reproductive ecology of Pinckneya bracteata which is found in isolated populations and very specific habitats. The study focused on three areas: the mating system, seed dispersal, and seed germination requirements of the species. The mating system is studied to determine if cross pollination is likely between scattered populations. Seeds, which are dispersed by wind, are examined to see what effects their morphology has on dispersal distance. Because this species is habitat specific, the seed germination requirements are examined to find out what part they play in determining the location of P. bracteata plants.

These three areas are addressed sequentially in each chapter. The history and a description of the plant are presented in this introduction as background material. This background is followed by a brief discussion of plant reproduction as it relates to P. bracteata.

HISTORY

In April of 1773 William Bartram sailed from Philadelphia "at the request of Dr. Fothergill, of London, to search the Floridas, and the western parts of Carolina and Georgia, for the discovery of rare and useful productions of nature, chiefly in the vegetable kingdom" (Bartram, 1791). In the course of this search, Mr. Bartram arrived at the Altamaha River in the vicinity of what was then Ft. Barrington, near present Jessup, upstream from Darien on the Old Post Road, where he was "greatly delighted at the appearance of two new beautiful shrubs, in all their blooming graces." One of these
turned out to be *Franklinia alatamaha*, Bartram's famous discovery that has not been found in the wild since 1803 (Ewan, 1969). Bartram (1791) described the other plant as follows:

"The other was equally distinguished for beauty and singularity; it grows twelve or fifteen feet high, the branches ascendant and opposite, and terminate with large panicles of pale blue tubular flowers, speckled on the inside with crimson; but what is singular, these panicles are ornamented with a number of ovate large bracteae, as white, and like fine paper, their tops and verges stained with a rose-red, which, at a little distance, has the appearance of clusters of roses, at the extremities of the limbs: the flowers are of the Cl. Pentandria monogynia; the leaves are nearly ovate, pointed and petioled, standing opposite to one another on the branches."

He later revisited the site and named this second plant *Bignonia bracteata* in reference to the large petaloid sepals which he referred to as bracteae. Bartram wrote that in all of his travels, he only saw the plant at two other places, "and there very sparingly."

Because of the poor communications of the time, Andre’ Michaux was not aware of Bartram’s discovery when he came to America from France in 1785 for a similar exploratory expedition (Ewan, 1969). Michaux found the same species, described it (Fig. 1), and named it *Pinckneya pubens* (Michaux, 1803). The genus was named in honor of Charles Cotesworth Pinckney (1746-1825) of South Carolina (Little, 1979). Pinckney was a general in the American Revolution and was three times the nominee of the Federalist party for the office of President of the United States, being defeated by Thomas Jefferson and James Madison. He was also a founder, in 1773, and the first curator of the Charleston Museum. Pinckney met and was helpful to Michaux while the latter was in South Carolina (Zahniser, 1967). Michaux had established a botanical garden about ten miles from Charleston (Ewan, 1969). The specific epithet, *pubens*, refers to the pubescence of the young twigs (Little, 1979).

Constantine Samuel Rafinesque realized that these two descriptions were of the same species and that this taxon did not belong in the genus *Bignonia*, nor did it belong in
the genus *Mussenda*, as some had suggested. He recognized Michaux's genus *Pinckneya*, and rejected the proposed generic name *Bartramia*, but because Bartram was the first to describe the species, Rafinesque retained the specific epithet *bracteata* (Rafinesque, 1827). Since this time both *Pinckneya pubens* and *Pinckneya bracteata* have been used almost interchangeably. Although *bracteata* has priority, *pubens* has been used by numerous authors (Elliott, 1816; Harper, 1906; Small, 1933; Radford et al., 1968).

Common names include Fevertree, Georgia Bark, Maiden's Blushes, and Pinckneya. *Pinckneya* is a monotypic genus in the family Rubiaceae.

John James Audubon painted *Pinckneya* with the Bachman's Sparrow in 1833 (Audubon, 1838). This painting is plate 165 of his famous *Birds of North America* and is reproduced as the frontice piece of this work. Audubon likely saw the plant somewhere in Florida.

*Pinckneya* is a close relative of the South American *Cinchona* tree that furnishes the antimalarial drug quinine. The former has been thought to contain quinine and to have the same properties as the latter for treating malaria (Coker & Totten, 1934). Elliot (1816) commented that it was "nearly allied to *Cinchona*" and other older writers remarked that the bark has an appearance and taste similar to *Cinchona* and shares its medicinal properties (Nuttall, 1818; Rafinesque, 1827; Torrey & Gray, 1841; Harrar, 1962). Legend has it that *Pinckneya* was used medicinally by the North American Indians, early southern settlers, and by the Confederate Army (Dr. John Boseman, Dir. Natural Heritage Trust, Georgia Dept. of Nat. Res., pers. comm.). In fact, Harrar (1962) states, "in early colonial days a decoction prepared from the bark of this plant was used successfully in treating victims of malaria." Prior to 1869, a Dr. Fauntleroi of Virginia claimed medicinal values other than those of quinine for *Pinckneya* (Coker & Totten, 1934). The literature describes the bark as having a very bitter taste (Harrar, 1962). I concur with this opinion, the taste is quite bitter and not particularly pleasant.
Interestingly, I have noticed that goats consider Pinckneya bark a delicacy, consuming it first in preference to all other available browse.

The Spanish conquerors of Peru learned of the curative power of Cinchona bark in about the year 1640. The powdered bark became so popular in Europe for the treatment of malaria that the demand was enormous. The Cinchona supply of South America was nearly exhausted by the middle of the nineteenth century. Dutch colonists introduced the tree into Java about 1850 and by the time of World War II, 90% of the world's quinine came from the Netherlands Indies (Indonesia). Seizure of this area by Japan in 1942 spurred research among the Allies for quinine substitutes. At least two studies investigated P. bracteata for antimalarial properties during this period. No cinchona alkaloids or beneficial effects were found (Sumerford, 1943; Cornatzer et al., 1944).

**DESCRIPTION**

*Pinckneya bracteata* favors wet and boggy soils (Elliott, 1816) such as low woods (Radford et al., 1968), branch swamps, and sand hill streams (Harper, 1906). The species is found at the edges of openings or throughout larger gaps where a disturbance has occurred that permits unfiltered sunlight, at least temporarily, to reach bare soil (personal observation). The range of *Pinckneya* extends from extreme southeastern South Carolina in Beaufort and Jasper Counties (Mellinger, 1966), through the coastal plain of Georgia and into northern Florida, as far west as Bay County (Godfrey, 1988). Harper (1906), wrote that it was more abundant in the Altamaha Grit region of Georgia than in all of the rest of its range combined. This region is a band about 75 miles wide and tapered to a narrow point on each end that stretches diagonally across the coastal plain from Screven County in the north east, to Decatur County in the south west. A list of
Pinckneya is a large shrub or small tree 3-7 meters tall with many stems from each root. The National Champion Pinckneya (1972) from Gadson County, Florida, measured 7 meters tall with a stem circumference of 25.4 cm and a spread of 4.5 meters (Godfrey, 1988). The national champion not withstanding, I have measured stem circumferences of 30 cm. Coker and Totten (1934) report stem diameters of up to six inches which indicate circumferences of up to about 48 cm and Harrar (1962) mentions diameters of 6 to 10 inches which indicate circumferences of nearly 80 cm and heights of up to 25 feet (7.62 meters).

The narrow rounded crown is composed of slender horizontal branches which originate from the several vertical trunks. The root system is shallow and fibrous, with new stems arising from lateral roots at distances of up to several meters from the parent plant. Young twigs of the current year, petioles, inflorescence axes, floral tubes, and other perianth parts are densely soft-pubescent. Twigs are greenish to tawny in color at first, becoming a rusty brown as the pubescence is lost. Limbs, trunks, and fruit have obvious gray, wart-like lenticles. A continuous white pith is present from the base of the trunk to the stem tips. The 4-20 cm long and 2-12 cm broad pinnately veined elliptic to ovate leaves are simple and deciduous. They are opposite on the stem and subtended by triangular stipules that quickly abscise, leaving a line-like stipular scar between the 1-3 cm petioles. Leaf bases are broadly cuneate and apices are obtuse to acute. The upper leaf surface has scattered short trichomes and the lower surface is densely short pubescent. Leaf scars are heart shaped and each has a narrow crescent shaped vascular bundle scar.

The loose cymose inflorescences are terminal or from the 1-2 most distal nodes. One or two of the five sepals of at least one flower (usually several) of each cyme are greatly enlarged and petaloid. These larger sepals are 4-8 cm long and 2-6 cm broad. The color ranges from a pure cream white to a deep rose pink and tends to darken over
the flowering season. The remaining sepals are linear and about one cm long. The trumpet shaped corolla is an obscure yellowish green color mottled with maroon to red spots. The five curling lobes are shorter than the 1.5 to 2.5 cm long tube. Five epipetalous stamens are exserted well beyond the throat, as is the mature, two lobed, capitate stigma. The two carpellate ovary is inferior and the ultimate length of the style is greater than that of the filaments.

The fruit is a subglobose to ovoid, two valved, hard, brown capsule that dehisces terminally, across the septum. The apical end is flattish and ringed by a perianth scar. Each capsule contains 26 - 123 tan flat seeds that are stacked in each locule. The seeds have variously shaped outlines and a thin, membranous, markedly reticulate testa extending out from the elliptic embryo. Seeds are 6.5 - 11 mm long by 3.5 - 7 mm broad. Seed release begins in October and continues throughout the winter. Most seeds are gone from the capsule by the following March or April but I have found a few persisting as late as July. The capsules do not open enough that the seed simply fall out from the pull of gravity, some disturbance is required to dislodge them.

Individual plants are difficult to age because stems that are killed by fire or mechanical injury are replaced with new stems from the root base. Because of this, the age of a particular stem, as represented by annual rings, may not be indicative of the age of the plant. This is further complicated by vegetative reproduction, which makes it difficult to arrive at an accurate age for a complete genet.

REPRODUCTION

Many woody perennials reproduce asexually. Pinckneya, as mentioned above, produces adventitious stems (suckers) from lateral roots. In an open environment, close clonal growth acts to "cover the flank" of central stems, choking out competitors and dominating adjacent space. This permits the plant to compete at a relatively short height.
The resources saved by reductions in stem tissue can then be invested in flowers and seed (Waller, 1988). The clustered growth pattern of *Pinckneya* may be this type of adaptation. In any event, plants in the open are not as tall as plants on an edge as plants competing for light.

In contrast, perennials growing in a shaded environment where the top of the canopy is out of reach, often adopt different forms of clonal growth. In these cases the clones arise from lateral roots some distance from the mother plant in a search for new light resources. If these daughter shoots (ramets) do not soon become self sufficient, resources from the overall plant colony (genet) will be withheld and the ramet will whither and perish. These plants tend to restrict their flowering to those parts that occupy areas of brighter light (Waller, 1988). *Pinckneya* often has numerous suckers growing from lateral roots in deeply shaded areas. These stems are not clustered as the stems in the open are, but are distributed randomly. Only those stems, or even branches, that receive full sun have been observed flowering. Stems growing in the shade are usually less than 1 meter tall, although some grow to a greater height but have a thin trunk. I have never seen a shaded plant bloom.

Asexual reproduction has advantages and disadvantages. Clonal growth is advantageous in expanding a genet in a particular area where it grows and is adapted. It allows indefinite survival of the genet. Offspring can be subsidized by the parent, allowing for faster growth than would otherwise be possible. New stems can be located in more favorable places. Asexual reproduction, however, does not allow for genetic recombination and vegetative propagation is not well suited to long distance dispersal. To respond to these problems we must turn to sexual reproduction.

The sexual reproductive process in plants involves the production, dispersal, and establishment of propagules. In the case of *Pinckneya*, these propagules are seeds. Seeds play an important part in four processes: reproduction to permit continuance of the
species, dispersal within the same community, expansion to new territories or other habitats, and survival of the germplasm through seasons or environmental conditions unfavorable for growth (Fenner, 1985). Like a reproducing animal, a plant that produces seed must undertake some sort of biological "ritual." This ritual is its mating system. A seed that successfully establishes a plant must be dispersed to a favorable site and germinate at the proper time before it can grow to maturity and produce seed of its own. This study examines the mating system, dispersal ability, and germination requirements of P. bracteata.

MATING SYSTEM

We know that Pinckneya produces seed. It follows from this observation that the species reproduces either sexually or apomictically.

One of the most common forms of asexual reproduction is apomixis, or agamospermy, which is the production and development of seeds without fertilization (Stern, 1982). A wide variety of plants, such as the dandelion (Taraxacum officinale), reproduce apomictically (Proctor & Yeo, 1972). Apomixis has the advantages and disadvantages of other forms of asexual reproduction, except, because the process results in seed, the potential for dispersal is normally greater than that for vegetative reproduction.

Sexually reproducing angiosperm plants potentially receive pollen from themselves or from other plants. Each of these scenarios has advantages and disadvantages.

The potential advantages of self pollination are similar to those of asexual reproduction. These advantages include (1) the propagation of a successful genotype, (2) reduced costs of mating with the savings allocated to increased fecundity, allowing the rapid exploitation of an open location, and (3) increased seed-set where or when
pollination is unreliable (Willson, 1983). Self compatible species are at a great advantage in long distance dispersal away from all conspecifics. This is because a single individual can give rise to a breeding population. Self fertilization is an evolutionary dead end however, because it prevents the development of radically new adaptive devices (Baker, 1955).

The chief advantages of outcrossing have long been thought to be the superiority of the heterogeneous offspring and the avoidance of inbreeding depression (Darwin, 1876). Offspring resulting from outcrossed matings can be expected to out compete offspring from selfed matings in limited environments (Lloyd, 1980). Outcrossing expands the advantages of sexual reproduction by increasing the genetic variation among the offspring (Willson, 1983). Rates of outcrossing can be increased by various morphological devices and by incompatibility systems.

Self pollination is reduced in many plants because of flower morphology when the pollen is removed from the vicinity of the stigma. In some plants (diclinous) the pistils and stamens are located in separate flowers or even in separate plants (dioecious). Plants with hermaphroditic (monoclinous) flowers sometimes separate the sexual functions spatially (herkogamy) or temporally (dichogamy) (Bertin, 1988). Dichogamy can take either of two forms, protandry or protogyne. In protandry the anthers mature and release their pollen before the stigma is receptive. Protandry is most common in plants that are pollinated by bees, flies and birds (Wyatt, 1983). In protogyne, the stigma completes its receptive period before the pollen is shed. Protogyne is most common in plants that are pollinated by wasps and wind (Wyatt, 1983).

Incompatibility systems are fairly common among angiosperms and can prevent self fertilization and also fertilization from relatives or plants that are genetically similar (Willson, 1983). These incompatibility systems can be classified as either prezygotic or postzygotic. Postzygotic events may be due to genetic problems in the embryo itself, such
as the expression of lethal genes, or they may result from abortions without regard to embryonic defects. Prezygotic events that prevent fertilization are considered true incompatibility. These events result in the failure of the sperm to fertilize an egg. This failure can take place at any time before fertilization, on the stigma or in the style, ovary, or ovule. Failure may be complete, partial, or fertilization may simply be delayed.

Two major mechanisms of prezygotic incompatibility are known, gametophytic and sporophytic incompatibility. Gametophytic incompatibility results from the expression of genes in the haploid pollen grain (gametophyte) and is widely distributed in both dicots and monocots (Pandy, 1960). In this method fertilization is inhibited, after the union of the gametes, if the pollen grain contains the same allele as the diploid maternal tissue at some particular locus. In sporophytic incompatibility, pollen failure is determined by the plant (sporophyte) that produces the pollen (Barrett, 1988). All pollen of a given male is rejected by the female organs of that individual. Multiple loci can be involved in this system and a variety of outcomes are possible, from complete to partial inhibition of germination.

Sometimes fertilization is not completely prevented, just delayed. In some species the pollen tubes of pollen from the same plant grow much more slowly than those of pollen from another plant (Mulcahy, 1983). This has the effect of allowing cross pollination if the pollen is available, while also allowing self pollination if foreign pollen is not available.

Flowers and flowering patterns have evolved mostly in response to selection for effective pollen transfer. Pollen can be transferred by abiotic vectors (wind or water) or biotic vectors (animals). In general, animal vectors increase the likelihood that some pollen will travel to some far removed location. This increases outcrossing with more distant individuals (Willson, 1983). *Pinckneya* flowers are of the type that utilize animal vectors.
A wide and diverse variety of animals serve as vectors, but hymenopterans, bees in particular, are the most common pollinators. Janzen (1971) showed that tropical Euglossine bees forage as far as 23 kilometers from their nest, regularly visiting the same set of plants, probably in the same order. He called this phenomenon "traplining" and suggested that *Bombus* and other large bees, some hummingbirds, and sphinx moths exhibit a similar behavior. Traplining species convey certain advantages to the plants that they pollinate. The following advantages are among those conveyed (Janzen, 1971). Effective outcrossing is possible at very low plant densities and between scattered populations. Large amounts of energy need not be expended on an excess of synchronized flowering, smaller amounts of energy may be used each day over a longer period of flower production. Since only a small number of flowers are needed at any time for effective outcrossing, a woody plant may reproduce at a smaller size or in a nutrient deficient habitat.

Traplining has been confirmed by other investigators in a wide variety of bees (Eickwort & Ginsberg, 1980) and in *Bombus* in particular (Heinrich, 1976). Feinsinger & Chaplin (1975) reported traplining in several species of nonterritorial hummingbirds. He also determined that in species of hummingbirds, such as the Ruby-throated Hummingbird (*Archilochus colubris*), which exhibit reverse sexual size dimorphism, the smaller males are usually nonterritorial while the larger females have a propensity to trapline. Hovering flight is cheaper for these larger females than for the smaller, nonterritorial males because their larger wings, relative to their body size, result in lower energy requirements of flight. "Traplining" hummingbirds have a much greater pollen dispersal distance than territorial hummingbirds (Linhart, 1973). In a few species of plants, pollen transfer is accomplished by a single pollinator species and the flower is highly specialized. Most animal pollinated plant species, however, have several species of pollen vectors (Willson, 1983) which may even belong to different phyla. The
differential contributions of the various vectors toward plant fitness influence the evolution of floral biology (Bertin, 1982). The floral biology of a particular plant species reflects the relative contributions of that species' pollinators. For example, hummingbirds meet their high energy requirements largely from floral nectar (Grant & Grant, 1968). If some plants have evolved costly floral adaptations, such as high nectar production, to attract hummingbirds, we might expect the birds to be more effective at pollen transfer, for those particular species, than other vectors.

Flowers that attract hummingbirds tend to produce copious amounts of nectar and to have long tubular corollas, one function of which might be to reduce nectar evaporation (Willson et al., 1979). These flowers are often a reddish color and not strongly fragrant (Grant & Grant, 1968). The anthers and stigmatic surfaces are exserted or located near the opening of the corolla tube. There is usually no landing platform at this opening. The flowers of Pinckneya fit this description.

Ruby-throated Hummingbirds are the only important bird pollinators in the range of Pinckneya (Grant & Grant, 1968), so any adaptations to bird pollination must be adaptations to pollination by this species. Ruby-throats are present in this range from about April through about October (Austin, 1975). Bertin (1982) has shown that these birds deposit as much as 10 times as much pollen per stigma per visit as honeybees (Apis spp.) and bumblebees (Bombus spp.). He considers this evidence that the birds are very effective pollinators. Bertin's studies showed that fruit production in Campsis radicans was greater where hummingbirds were common. Many floral traits of C. radicans are similar to those of Pinckneya. DePamphilis and Wyatt (1989) attributed pollen dispersal distances of tens to hundreds of kilometers to migrating Ruby-throated Hummingbirds.

Ruby-throats increase the seed set of plants at which they feed but no obligate relationship exists between the bird and any plant species (Bertin, 1982). One reason for this lack of specialization may be the presence of only one bird in this simple system.
Another reason may be that, because of the relatively recent ice age, the bird and plants have not been interacting long enough for such specialization to evolve. Although some plant characteristics appear to have evolved in response to Ruby-throats, there is no evidence that any Ruby-throat characteristic has evolved in response to particular food plants (Bertin, 1982). This asymmetrical evolution of the mutualistic partners may reflect the small number of major pollinators servicing each plant and the much larger number of nectar sources that each bird must have. Also, one day without food would mean death to a hummingbird, but to a plant one day without a pollinator visit would only be an inconvenience.

The reproductive biology of Pinckneya was undocumented at the outset of this project. The research described here was undertaken to delineate these requirements and to begin to understand their significance. Two questions summarize this goal.

**A. What is the mating system of Pinckneya and does this system favor selfing or outcrossing?**

I addressed this question by observing the flowering phenology of numerous individuals and by conducting a series of pollination experiments. In these experiments flowers were self pollinated, cross pollinated, and pollination was prevented in an attempt to elucidate the mating system. Some inflorescences were also bagged and compared with unbagged inflorescences after the flowering season.

**B. What are the primary pollinators of Pinckneya and how does the potential of these pollinators for pollen dispersal relate to the mating system?**

I addressed the second question by observing flower visitors, capturing the insect visitors, identifying these insects, and inspecting them for pollen. The literature relating to likely pollinators was consulted for estimates of their potential for pollen dispersal.
DISPERAL

Seed dispersal, an important stage in the life history of a plant, influences recruitment on a spatial and temporal scale and ultimately influences the structure and dynamics of a plant community. Patterns of dispersal are determined by the dispersal vector and the dispersal related fruit or seed characteristics (Sinha & Davidar, 1992). Because the seeds of most species are deposited near the parent, a leptokurtic seed distribution with a peak near the parent results (Janzen, 1970). Most of these seeds do not survive because of density dependent factors such as predators, pathogens, and competition (Willson, 1992). Seed dispersal away from the adult may reduce density dependent mortality and help to colonize new areas (Janzen, 1970). Dispersal ability exerts it's greatest influence when sites suitable for establishment and growth are scarce and unpredictable or when populations invade open habitats (Bazzaz, 1986). In spite of the widely recognized importance of the phenomenon, knowledge of the process of dispersal remains rudimentary. Although wind dispersal is one of the most common modes of seed dispersal, for example, no adequate theoretical models have been developed to deal with this type of seed transport (Anderson, 1991).

The seeds of Pinckneya are winged and are dispersed by the wind. In wind dispersal, the dispersal distance is influenced by seed morphology and release height, which are determined by the parent, and wind speed and surrounding vegetation, which are not determined parentally (Augspurger, 1986). Subsidiary factors include secondary transport and the threshold wind velocity for seed release (Anderson, 1991). Sheldon and Burrows (1973) write that the velocity with which a seed falls through the air is perhaps the single most important factor determining it's dispersal efficiency. However, Sinha and Davidar (1992) state that wind speed is most important in predicting dispersal distances and Lamont (1985) asserts that plant height is more important than all of the other traits in accounting for dispersal distance.
Particle diffusion models have been used to model wind dispersal and may be appropriate for very small seeds or spores (Augspurger, 1986; Solbreck & Anderson, 1987; Okubo & Levin, 1989; Green & Johnson, 1993). In addition to these, models have been made, based on settlement velocity and wind velocity, which attempt to predict the dispersal behavior of larger winged and plumed seeds (Cremer, 1977; Green, 1980; Augspurger, 1986; Matlack, 1987; Augspurger & Franson, 1988; Okubo & Levin, 1989; Matlack, 1992; Green & Johnson, 1993; Benkman, 1995). There have also been a number of studies conducted in the field that captured, in one way or another, dispersed seed and measured the “seed shadow” and dispersal potential of selected plants (McQuilkin, 1940; Cremer, 1966; Spring et al., 1974; Lamont, 1985; Drake, 1992; Lee, 1993).

A number of studies have attempted to relate some aspect of the morphology of winged seed to the terminal velocity of fall. The most basic of these is probably seed mass (Green & Johnson, 1993; Benkman, 1995), followed by wing area (Siggins, 1933). Other factors that have been investigated are disk loading, which is seed mass divided by the area of the circle transcribed by the spinning samara (Benkman, 1995); wing loading, which is seed mass divided by wing area (Green, 1980; Augspurger, 1986); and the shape of the diaspore (Matlack, 1992). A diaspore is a seed or spore that functions in dispersal (propagule).

This study examines the relative importance of these and other morphological variables of *Pinckneya* seed to the terminal fall velocity. It also establishes the statistical distribution of seed fall velocity for the species.

In the studies concerning the winged seeds, dispersal distance is estimated by multiplying the fall velocity (settling velocity) of the diaspores by their height above the ground to determine the time of flight. If a wind speed is multiplied by this product, the result is the dispersal distance for those parameters (Augspurger, 1986; Matlack, 1987;
Okubo & Levin, 1989; Green & Johnson, 1993). Although most models are based on a steady rate of descent, gusty winds have been shown to slow the rate of descent and increase the dispersal distance (Sinha & Davidar, 1992). Turbulence prolongs the flight time of seeds by carrying them above their release height, the seeds then glide from the advantage of this increased altitude (Sharpe & Fields, 1982). Webber (1934) describes Meranti fruit being carried 150 meters vertically into the air by high altitude turbulence when the air near the ground was still. He concluded that storms could distribute seed over many kilometers. Reports of seed being carried great distances by storms and turbulence are on record (Ridley, 1930; Thoreau, 1993). Ridley (1930) reports finding maple samaras 1 km above and 4 km distant from the nearest maple tree. He also reports ears of corn carried 3 km and beech leaves carried 20 km. Although I would like to have included the effects of turbulence on seed dispersal, too little is known about the phenomenon for objects as heavy as seeds to make quantitative generalities (Moen, 1974; Bergen, 1975).

I established confidence intervals for number of seed per capsule and number of capsules per plant for Pinckneya by counting. The mean number of seeds per plant is, of course, the product of these two measures. So, by constructing a model with accurate values for fall velocity, fall distance, wind velocity, wind direction, and seed number, a reasonable, if conservative, estimate of dispersal distance and seed shadow was made.

GERMINATION

At the small scale of a seed, the environment is heterogeneous (Harper, 1977) and varies tremendously both spatially and temporally (Cook, 1979). If a plant is to establish from seed, the immediate environment at the final location of the seed must meet the needs of that seed. These needs include a suitable surface for the submergence of the radical, adequate moisture, appropriate temperature, and whatever conditions are needed
to overcome any seed dormancy mechanisms (Street & Opik, 1984). Each species of plant has a unique set of requirements for germination and seedling establishment. If the site where a seed is finally deposited meets all of these requirements, it is considered a "safe site" (Fowler, 1988). Because seedling establishment is possibly the most vulnerable phase of a plant's life history, the success or failure of the plant is determined to a large degree by the final location of the seed (Fenner, 1987) and the time of its germination (Silvertown, 1988).

Since dispersed seed do not have the option of moving to a better site, germination must occur at their final location. If we recall that habitat varies temporally as well as spatially, it becomes clear that seed do have the option of waiting on better conditions, rather than germinating immediately. If this option is to be exercised, the seed must possess a method of recognizing the state of some important environmental variable and a method of regulating germination on that basis. When the other requirements for germination are met, the dormancy mechanism(s) mentioned above serve this purpose (Vazquez-Yanes & Orozco-Segovia, 1993). It is self evident that in order for a dormancy strategy to be successful, the dormant seed must have some measure of longevity and the strategy must be keyed to some reliable environmental cue. Environmental variability and uncertainty is the essence of most evolutionary explanations of dormancy (Silvertown, 1988).

Light is a critical resource for plants and competition for light can be intense. Individual plants must cope with the complex, changing, heterogeneous light environment of forests (Schmitt & Wulff, 1993). Light distribution is determined by forest geometry so defining light habitats by the geometry of the major light sources explains much of the variation in forest light (Endler, 1993).

Endler (1993) divides forests into four light habitats: forest shade, woodland shade, small gaps, and large gaps. These habitats differ in the relative angles subtended
by vegetation, skylight, and direct sun; forest shade and small gaps receive little if any
skylight, whereas woodland shade and large gaps receive much skylight. *Pinckneya* is
found in large gaps which receive a preponderance of light directly from the sun and from
the open blue sky. The color of the light in large gaps is white. Under plant shade those
wavelengths of light that are most reduced are those that are important in photosynthesis
and therefore are adsorbed by the plants in the canopy. The absorbed wavelengths are
photosynthetically active radiation (PAR) and represent that part of the light spectrum
with wavelengths between 400 and 700 nm (Street & Opik, 1984).

Dense shading by green leaves in forest shade can reduce the intensity of the
radiation to about 2% of direct sunlight (Frankland, 1981). Also, because of absorption
by chlorophyll, the blue and red wavelengths are reduced much more than the far red
wavelengths. Far red is transmitted more readily by leaves because it is out of the
photosynthetically active spectrum (Frankland, 1981). This results in the red/far red ratio
(R:FR) of ambient light under the canopy being dramatically reduced compared to values
in full sunlight (figure 1). Full Sunlight (large gaps) has a R:FR ratio of 1.2:1 but beneath
green canopies the ratio may be reduced to levels as low as 0.08:1 (Frankland, 1981).
This ratio varies widely as a function of canopy depth and age. Thus, R:FR is an
important clue by which seed may detect shading by other plants (Schmitt & Wulff,
1993).

Seed which require light to overcome dormancy are called positive photoblastic
seed (Vazquez-Yanes & Orozco-Segovia, 1993). The plant pigment phytochrome is a
physiological sensor of light in seed (Frankland, 1981). Light controlled seed
germination has been associated with this pigment since the pioneer studies on lettuce
seed (Borthwick et al., 1952). The term phytochrome refers to a family of
photoreceptors, each consisting of a low molecular weight protein covalently attached to
a photoreversible pigment (Schmitt & Wulff, 1993). These photoreceptor molecules
exist in two main forms that are interconvertable by exposure to different wavelengths of light. The active form, called Pfr, is converted from the inactive form, Pr by exposure to red light (R=655-665 nm). Far red light (FR=725-735 nm) converts Pfr to Pr, thus reversing the effect of the red exposure (Vazquez-Yanes & Orozco-Segovia, 1993). In the dark, Pfr is converted to Pr by a thermal, rather than a photochemical, process with a half life of about five hours (Fig. 2).

In a little understood phenomenon, known as inverse dark reversion, (IDR) Pr is converted to Pfr in the dark (Rollin, 1972). It has been observed in dry and imbibed seed. The positively photoblastic seed of *Rumex obtusifolius* have been found to germinate in complete darkness if they are subjected to temperatures of 40°C for one hour (Vincente et al., 1968). This effect is reversed by a short exposure to far red light at 20°C. Rollin (1972) suggests that the short exposure to 40°C induced IDR. "Grand Rapids" lettuce seed will not germinate in darkness at 30°C but a short exposure to temperatures between 5°C and 25°C can overcome the dark imposed dormancy of the higher temperature (Berrie, 1966). If the exposure to low temperature is from the beginning of germination it is less effective. Low temperature induced germination is not reversed by far red light, suggesting that at low temperatures an alternative metabolic pathway predominates.

In contrast to these examples of temperature overcoming phytochrome imposed dormancy and inducing germination, prolonged exposure to elevated temperature has been shown to inactivate Pfr and prevent germination. Scheibe and Lang (1969) demonstrated reduced germination in photosensitive lettuce seed that were exposed to temperatures of 37°C for a period of several hours.

Increases in R:FR of the light experienced by plants produce a continuum of proportional increases in the relative concentration of Pfr and thus in the phytochrome photoequilibrium, Pfr:P (Schmitt & Wulff, 1993). It is this Pfr:P photoequilibrium that influences actions such as germination. It is postulated that Pfr interacts with some
"reaction partner" X, therefore the greater the photoequilibrium, the more Pfr is available for reaction. Since red light is known to induce germination in some species, this Pfr:P photoequilibrium and the reaction with X is indicated to be essential for some step in the breaking of dormancy in these species. (Frankland, 1981) Phytochrome dependent germination has been demonstrated both in the field and in the lab (Vazquez-Yanes & Orozco-Segovia, 1989). See figure 2 for a diagram of the phytochrome system.

When we consider the mosaic of various intensities and colors of light to which a seed may be exposed, along with the ability of the phytochrome system to discriminate between particular light environments, we begin to appreciate the potential of the seed to selectively germinate where it encounters conditions favorable for establishment. The seeds of many species fail to germinate at the soil surface when shaded by other plants. This ensures that seedlings are not produced under conditions where the photosynthetic rate would be inadequate for growth. (Vazquez-Yanes, 1982; Vazquez-Yanes & Smith, 1982; Bliss, 1985; Silvertown, 1989; Vazquez-Yanes, 1989) This discrimination can occur either spatially or temporally.

Light controlled germination has been related to establishment in canopy gaps. Because the R:FR ratio of the ambient light at a particular site changes with the angle subtended by the sun when the sun is a significant part of the spectra, a seed may be able to determine the size of a gap and its position within a gap (Vazquez-Yanes & Smith, 1982). Seedlings in canopy gaps receive a greater amount of radiant energy, and hence have higher growth rates and are more likely to survive by out competing slower growing individuals. Most fast growing species are strict heliophytes. (Vazquez-Yanes et al., 1990).

The seed germination requirements of Pinckneya, like those of many coastal plain plants, were unknown when this project began. The research described here was undertaken to elucidate these requirements and to begin to understand their ecological
significance in light of the life history of the plant. This goal was approached by asking the following questions.

A. **What percentage of the seed are viable?**

A sample of seed was tested with tetrazolium to determine what proportion was living and what proportion was dead or contained dead material.

B. **What effect does light have on germination?**

Preliminary experiments in which seed were placed in growth chambers at various temperatures with some in the dark and some exposed to light indicated that *Pinckneya* seed are positively photoblastic. All lighted experiments were replicated in the dark and these results were confirmed. With this and the possibility of a phytochrome dormancy mechanism in mind, more specific questions were asked.

Does the photoblastic response indicate that the seeds are adapted to germinate with a single sudden exposure to light or does the response indicate that the seeds are adapted to recognize light quality changes? Germination after a single short exposure or a few intermittent exposures indicates response to conditions similar to those that occur when a buried seed is temporarily uncovered. If multiple photoperiods are needed to induce germination, it seems increasingly likely that the seed is responding to qualitative changes in the ambient light. This could enable a seed to differentiate a small sun fleck from a substantial light gap. To test this response in *Pinckneya*, a growth chamber was set at twelve hour photoperiods, a set of seeds was removed from the light to permanent darkness at the end of each light period for 5 days. The seeds remained in darkness until twenty eight days after imbibition, at which time they were removed and germination was checked. The effects of continuous exposure was tested by removing seeds from white light at 1,4,8,16,24, and 48 hours and keeping them in the dark until 28 days after imbibition.
Does far red light inhibit or enhance germination? Do the results of field experiments support conclusions drawn from laboratory experiments? During a twelve hour photoperiod, at 30°C in a growth chamber, sets of seed were moved from red to far red light, and other sets from far red to red light halfway through the photoperiod. Each was returned to its initial location at the end of each photoperiod. Additional seed were left in the red, far red, and white light, and continuous darkness.

In the field, sets of seed were placed in the center of a 0.2 hectare light gap and on the northern and southern edges. Another set was placed under the cover of a fully closed canopy of vegetation. Illumination ranged from full sun in the center of the gap to full shade under the canopy. The edges of the gap were in sun for part of the day and shade for part of the day with the southern exposure of the northern edge receiving much more direct sun than the northern exposure of the southern edge. The amount of direct sunlight received by either edge was intermediate to that received by the other two locations. These seed remained in place for one month and germination was checked periodically as they were watered. Two Pinckneya seedlings were placed in pots at each location and observed throughout the month of the experiment.

C. What effect does temperature have on germination?

Seed were germinated in four temperature regimes in two experiments that were conducted 3 months apart. The rates of germination and the end percent germination tell us which of those temperature regimes tested is more favorable. In a third experiment seeds were imbibed and kept in the dark for two days. One third of the seed were exposed to 40°C and one third to 50°C for two hours and then returned to the optimum temperature with the final third, which were control plates. All seed were kept in the dark throughout this procedure. This tested the ability of the seeds to withstand somewhat higher temperatures and the ability of heat to overcome any photoblastic dormancy. A fourth experiment was conducted to determine if long term, more moderate temperature
change can overcome positive photoblastism. Seed were kept in the dark at 10°C for 28
days and then moved to 30°C where, still in the dark, they remained for another 28 days.
Controls were kept in the dark at 10°C and 30°C for the entire 56 day period.

D. What effect does the seed coat have on germination?

A tannic substance was observed leaching out of the seed coat of Pinckneya. The
coats of these seeds were noticeably lighter after rinsing. To determine if this leechate or
some other attribute of the seed coat contributes to dormancy, seeds were prepared
according to four treatments: rinsed with tap water; rinsed with tap water and the seed
coats removed; seed coats removed only; no modification, the condition in which
collected. The prepared seed were then germinated in a growth chamber and germination
percentages compared between the treatments.
MATERIALS AND METHODS

Seeds were collected from twelve different Pinckneya populations in a 60 km radius of Statesboro, Georgia during September, 1995 (Table 2). An approximately equal number of seeds from each population was selected at random and mixed. Seeds from this mixed sample were used in each experiment. The JMP™ statistics program for the Apple Macintosh™ computer was used for all statistical analysis.

MATING SYSTEM

The study site for the mating system experiments was a large population of Pinckneya located on private property on the south side of US 80 about four miles west of the intersection of US 80 and Georgia 23 in Twin City, GA (location 4, Table 2). This population was growing near a stream branch or tributary that had been cleared and planted in slash pine, Pinus elliottii, seven years earlier. The soil conditions were apparently too wet for the optimum growth of the pines. Pinckneya, although it did not form a true canopy, was the dominate woody species present at the time of this study.

Bridal veil was sewn into bags that averaged about 10 cm square, but varied in size, and were open at one end. These bags were used to isolate flowers from pollinators and were of adequate size for this purpose. They were attached to the plant by slipping the flower or inflorescence into the bag and tying the bag tightly around the pedicel or stem with colored flagging. The color of this ribbon identified the particular treatment. To facilitate the attachment of the bags and to reduce abortions due to resource limitations, buds and flowers other than the ones subjected to each treatment were
removed from the immediate peduncle of each experimental flower.

Flowers were subjected to each of the four treatments described below. Each flower, after the assigned treatment was performed, was bagged as described in the previous paragraph. Thirty flowers were emasculated by opening the nearly mature bud and removing all of the undehisced anthers. Thirty flowers were simply bagged before they opened to test the ability to self pollinate without assistance. Thirty flowers were self pollinated by removing open anthers from other flowers on the same plant and placing this pollen on the open stigmas of these experimental flowers. This was accomplished by rubbing the anthers against the stigmas until pollen could be seen clinging to the stigma. Thirty flowers were out-crossed, in a similar manner, by pollinating with anthers from other plants from the same population, and likewise, thirty more flowers were pollinated by anthers from another population. This second population (location 5, table 2) was located about 8 km by air from the first, on the west side of Georgia route 23 approximately halfway between Twin City and Garfield. Seventy unopened but nearly mature buds were marked as controls, but no bags were applied. As in the experimental treatments, competing buds and flowers were removed from the immediate peduncle.

The due date of this thesis is several months before *Pinckneya* fruit normally matures, for that reason, some indicator of pollination success other than mature seed production was needed. Using the logic that fruit containing viable seed would be larger than fruit without a minimum number of viable seed, I decided to use fruit width as an indicator of the presence of viable seed. This approach does not damage developing fruit, which will be collected at maturity to confirm these results. One month after the pollinations were completed the width of the ovaries of each of the experimental and control flowers was measured. A large sample of ovaries, ranging in size from the smallest to the largest found, was collected at this time. These ovaries were dissected and
their contents were microscopically examined in an attempt to determine the minimum size ovary that contained recognizable seed.

In a second, simultaneous experiment, thirty inflorescences that contained no previously opened flowers were bagged with large bridal veil bags, and thirty similar control inflorescences were marked but not bagged. One month later the number of ovaries exceeding 7mm in width were counted on all sixty inflorescences and the numbers found were analyzed for the presence of a statistical difference between the treatments.

Numerous hours were spent observing the flowers and flower visitors of *Pinckneya*. The sequence of events related to flowering was noted. Examples of most of the observed insect visitors were collected and identified by the use of Peterson's key. All insects collected were examined microscopically for the presence of pollen.

**DISPERsal**

Fifty seeds from the sample discussed above were measured and described in the following eight ways:

1. Wing Area (Aw) = planeform area of wing in mm², determined by grid under a dissecting microscope.
2. Length (L) = maximum length of wing
3. Width (W) = maximum width of wing perpendicular to L
4. Short Axis (Xs) = length of shortest axis of wing
5. Shape = general shape of wing, classified as either elliptic, circle, seem-circular, quarter-round, oval, comma, triangular, or tetrahedral
6. Mass (m) = mass of seed in mg
7. Embryo Longitude (El) = embryo location on the longest axis of the wing, computed by dividing the distance from the center of the embryo to the nearest end of the wing
by the length of the wing, a value of 0.5 means that the embryo is centered with respect to the L axis, an uncensored embryo has a value less than 0.5

8. Embryo Latitude (Ew) = embryo location on the axis perpendicular to the longest axis of the wing, computed by dividing the distance from the center of the embryo to the nearest edge on the axis perpendicular to the longest axis of the wing, a value of 0.5 means that the embryo is centered with respect to the W axis, otherwise the value of Ew is less than 0.5

Eight other descriptive variables were derived from the eight measurements above. The derived variables included:

1. Embryo Location (Ec) = composite embryo location. Ec = El x Ew.
2. Length/Width ratio = length divided by width: L/w
3. Length/Short Axis ratio = length divided by shortest axis: L/Xs
4. Aspect ratio = L^2 / Aw
5. Circular form = $2 \sqrt{\frac{Aw}{\pi}}$ / L
6. Disk loading = m/π((1-El) x L)^2
7. Wing loading = m/Aw

These fifty seeds were then dropped individually, with a specially constructed mechanism, from a height of 4.47 meters in an enclosed area that was protected from external influences. The fall time of each of these seed was recorded and each rate of fall was computed. In addition to the 15 factors already described, fall type was described as either "flutter," "spin," "tumble," or "helical."

To remove any wind influence, the seeds were dropped inside of a closed corrugated steel grain bin. The tests were conducted at night when wind was least and
the internal and external temperatures of the bin were equalized to minimize thermal
effects such as updrafts. Seed to be timed were placed on a mechanism based on a heavy
iron barn door hinge. This mechanism was constructed so that when a string was pulled,
one side of the hinge would swing out from under the seed that was resting on top of it.
This seed would then fall to the floor of the bin. The surface on which the seed rested
was covered with duct tape, with the slick side toward the seed, to prevent the seed from
sticking to the hinge. The floor of the bin was covered with a white sheet to improve the
visibility of the seed.

A person on the floor with a stop watch would call out, in a regular cadence, "one,
two, three, go," and would start the stopwatch on "go." A second person would pull the
string of the mechanism on "go," starting the fall of the seed. The person with the watch
would stop it when the seed touched the sheet. To be consistent, the same person was
used in each position for all trials. If there was any ambiguity in the coordination of
either the starting or stopping of the watch, the results in question were not recorded.
Both people, by consensus, decided the proper category of fall type for each seed.

The fall time was divided by the 4.47 meter fall distance to determine the rate of
fall. This rather large fall distance acted to minimize the proportion of the time that was
spent in acceleration. Terminal velocity was reached within a few centimeters of fall.
Each seed was only measured once because earlier experiments showed that the results of
multiple drops were substantially the same (within 8%) as long as the seed was not
damaged. Additionally, seeds were often damaged on impact or by excessive handling,
making repeated tests of the same seed impractical.

The fall times were analyzed statistically to determine what morphological factors
most influenced fall velocity. Fall velocity was used in conjunction with wind data from
the National Oceanographic and Atmospheric Administration (NOAA) to estimate the
seed dispersal pattern. The wind data used were an average of the yearly average figures from Savannah, Albany, and Valdosta, Georgia.

GERMINATION

Unless otherwise noted, all seed tested were from the sample described at the beginning of this Materials and Methods section. One hundred seed were tested for viability by cutting each in half, across the embryo, and placing the halved seeds in petri dishes with a 1% tetrazolium solution as described by Lakon (1949). After a period of 24 hours in a dark cabinet, the seeds were checked for the red color that indicates that respiration is occurring.

Laboratory experiments were carried out in Lab-Line Biotronette Plant Growth Chambers lighted by wide spectrum fluorescent bulbs. A dish consisted of two sheets of filter paper in the bottom of a petri dish. Twenty five seeds picked at random from the mixed sample were evenly distributed in each dish and hydrated with tap water. The edge of each petri dish was wrapped with parafilm to conserve moisture. The dishes were then immediately placed in their assigned location, according to the treatment, before imbibition occurred. Germination was considered to have occurred when the hypocotyl emerged from the seed coat.

In the initial experiment, during the month of December, 1995, 12 dishes were prepared as described above and placed in three light proof stainless steel petri dish containers, four to each container. These containers were each placed in a separate growth chamber, along with an identical number of similar dishes that were not in light proof containers. All growth chambers were set to have a 12 hour photoperiod (12 hours of darkness followed by 12 hours of light) and were each operated at a different temperature: 20°C, 30°C, and 20/30°C. All dishes remained in the chambers concurrently for the entire 28 day period. The 20°C and 30°C temperatures were constant and the
20/30°C temperature alternated at twelve hour intervals, synchronized with the 12 hour photoperiod. At the conclusion of the 28 day period, all of the dishes were removed from the dark containers and checked for germination.

This experiment was repeated during the months of March and April, 1996. The repetition was performed as described above except that the temperature regimes were changed to 10°C, 20°C, and 30°C constant temperature.

Twenty dishes were prepared and immediately placed in a growth chamber set at 30°C with a 12 hour photoperiod. Four dishes from this group were triple wrapped with aluminum foil at the end of the lighted period each day for five days and returned to the growth chamber. All of these dishes remained within the dark foil wrapping until 28 days after the start of the experiment, at which time they were opened and checked for germination.

Twenty four dishes were prepared and immediately placed in a growth chamber set at 30°C with a 24 hour photoperiod (constant light). Six subsets of four dishes each from this group were triple wrapped with aluminum foil at 1, 4, 8, 16, 24, and 48 hours following the start of the experiment and returned to the growth chamber. All 24 of these dishes remained within the dark foil wrapping until 28 days after the start of the experiment, at which time they were opened and checked for germination.

Twenty four dishes were prepared and immediately placed in a growth chamber set at 30°C with a 12 hour photoperiod. Four dishes from this group were placed in a light proof stainless steel container where they remained for the duration of the experiment. Four dishes were placed on a shelf in the white fluorescent light. Four dishes were placed in a plastic tray that was covered with a polycarbonate light filter. This filter only admitted light of the red wavelengths. Four dishes were placed in another plastic tray that was covered with a polycarbonate light filter. This filter only admitted light of the far red wavelengths. Because fluorescent bulbs do not emit far red light,
incandescent bulbs were used above this container. Temperatures increase in the far red container under the incandescent light. This necessitates a compensatory lower temperature setting in the growth chamber. Because of this required temperature setting differential, it was necessary to use two growth chambers for this experiment, one for the far red treatment, and one for the other treatments. A fifth subset of four dishes from this group was placed in the red container prior to the start of the lighted period each day and transferred to the far red container six hours into the 12 hour photoperiod. Finally the remaining set of four dishes was placed in the far red container prior to the start of the lighted period each day and transferred to the red container six hours into the 12 hour photoperiod. This was continued daily for 10 days. At the end of the tenth photoperiod, all of the dishes were placed in light proof stainless steel cylinders where they remained until 28 days after the start of the experiment. Each of the cylinders was opened and the seeds were checked for germination at that time.

Twelve dishes were prepared and immediately placed in three light proof containers, four dishes to each container. These containers were placed in a 30°C growth chamber. Two days later, one of the cylinders was placed in a 40°C incubator for 2 hours and one in a 50°C incubator for 2 hours. At the end of the 2 hour period, both containers were returned to the 30°C growth chamber with the third cylinder. Twenty eight days after the elevated temperature treatments, the three cylinders were open and the seeds were checked for germination.

Four sets of four dishes each were prepared and placed in four stainless steel light proof containers. Two of these sets were kept in a 10°C growth chamber for 28 days, at which time one was exposed to sunlight for five minutes and one remained in complete darkness. Both sets were placed in a 30°C growth chamber for another 28 days. One of the remaining two sets of dishes was kept in the 10°C growth chamber and one in the
30°C growth chamber in complete darkness for the entire 56 day period. At the end of this period all were checked for germination.

To determine if the seed coat played a part in dormancy, four different treatments were prepared. In treatment one all seed were rinsed for two minutes in 10% Clorox™ bleach solution to kill any harmful fungi that may have infected the seed and immediately rinsed with tap water for 10 minutes. In treatment two all seed were subjected to the same treatment as treatment one after which the seed coats were removed. In treatment three nothing was done to the seed, they were placed in the petri dishes in the condition in which they were collected. In treatment four the seed coats were removed and nothing else was done. One dish of each of these treatments was subjected to each temperature and light regime for 28 days.

In January, 1996, five dishes of twenty seed each were prepared and placed in a 30°C growth chamber, under a 12 hour florescent photoperiod, for 40 days. Germination was assessed at the end of these 40 days. These seeds were from a sample that was collected in November of 1973 by Dr. Donald Drapalik. Seeds from this sample were also tested for viability with tetrazolium as described above.

In the field germination experiment, four inch plastic flower pots were filled with wet vermiculite and 10 Pinckneya seeds were placed on the surface in each pot. Ten of these pots were placed in a plastic tray in which water was maintained to prevent the vermiculite from drying out. Two potted Pinckneya seedlings, each about 10 cm tall, were placed in each tray with the pots of seed. One tray was placed at each location described below. In a forest clearing of about 0.2 hectares in size, one location was in the geometric center of the clearing. The tray was buried so that its top was roughly flush with the ground in an attempt to moderate the temperature. Two other locations were on the northern and southern edges of the clearing. A third location was in the deep forest under the cover of a complete canopy of Persea borbonia and Vaccinium spp. All light
reaching the forest floor at this location was filtered through foliage. A final location was the center of a small light gap of about 3-4 meters in size. This experiment was started on the evening of May 21, 1996 and stopped on the morning of June 25, 1996. Germination was assessed on the ending date.
RESULTS

MATING SYSTEM

A. What is the mating system of Pinckneya?

Observations of flowering phenology: Pinckneya is protandrus. The anthers dehisce about the time that the buds open. Pollen is presented beginning at various times from just before the bud opens to just after opening. At flower opening, the distal ends of the petals roll back on themselves, causing the anthers to be exserted. The style, at this time, is only about one half as long as the filaments so the stigma, which is closed and apparently unreceptive to pollen, is well within the confines of the corolla tube. After the pollen is gone, the stamens "wilt" so that they are not obstructing the opening of the corolla tube. The style now grows longer, reaching a length that is slightly longer than that previously reached by the filaments. Once the style is grown, the stigma is fully exserted and alone in the space beyond the corolla opening. It is only at this point in the flowering sequence that the stigma finally opens and becomes receptive to pollen. I concluded that it is receptive because pollen readily sticks to it at this stage of development but not at earlier stages. Once the stigma is open, the receptive surface appears very different from the other surfaces. Because of this sequence, pollen from the anthers of a flower is normally not available to the stigma of that same flower.

Pollination experiments: The ovaries of opening flowers measure 4 to 6 mm in width. One month after the pollinations were completed, a cross section of fruit, ranging from 4 to 20 mm in size, were dissected and examined for the presence of seed. All fruit of widths of 6-7 mm and greater were found to contain recognizable multicellular seed. Fruit smaller than this size contained structures that appeared to be embryo sacs. I could
not be sure that these smaller structures will not develop into seed, but ovaries above 6-7 mm had begun to swell, taking on a rounded fruit like appearance, while those smaller than this size were cone shaped and resemble the receptacle - ovary of the flower. Two weeks later, the larger fruit had continued to grow but the smaller ones had not grown. Some of the smaller ovaries had begun to wither and fall off.

Ovary width was significantly correlated with pollination treatment (Fig. 3). The ovary widths of the emasculated flowers, the flowers, that were bagged and left alone, and the self pollinated flowers, were not significantly different and all averaged smaller than the 6 to 7 mm size range. Ninety percent of the fruit in these categories was smaller than this size range or borderline. The mean widths were 3.77 (SE=0.30) for the emasculated flowers, 4.07 (SE=0.37) for the flowers, that were simply bagged, and 4.48 (SE=0.42) for the self pollinated flowers. The ovary widths of the two outcrossed treatments, one from the same population and one from a different population, were not significantly different from each other, but the outcrossed treatments had ovaries that were significantly larger than the other three treatments, and well above the 6-7mm range. Seventy five percent of the fruit of outcrossed flowers are of a size that contains recognizable seed. The mean widths were 10.08 (SE=0.89) for those outcrossed from the same population and 11.17 (SE=0.90) for those outcrossed from a different population. The mean width of the control flowers was 6.87 (SE=0.48), this width is significantly different from both of the other two groups and it falls within the range where seeds become recognizable. All tests of significance used a Tukey Kramer nonparametric test and a 0.05 level of significance.

By this same test, the numbers of fruit larger than 7 mm in the bagged inflorescences and in control inflorescences were significantly different. The mean number of large fruit in a control inflorescence was 4.67 (SE=0.49) and 0.14(SE=0.50) in
a bagged inflorescence (Fig. 4). Only 10% of the bagged inflorescences had any fruit larger than 7 mm but 93% of the control inflorescences contained large fruit.

**B. What are the primary pollinators?**

Most of the insects seen or captured at *Pinckneya* flowers were bees. Members of two genera, *Bombus* and *Apis*, were present among the bees. Approximately 10 bumblebees (*Bombus*) were found for each honeybee (*Apis*). Three size classes of bumblebees were present in roughly equal numbers: Small - about 12 mm in length; Medium - about 18 mm; Large - about 25 mm. I found pollen on the bodies and in the pollen baskets of the honeybees and bumblebees in all three size classes. The smaller bees were normally active around the open end of the corolla in the vicinity of the anthers, but the large bumblebees spent a considerable amount of time on the outside of the corolla tube. I often observed them facing the proximal end of the flower, apparently exploring the region where the calyx meets the corolla. Although I never saw a bee puncture a perianth, I did find some puncture marks in this part of the flower that may or may not be attributable to bumblebees. A small amount of nectar occasionally was available between the calyx and the corolla, but other insects seemed to ignore it.

The insects other than the bees included stinkbugs (*Pentatomidae*), leaf footed bugs (*Coreidae*), ants (*Formidae*), small flies (*Diptera*), beetles (*Coleoptera*), grasshoppers (*Acrididae*), and a few butterflies (*Papilionoidea*). Out of this motley assortment, only the occasional butterfly had an appreciable amount of pollen on its body.

I also observed the Ruby-throated Hummingbird visiting flowers. Contrary to what is written in much of the literature, I perceived this bird to visit only one or two flowers per plant and then to quickly move to another plant. In this way it moved rapidly through the population.
DISPERAL

Samples of seed capsules from 11 populations averaged 76.6 (SE=3.52, n=50) seeds per capsule. Ninety five percent of the capsules contain between 69 and 83 seeds. There were 205 (SE=12.6) capsules per plant and the plants averaged 3.54 (SE=0.21) meters tall. Ninety five percent of the flowering individuals were between 3.1 and 4.0 meters in height. Inflorescences were located from the top of a plant to within a meter of the ground. Inflorescences were rarely lower than 1 meter.

The mean fall velocity for all measured seed was 1.13 meters/second (SE=0.03). The 49 measured velocities were normally distributed (Shapiro-Wilk W test - p = 0.507; 95% C.I. = 1.07 - 1.19).

Of the measured variables, wing area, length, width, short axis, and embryo longitude were found to significantly affect with fall velocity (ANOVA, p>0.05). Of the derived variables, only embryo location and wing loading were significant (Table 3). Wingloading was much more correlated with mass (0.81) than area (-0.17) so it can be considered reasonably independent from area, as a variable. Embryo location was closely correlated (0.75) with embryo longitude (0.55 with embryo latitude) and so is not independent. Embryo longitude, which explained 29% of the variation in fall rate, is most likely the better of the two as an indicator. The seven measured variables, together explained 59.2% of the variation in fall velocity (multiple regression). All of the measured and derived variables combined explain 73.1% of this velocity variation.

I conducted a principal component analysis in order to assess the relationships among the seven measured variables (Table 4). The first three principal components (PC) accounted for over 81% of morphological variability among the seeds. PC1, accounting for 47.83% of the variation, describes a gradient of increasing size. PC2, accounting for 21.79% of the variation, represents positive covariation in the closeness of the embryo to
the center of the longest axis and seed mass. PC3 accounts for 11.63% of the variation and represents a gradient of increasing embryo latitude and decreasing mass.

A multiple regression showed that these three principal components accounted for 52.4% of the variation in seed fall velocity, PC1 accounted for 31.3%, PC2 accounted for 5.0%, and PC3 accounted for 16.1% of the fall velocity variation (Table 5). Individually, PC1 and PC3 were significantly correlated with fall velocity (ANOVA, p<0.05) but PC2 was not (p=0.12).

Wingloading explains more of the variation (33%) than any other single variable. Together, PC1 and PC2 explain 47.4%, more than any other two variables.

GERMINATION

A. What percentage of the seed are viable?

The tetrazolium test indicated that 100% of the 1995 mature seed contained respiring embryo axes. Seedlings are epigeal.

B. What effect does light have on germination?

Do seed have a photoblastic response? In the initial experiment conducted during December 1995, the mean percent germination of the seed in the light across all temperatures was 55.83% (SE=6.79) and of those in the dark was 0.83% (SE=0.83). In the favorable 30°C temperatures, the germination percentages were 68.75% (SE=4.79) for the seed in the light and 1.11% (SE=1.11) for the seed in the dark (Fig. 5). These results, in both cases, are significantly different at a 0.05 level of significance with a Tukey Kramer nonparametric test.

In the second, similar experiment, conducted during March and April of 1996, the results were essentially the same. With all temperatures pooled, 43% (SE=9.86) of the seed in the light germinated compared to 1% (SE=0.52) of the seed in constant
darkness. At 30°C, the best of the temperatures tested for germination, 73% (SE=6.40) of the seed in the light germinated versus 2% (SE=1.15) of the seed in the dark.

Does the photoblastic response indicate that the seeds are adapted to germinate with a single sudden exposure to light or does the response indicate that the seeds are adapted to recognize light quality changes? The two experiments that tested for the minimum time of exposure to white light were conducted in response to this question.

The germination percentages for seeds exposed to from 0 to 5 photoperiods of 12 hours each were: No light, 2% (SE=2); 1 photoperiod, 0% (SE=0); 2, 6% (SE=4.0); 3, 34% (SE=6.0); 4, 32% (SE=7.3); 5, 58% (SE=5.8) (Fig. 6). Germination increased linearly with increasing numbers of days exposure to light (Fig. 7). This relationship is described by the formula: \( \% \text{ germination} = 11.54 \times (\text{number of photoperiods}) - 6.857; r^2 = 0.716 \) (p<0.001).

The germination percentages for seeds exposed to from 0 to 48 hours of continuous white light were: no light, 2% (SE=2); 1 hour, 2% (SE=2); 4 hours, 4% (SE=2.45); 8 hours, 2% (SE=2); 12 hours 10% (SE=3.16); 24 hours, 20% (SE=5.47); and 48 hours, 28% (SE=3.74) (Fig. 8). Germination increased linearly with increasing time of continuous exposure to light (p<0.001) (Fig. 9). This relationship is described by the formula: \( \% \text{ germination} = 0.588 \times (\text{number of hours constant light}) + 1.564; r^2 = 0.648 \).

Does far red light inhibit germination and does red light enhance germination? In the colored light experiment, the following germination percentages were recorded for each treatment: Dark, 11% (SE=3.0); Far Red, 7% (SE=3.4); Red - Far Red, 17% (SE=1.9); Far Red - Red, 41% (SE=4.4); Red, 67% (SE=8.4); White, 74% (SE=6.2) (Fig. 10). The Dark, Far Red and Red - Far Red treatments were not significantly different from each other (Tukey-Kramer, p<0.05). The red and white treatments were not significantly different from each other, but the latter group had significantly higher germination than the former group. The Far Red - Red treatment was significantly
different from both groups and was intermediate between them. Far red light inhibited germination and red light promoted germination.

In the field germination trial, the following germination percentages were recorded: Full Sun, 3.8% (SE=1.8); Southern exposure, 8.3% (SE=4.8); Northern Exposure, 7% (SE=4.2); Full Shade, 21% (SE=5.5). The location in the small light gap was destroyed by a marauding armadillo. Germination in the full shade was significantly greater than all of the other locations, which were not significantly different from each other (Tukey-Kramer, ps<0.05) (Fig. 11). The following temperature ranges were recorded at the four locations: Full Sun, 14°C -55+°C; Southern Exposure, 16°C - 55+°C; Northern Exposure, 14°C - 43°C; Full Shade, 18°C - 38°C. The seedlings survived in all four locations.

C. What effect does temperature have on germination?

Differences in % germination and germination rate between temperature regimes were statistically significant (Fig. 12). In the initial December experiment, the mean percent germination after 28 days of each temperature was: 20°C = 30.0% (SE=8.2); 30°C = 62.5% (SE=7.5); 20-30°C = 75.0% (SE=5.0). Temperature had a significant effect on germination among all treatments (Tukey-Kramer, ps<0.05). Among the treatments there was a significant difference between the 20°C treatment and the other two but the difference between the 30°C and the 20-30°C treatments was not significant.

In the March - April temperature experiment, temperature was again found to have a significant effect on germination (Tukey-Kramer, ps<0.05), however, at this time, the difference between the 20°C and the 30°C treatments was not quite significant. The 10°C treatment had a significantly lower germination percentage than the other two treatments. Mean final germination percentages were: 10°C, 0% (SE=0); 20°C, 56% (SE=7.5); 30°C, 73°C (SE=6.4). Germination at 20°C was significantly higher in March/April than in December.
There was no germination in any of the treatments in the experiment in which seeds were exposed briefly to 40°C and 50°C.

In the fourth temperature experiment, it was discovered that a change in temperature could overcome dark germination inhibition (Fig. 13). The seeds kept in the dark at 10°C for 56 days had 0% germination. The seeds kept at 30°C in the dark for 56 days had 8% (SE=3.3) germination. The difference between these two treatments was not statistically significant (Tukey-Kramer, ps<0.05). The seeds moved from 10°C to 30°C and not exposed to light had a 28% (SE=5.2) germination. The seeds moved from 10°C to 30°C and briefly exposed to sunlight had a 35% (SE=6.8) germination. The difference between these two treatments was not statistically significant. However, the germination percentages of those seed which changed temperature were significantly higher than those seed which remained at a constant temperature, either 10°C or 30°C.

D. What effect does seed coat have on dormancy?

There was no statistically significant difference between any of the four seed coat treatments (Tukey-Kramer, ps<0.05) (Fig. 14).

Germination of old seed

In the germination test on the seed that were kept in cold storage for 22 years, no seed germinated. The tetrazolium test indicated that the cotyledons were still metabolically active but the embryo axes of all seed tested were dead.
DISCUSSION

MATING SYSTEM

_Pinckneya_ is clearly protandrus. This reduces or eliminates the possibility of pollen from a particular flower being deposited on the stigma of that same flower. However, it does not eliminate the possibility that pollen from another flower of the same plant may be deposited on the stigma. Therefore, although protandry increases outcrossing, it does not necessarily eliminate self-fertilization.

Heterostyly is a polymorphism of style and filament length that has the effect of ensuring outcrossing. It is usually expressed as distyly which is the presence of roughly equal numbers of two different floral types. Heterostyly is particularly common among members of the Rubiaceae, which include _Pinckneya_ (Darwin, 1877). Cleistogamy is the presence of flowers that shed pollen onto receptive stigmas without opening and it has the effect of ensuring self pollination.

In _Pinckneya_ I found examples of shortened filaments, abnormal pistils, and pollen shedding within the unopened bud, particularly late in the flowering season. These examples, however, are rather isolated and appear to be abnormalities rather than any type of polymorphism. For example, out of the many fruit examined, one specimen with three carpels was found. All of the others had two carpels. Incidents of this nature show up relatively frequently but the individual abnormalities are not consistent. This may be evidence of heterozygosity or multiple allelism. At any rate, it seems inconsistent with a selfing, homozygous population and may be indicative of outcrossing. The many shades of sepal color also fit well within the outcrossing paradigm. Plants with vastly different sepal color grow juxtaposed to one another, so the variation appears to be genetic rather than environmental.
Ornduff (1969) listed many characteristics that are common among outcrossing plants. *Pinckneya* exhibits many of these characteristics (Table 6).

The pollination experiments indicate that *Pinckneya* is self-incompatible. The lack of seed production in the emasculated and self pollinated treatments, in contrast to the substantial seed production of the outcrossed treatments, is indicative of a nonapomictic, obligatory outcrossing mating system. This evidence is supported by the lack of seed production in the bagged inflorescences, as distinguished from the near universal seed production of the control inflorescences.

A single, small, transplanted *Pinckneya* plant in Vidalia, Georgia offers further evidence that supports outcrossing. This plant was set out in a residential yard in the winter of 1995-96, and bloomed the following summer. There were no other flowering individuals of this species known to be within several miles of this plant, which only produced one inflorescence. No seed or fruit resulted from this inflorescence.

The fact that fruit size was not significantly different between flowers pollinated from the same population and flowers pollinated from a different population suggests that the pollen source is not important for fruit set and seed production, as long as the source is a different plant. This observation is consistent with a sporophytic incompatibility mechanism.

One may wonder why a plant that is genetically self-incompatible is also protandrus. In plants that are self incompatible, the primary utility of protandry may be the avoidance of interference of the male and female functions with each other (Lloyd & Yates, 1982). In other words, the pistil does not interfere with pollen removal and the stamens do not interfere with pollen deposition. Also the stigma is not cluttered with useless self-pollen. The advantages of this to *Pinckneya* are apparent because of the relatively small stigma (about 2 mm$^2$ in area) and the necessity of enough viable pollen to produce a mean of 77 seeds.
Fruit production in many species is resource limited, but research shows that some species, in particular hummingbird pollinated species such as Trumpet creeper, *Campsis radicans*, may be pollinator limited (Bertin, 1982a). The lower rate of fruit production of the control flowers in comparison to the outcrossed flowers in this study (Fig. 3) suggests that pollination is limiting seed production in *Pinckneya*.

Because *Pinckneya* is an obligate outcrosser, it must attract pollinators that have the capacity to transfer pollen among plants and that habitually make this transfer. An isolated plant needs pollen from another population in order to produce seed so pollinators must be capable of supplying this need. Bees, which are *Pinckneya*’s primary insect visitors, and hummingbirds are known to transport pollen over long distances.

All flower visitors, of course, are not necessarily pollinators. Many species of large bees, including members of the genera *Bombus* and *Xylocopa*, are regular nectar thieves (Hendrix, 1988). The large bumblebees that frequented the outside of the perianth may have been nectar thieves. Likewise, the smooth insects that carried no pollen on their bodies were probably not pollinators.

In *Pinckneya*, nectar is secreted inside of and at the base of the floral tube. Because of the length of this tube, and because of a region of protective pubescence inside of the corolla, smaller bumblebees and possibly the larger ones are probably unable to reach the nectar. These species most likely visit flowers primarily for the purpose of collecting pollen. The contents of their pollen baskets provide evidence that supports this hypothesis. Although same flower pollen is not available at the same time as a receptive stigma, pollination could still occur as these bees searched a functionally pistilate flower for pollen.

The June flowering period of *Pinckneya* coincides with the nesting period of the Ruby-throated Hummingbird (Bertin, 1982b). During this period, female Ruby-throats would be unable to migrate a long distance to a new feeding site, obligating them to visit
suitable flowers within range of their nest. The low number of plants, and therefore flowers, of most *Pinckneya* populations should preclude such large pollinators as hummingbirds, with their high energy requirements, from establishing a protected territory around a particular population. However the high nectar production and the presence of flowers over an extended period of time makes it a valuable resource while it is available. This combination is attractive to traplining pollinators such as hummingbirds or large bees. Effective pollination by these traplining pollinators is favorable to outcrossing with other populations a considerable distance away.

Bertin (1982a) counted the number of pollen grains deposited by each of three pollen vectors on the stigma of *C. radicans*. He reported the following means: Ruby-throated Hummingbird, 386 (SE= 123); Honeybee, 44 (SE= 27); Bumblebee, 33 (SE= 10). These were the three vectors that carried the largest pollen loads. *Campsis radicans* pollen is about 50% larger than *Pinckneya* pollen, about 30 microns in diameter (Jones et al., 1995) verses about 20 microns for *Pinckneya*. The stigma of *C. radicans* is about ten times as large as the stigma of *Pinckneya*. This means that if all else is equal, the capacity to deposit approximately 6 pollen grains on *C. radicans* is needed for a pollinator to have the capacity to deposit 1 pollen grain on *Pinckneya*. Using these figures, we should expect the following means for the number of pollen grains that these three animals deposit on a *Pinckneya* stigma: Ruby-throated Hummingbird, 64; Honeybee, 7; Bumblebee, 6.

The mean number of seed found in *Pinckneya* fruit was 77 (SE=3.5). If every pollen grain that arrived at a stigma successfully produced a seed, at least 77 pollen grains would be needed, on average. Often over 100 would be necessary to account for the seed set. It is obviously unlikely that every pollen grain would produce a seed, so the requirement should be somewhat higher. Only the hummingbird is capable of regularly depositing close to this volume of pollen in one visit. Of course other pollinators, such as
the bees, could make multiple visits and accomplish the same end. However, because pollination appears to be limiting fruit production in *Pinckneya*, the probability that a flower will receive a large number of pollinations seems unlikely.

The pollen grain to seed ratio is unknown for *Pinckneya*, but is known for some other plants such as Pavonia, *Pavonia dasypetala* and Passion Flower, *Passiflora vitifolia*, two primarily hummingbird pollinated species. *Pavonia dasypetala* has been found to average 10.8 pollen grains on the stigma for each seed produced (McDade & Davidar, 1984). Seed set in this species, however, is not pollen limited and it only produces 1-5 seed per fruit. The 10.8 pollen grains per seed undoubtedly represents an excess of pollen because seed production is resource limited. A better comparison may be *Passiflora vitifolia*, a pollen limited species with a many seeded fruit. Snow (1982) reported 1.6 pollen grains per seed in this species. At this rate a hummingbird could supply enough pollen in a single visit to produce a viable fruit but would need to make two visits to produce an average fruit. A bee would need to make about six visits to produce a viable fruit and 20 visits to produce an average fruit. Although this logic may contain unwarranted inferences, the principal of the superiority of hummingbirds as pollinators holds. In light of the fact that *Pinckneya* is pollinator limited, this principal supports hummingbird pollination.

Hummingbirds apparently have a very limited olfactory capability (Grant & Grant, 1968), but insects rely heavily on scent in locating food (Barth, 1991). The flowers of *Pinckneya* have very little scent, a property that is consistent with hummingbird pollination. Other hummingbird consistent floral traits of *Pinckneya* are reddish coloration, deep floral tube, high nectar production, nectar protecting hairs on the inside of the calyx, no landing platform, and exserted anthers and stigma.

*Pinckneya* is likely pollinated primarily by bumblebees and Ruby-throated Hummingbirds. There is evidence that the flowers are adapted to hummingbirds, and
hummingbirds may be necessary as pollinators to explain the levels of seed production found in many populations. Both bumblebees and female Ruby-throated Hummingbirds exhibit traplining behavior which could transfer pollen among distant populations. A territorial male hummingbird could transfer pollen between plants of a population, and occasionally between populations. These species of pollinators support the outcrossing mating system very well.

DISPERsal

The single best indicator of fall velocity in Pinckneya is wingloading, even though it explains less of the velocity variation in this species than in many others (Augspurger, 1986). Wing area is nearly as good an indicator as wing loading, and the related measures of length and width are also significantly correlated with fall velocity. Mass, which is 81% correlated with wingloading is an insignificant variable. These significant variables, with the exception of wingloading, are measures of diaspore size, independent of mass (Fig 15). Fall speed varies inversely with size. Wingloading is an inverse indicator of size and a direct indicator of mass, so as wingloading increases, fall velocity increases (Fig. 16).

PC1, which explains about 48% of the morphological variation of the seeds, is also a measure of size. As PC1 increases, fall velocity decreases. This consistent decrease in fall velocity with increasing size is an indication that wing area increases faster than mass as seed grow larger. This relationship is counterintuitive because volume and mass increase much faster than area when an object of constant proportions is made larger (Green & Johnson, 1993). Larger seeds, therefore, are proportionally different from smaller seeds. A greater part of the mass of the larger seeds must be devoted to wing than is normal for the smaller seeds. Because this changing
proportionality is contrary to the laws of nature, it does not happen automatically and must be the result of selective pressures.

Seed size is normally seen as a compromise between the demands of dispersal, which favors smaller seed, and nutrient supply for the seedling, which favors larger seed (Green & Johnson, 1993). By altering the wing/embryo ratio of larger fruits, Pinckneya has turned conventional wisdom upside down. The larger seed fall slower and can therefore be dispersed farther, while at the same time they supply more resources to the seedling. What limits this system and prevents the seed from growing ever larger? Although larger seed may be superior to smaller seed, each size increase is more expensive than the one before it. The increasing cost of these larger seed must be balanced against the smaller number that can be produced from the allocated resources. A very habitat specific species such as Pinckneya can not afford to put too many eggs in too few baskets, so to speak.

Embryo longitude was the third most significant variable. The location of the embryo along the lateral axis had no effect on the fall velocity of the seed but the embryo location along the longest axis accounted for 29% of the variation in fall velocity. Seed with centered embryos fell slower than seed with acentric embryos (Fig. 17). The location of the embryo greatly affects the balance and center of gravity of a seed. This balance presumably affects the fall pattern of the seed. I found no relationship between fall pattern and embryo location but the differences may have been too subtle for me to notice or of a type that I did not recognize. Fall pattern is considered important, none the less, in determining fall velocity (Augspurger, 1986) and, in Pinckneya, is the most probable explanation for the effect that embryo location has on fall velocity.

The mean fall velocity of 1.13 meters per second (m/s) is well within the range of velocities described for winged diaspores (Green, 1980; Augspurger, 1986; Matlack, 1987). The 15,785 seeds produced by the average plant are located between 1 and 3.5
meters above the ground. An even distribution gives an average height of 2.25 meters. A seed falling at 1.13 m/s over a distance of 2.25 meters is airborne for about 2 seconds. The mean wind speed for the coastal plain of Georgia is 2.7 m/s. An average seed released in an average wind would disperse 5.4 meters from the parent plant before contacting the ground. If this seed were released in the maximum normal wind of 34.5 m/s, it would travel 69 meters. A slow falling seed (0.8 m/s) released from the top of a 7 meter tall plant into this same wind would travel 302 meters. This represents the maximum dispersal distance that can be reasonably expected with these assumptions.

Actual dispersal distances are affected by a number of factors such as turbulence, wind speeds that are not constant, and the wind speed necessary to cause the seed to be released. It is possible that fall velocity is different in wind than in a still air column. Also, no estimate can be made of the effects of storms, thermal updrafts and other similar events. The environment of each location is different from that of every other location so generalities are dangerous to make. Environmental factors such as the surrounding vegetation play an important role in the final location of the dispersed seed. For example, seed which are blown toward the dense forest are likely stopped at the edge.

The thin dispersal of *Pinckneya* populations across the landscape, usually at intervals of several kilometers, is empirical evidence that dispersal occurs over greater distances than 300 meters. Many authors have recognized that greater dispersal distances occur than can be accounted for in the prevailing model. This distant dispersal is often considered an unusual occurrence. Some authors have suggested that it may not be so unusual (Matlack, 1992) but, so far, no one has presented an alternative (to the prevailing dispersal model) which accounts for this phenomenon. Unfortunately, I am not able to offer an alternative either, but I believe the current model is inadequate. There is much more involved in dispersal than simply time of flight, as a function of fall velocity in still
air, and mean horizontal wind speed. The forces involved are perhaps chaotic, and may be impossible to adequately model.

GERMINATION

*Pinckneya* seed obviously have a positive photoblastic response (Fig. 5). Germination does not occur after a few hours exposure to light (Fig. 8). Three 12 hour photoperiods are necessary before a meaningful amount of germination occurs (Fig. 6). These results are to be expected if the seeds are responding to qualitative changes in the light environment and not simply responding to the sudden presence of light, as in the uncovering of a seed. An example of a qualitative change is the difference in the illumination of a large light gap and a small sun fleck. In this example, I would expect the *Pinckneya* seed to germinate more readily in the large gap than in the small sun fleck.

In the monochromatic treatments red light permitted germination and far red light did not permit germination. The red and white light treatments were not significantly different in germination percentage indicating that the red wavelengths are a part of the light spectrum that overcome dormancy. The far red treatment was not significantly different from the dark treatment. These results only prove that the far red wavelengths are not among those that induce germination, but they do not prove that these wavelengths have any inhibitory effect on germination.

When red light followed far red light, the red overcame any effects of the far red and induced germination. In this instance, however, the germination percentage was significantly lower than that for the full photoperiod of red light, indicating that more than six hours of the red wavelengths per day are needed for full germination (Fig. 10).

When far red light followed red light, germination was inhibited. This demonstrated conclusively that the far red wavelengths actively inhibit germination. The red - far red treatment and the far red - red treatment were both exposed to the same
amount of each color light, but the percent germination was significantly different between the treatments. Regardless of color, the second exposure overcame the effects of the first exposure and determined the outcome of the experiment. All of these results are consistent with the phytochrome dormancy mechanism.

Light dependent dormancy has important ecological consequences. In the competition for resources, a size advantage, even if relatively small, can have profound effects on the outcome of the competition. Larger individuals generally out compete smaller individuals regardless of species (Fenner, 1987). Because a landscape is usually filled with large, established individuals, a seedling is presented with a great disadvantage. Established individuals are hard to displace so disturbances are often necessary for seedlings to develop (Harper, 1977). Light controlled germination has been related to establishment in canopy gaps. Seedlings in canopy gaps receive a greater amount of radiant energy and hence have higher growth rates and are more likely to survive by out competing slower growing individuals (Vazquez-Yanes et al., 1990). Because the R:FR ratio of the ambient light in a large gap changes with the angle subtended by the sun, a seed may be able to determine the size of a gap and the position within a gap through the phytochrome mechanism (Vazquez-Yanes & Smith, 1982).

When we consider the mosaic of various intensities and colors of light that a seed may be exposed to along with the ability of the phytochrome system to discriminate between particular light environments, we begin to appreciate the potential of a seed to selectively germinate where it encounters conditions favorable for establishment. The seed of many species fail to germinate at the soil surface when shaded by other plants. This ensures that seedlings are not produced under conditions where the photosynthetic rate would be inadequate for growth. (Vazquez-Yanes & Orozco-Segovia, 1982; Vazquez-Yanes & Smith, 1982; Bliss & Smith, 1985; Silvertown & Smith, 1989;
Vazquez-Yanes & Orozco-Segovia, 1989) This discrimination can occur either spatially or temporally.

Germination temperature is an ecologically important variable. Germination was significantly greater in Pinckneya at 30°C and 20/30°C than at 20°C and 10°C. Other investigators have reported increased germination of wetland species at higher temperatures. Barnyard grass, *Echinochloa civis-galli*, germinated best at 30°C (Brod, 1968). The highest mean germination for seven wetland species occurred in a 20/30°C regime (Galinato & van der Vaulk, 1986), and Blue flag, *Iris virginica*, only achieved high germination rates under a 20/30°C regime (Morgan, 1990). Baskin & Baskin (1988) reported similar results for four other wetland species.

The 20/30°C thermoperiod would be common at the surface of black exposed organic substrate in spring but probably would not be common during the winter, at least not with the 7 to 10 day duration that is needed for germination. This could provide a seasonal constraint on germination when other conditions are met (van der Vaulk & Davis, 1978).

Seed of many wetland species only germinate on mud flats that are devoid of vegetation and litter (Kaldec, 1962; Harris & Marshall, 1963; van der Vaulk & Davis, 1978). Van der Vaulk (1986) found that the removal of litter in an experimental marsh significantly increased seedling recruitment for all species tested. Field studies suggest that the effects of litter on seed germination are strictly physical (Morgan, 1990). An important physical factor regulating germination of wetland species under litter or plant canopy is the lower surface temperature relative to those found at exposed sites, which significantly limit germination (Galinato & van der Vaulk, 1986).

Germination at 20°C was significantly better in April than in the previous December. Perhaps some form of after ripening takes place that allows germination at a lower temperature; I only offer this as a possible explanation.
After the second germination experiment was concluded, seeds were moved from 10°C dark to 30°C dark with an exposure to light of less than one minute. These seeds were serendipitously found to have a 28% mean germination rate. This rate was significantly greater than anything seen before in the dark and the search for an explanation led to the forth temperature experiment. In this experiment, seeds that were moved from 10°C to 30°C, always in the dark, germinated significantly better than seeds maintained constantly at either temperature (Fig. 13). This phenomenon seems similar to the process of inverse dark reversion and may be attributable to that process. Regardless of the cause, temperature fluctuations appear to overcome the dormancy mechanism and induce dark germination.

Cooler temperatures are required for IDR (Berrie, 1966). In the experiment that placed seed from a 30°C growth chamber briefly in a 40°C or 50°C incubator, the temperature increase did not induce germination. This could mean that a cool temperature is a necessary ingredient in the dark germination of Pinckneya. Further research is needed.

IDR, or whatever causes this temperature fluctuation inducement of germination, may be behind the surprisingly high germination in the full shade in the field experiment (Fig. 11). Temperatures in the shade ranged from 18°C to 38°C compared to 10°C to 30°C in temperature experiment 4, a 20°C differential in both cases, but 8°C higher in the field. The germination percentages in these two cases were not significantly different. In both instances, germination was lower than under optimum conditions.

In the field experiment the temperatures that the seeds in the full and partial sun were exposed to were hotter than temperatures which prevent germination in phytochrome systems, after prolonged exposure (Scheibe & Lang, 1969). This is my best explanation of the low germination rates of these treatments.
Although I was unable to find germinating seed in the wild, these results indicate that the natural germination period is earlier in the year than the May - June period of the field experiment. Some seed that remain dormant until summer may germinate in the shade and succumb to the competition. Presumably, the seed in the sun would have germinated earlier, and thus the high temperatures of summer would have been of no consequence.

The presence or absence of the seed coat or whether or not it was rinsed had no effect on the germination of *Pinckneya* (Fig. 14). The apparent unimportance of the seed coat to germination is consistent with a phytochrome dormancy mechanism because the phytochrome that is involved in dormancy is located in the embryo axis of the seed.

The most important environmental factors controlling seed germination are temperature, light and soil moisture. Baskin and Baskin (1988) propose that temperature is frequently the overriding factor in a temperate mesic climate. They found that seed of species common to mud flats (sunny wet areas similar to those inhabited by *Pinckneya*) germinate at higher temperatures and often have a light requirement. Higher temperatures often correspond with seasonally falling water levels which may expose soil for seedling establishment. Thus, in a variable habitat, germination should occur in an open area when water levels recede, light is not limiting, and temperatures increase indicating the beginning of the summer growing season. These conditions maximize the chances of successful establishment.

Temperature undoubtedly has the potential to be the overriding factor in *Pinckneya*. A preponderance of red, rather than far red, wavelengths in the ambient light spectrum, however, is necessary for germination to reach full potential. Adequate soil moisture should not be a problem in the habitats where *Pinckneya* is normally found, but it may be a limiting factor in less suitable habitats. In general, the temperature and light
requirements of this species are well suited to the exploitation of the specific habitat in which it is normally found.

**SUMMARY**

*Pinckneya* is adapted to sunny wet areas that occur in certain disturbed gaps in the forest canopy. The gaps must be in wet areas such as branches, branch heads, bays, bogs or other similar areas. This species is not an emergent plant so soil must be above the water level. Its seed germination requirements are well suited to colonizing this type of habitat. Wind dispersal is also favorable to seeding forest gaps. Seeds that are dispersed by the wind fall into forest gaps at a greater rate than they fall into wooded sites (Augspurger & Franson, 1988). The proportion of seeds falling into gaps is greater than would be expected from the land area in the gaps.

Disturbance acts to reset the successional clock of an ecosystem without influencing the potential biomass supportable on that site. For a species that is dependent upon gaps, new sites must be colonized at a rate that is at least equal to the rate at which they are competitively excluded from old sites.

As a tree increases in size its efficiency at transporting water, nutrients, and photosynthate decreases. The roots must support a proportionally greater above ground biomass, and the photosynthetic tissue must support a proportionally greater mass of nonphotosynthetic tissue. These factors plus the tendency of trees to develop larger and more massive crowns which make them more "top heavy", cause them to be increasingly susceptible to smaller and more frequent disturbances. Because of these factors, forest disturbance rates are constrained to a fairly small range of potential values. In temperate deciduous forests dominate trees usually live to a maximum age of 300 to 500 years (Pickett & White, 1985). The rate of forest disturbance shows little variation, most
forests have an average disturbance rate of 1% per year. This means that any particular site is disturbed on average once every century.

If this disturbance rate is accurate for the range of Pinckneya, it is feasible that suitable sites would be available within the seed dispersal distance and life span of most individuals. Of course, these conditions are historical, the range of Pinckneya has been so modified that the species now primarily occurs in areas of human disturbance. Because of the increasing frequency of disturbances, such as logging, road building, agriculture, etc., Pinckneya may benefit from the more frequently altered landscape.

Species, such as Pinckneya that have a self incompatible mating system are at a disadvantage in forming breeding populations from a single seed. Traplining pollinators probably compensate for this. No seed is likely to establish a plant that is so disjunct as to be out of the range of pollen carried by the pollinators which it as adapted to attract. This combination assures outcrossing, along with its benefits, in a species that has populations widely scattered across the landscape.

Here we see a dispersal system and germination system that work together to establish plants in a habitat for which it is adapted. The mating system is one which maintains outcrossing between twidely separated populations and habitats. All three systems - mating, dispersal, and germination - compliment one another and contribute to the success of Pinckneya in exploiting its ecological niche.
LITERATURE CITED


Michaux, A. 1803. Flora Boreali-Americana Paris


Ridley, N. H. 1930. The Dispersal of Plants Throughout the World Ashford, Kent, L. Reeve and Company Ltd.


Table 1. Plants found growing with *Pinckneya bracteata*. Partially from Mellinger (1966).

<table>
<thead>
<tr>
<th></th>
<th>Genus</th>
<th>specific epithet</th>
<th>authority</th>
<th>common name</th>
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<td>(Walt.) Muhl.</td>
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<td>Walt.</td>
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<td>----------------</td>
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Table 2. Longitude and latitude of the *Pinckneya bracteata* populations that provided the seed for this study. Location 4 was the population used for the germination experiments.

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<th>Latitude</th>
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<td>82° 07.23 W</td>
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<td>6</td>
<td>32° 33.62 N</td>
<td>82° 04.63 W</td>
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<td>8</td>
<td>32° 13.86 N</td>
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<td>9</td>
<td>32° 12.62 N</td>
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<td>81° 46.32 W</td>
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<tr>
<td>12</td>
<td>32° 10' ?? N</td>
<td>82° 01' ?? W</td>
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</table>
Table 3. Variables tested for effect on fall velocity of seeds. Those in bold type have a significant effect at a 0.05 level of significance (ANOVA).

<table>
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<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard Error</th>
<th>$r^2$</th>
<th>ANOVA p value</th>
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<td>1.11</td>
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<td>$&lt;0.001$</td>
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<td>5.5 mm</td>
<td>0.10</td>
<td>0.14</td>
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<td>short axis</td>
<td>4.5 mm</td>
<td>0.09</td>
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<td>n/a</td>
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<td>n/a</td>
<td>n/a</td>
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Table 4. Results of the principal component (PC) analysis on morphological variation of *Pinckneya bracteata* seed.

<table>
<thead>
<tr>
<th></th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing Area</td>
<td>0.51852</td>
<td>0.07907</td>
<td>0.04891</td>
</tr>
<tr>
<td>Length</td>
<td>0.45992</td>
<td>0.19469</td>
<td>0.17473</td>
</tr>
<tr>
<td>Width</td>
<td>0.43832</td>
<td>0.03132</td>
<td>-0.29193</td>
</tr>
<tr>
<td>Short Axis</td>
<td>0.38794</td>
<td>-0.39133</td>
<td>-0.11768</td>
</tr>
<tr>
<td>Longitude</td>
<td>0.36206</td>
<td>-0.30949</td>
<td>0.49671</td>
</tr>
<tr>
<td>Latitude</td>
<td>-0.00016</td>
<td>0.62759</td>
<td>0.59050</td>
</tr>
<tr>
<td>Mass</td>
<td>0.21425</td>
<td>0.55863</td>
<td>-0.52210</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigen value</td>
<td>3.3482</td>
<td>1.5252</td>
<td>0.8141</td>
</tr>
<tr>
<td>% variation</td>
<td>47.83</td>
<td>21.79</td>
<td>11.63</td>
</tr>
<tr>
<td>Cumulative</td>
<td>47.83</td>
<td>69.62</td>
<td>81.25</td>
</tr>
</tbody>
</table>
Table 5. Correlation of principal components (PC) and seed fall velocity in *Pinckneya bracteata*

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r^2$</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>0.313</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PC2</td>
<td>0.050</td>
<td>0.123</td>
</tr>
<tr>
<td>PC3</td>
<td>0.161</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Table 6. Floral Characteristics of *Pinckneya bracteata* that are common among outcrossing species.

<table>
<thead>
<tr>
<th>Characteristics of Outcrossers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcrossing</td>
</tr>
<tr>
<td>Self incompatible</td>
</tr>
<tr>
<td>Many flowers</td>
</tr>
<tr>
<td>Long pedicels</td>
</tr>
<tr>
<td>Large sepals</td>
</tr>
<tr>
<td>Nectaries present</td>
</tr>
<tr>
<td>Long stamens</td>
</tr>
<tr>
<td>Extrace anther dehiscence</td>
</tr>
<tr>
<td>Anthers distant from stigma</td>
</tr>
<tr>
<td>Many pollen grains</td>
</tr>
<tr>
<td>Pollen presented</td>
</tr>
<tr>
<td>Long pistil</td>
</tr>
<tr>
<td>Stamens and pistil of different length</td>
</tr>
<tr>
<td>Exserted style</td>
</tr>
<tr>
<td>Well defined, papillate stigmatic area</td>
</tr>
<tr>
<td>Asynchronous stigma receptivity and anther dehiscence</td>
</tr>
<tr>
<td>Many ovules per flower</td>
</tr>
<tr>
<td>Many ovules not maturing to seed</td>
</tr>
<tr>
<td>Some fruits not maturing</td>
</tr>
<tr>
<td>Narrow distribution</td>
</tr>
</tbody>
</table>
Figure 1. Sketch of *Pinckneya bracteata* inflorescence from Michaux (1803)
Figure 2. Schematic diagram of phytochrome system. Adapted from Rollin (1972). Red light converts P(red) to P(far red) and far red light converts P(far red) to P(red). Under the proper conditions, the reaction can move in either direction in the dark.
Figure 3. Fruit widths one month after six pollination treatments in *Pinckneya bracteata*. Outcrossed treatments had significantly larger fruit than self-pollinated treatments (ANOVA, p<0.05), control fruit were intermediate in size and significantly different from the other two groups. Error bars indicate standard error.
Figure 4. Number of fruit per inflorescence larger than 7 mm in width one month after flowering in *Pinckneya bracteata*. Bagged inflorescences were bagged prior to flowering, control inflorescences were not modified. Control inflorescences had significantly more large fruit (ANOVA, p<0.05). Error bars indicate standard error.
Figure 5. Germination percentages of *Pinckneya bracteata* seed in total darkness and 12 hours of light per day, after 28 days. Seeds exposed to light germinated significantly better than seeds in constant darkness (p<0.05). Error bars indicate standard error.
Figure 6. Germination percentages of *Pinckneya bracteata* seed after exposure to 0 to 5 12 hour photoperiods of white light. All seed were placed in darkness after the indicated number of photoperiods was reached. Germination was measured 28 days after the start of the experiment, and increased with increasing numbers of photoperiods. Error bars indicate standard error.
Figure 7. Linear relationship between number of 12 hour photoperiods and germination percentage in *Pinckneya bracteata* ($r^2 = 0.716$). Germination increased with increasing numbers of photoperiods.
Figure 8. Germination percentages of *Pinckneya bracteata* seed after exposure to 0 to 48 hours of constant white light. All seed were placed in darkness after the indicated number of hours was reached. Germination was measured 28 days after the start of the experiment and increased with increasing length of time exposed to light. Error bars indicate standard error.
Figure 9. Linear relationship between number of hours of constant light and germination percentage in *Pinckneya bracteata* ($r^2 = 0.648$). Germination increases with increasing exposure to light.
Figure 10. Germination percentages of *Pinckneya bracteata* seeds at 28 days after exposure to 7 different light regimes. The red and far red treatments were constant light colors for 12 hours each 24 hours. In the red - far red treatment, 6 hours of red light was followed by 6 hours of far red light and then 12 hours of darkness. Likewise, in the far red - red treatment, 6 hours of far red was followed by 6 hours of red and then by 12 hours of darkness. The dark treatment was never illuminated. The red and white treatments germinated significantly more seed than the other treatments (p<0.05). The red - far red treatment was significantly different from and intermediate to the other treatments (p<0.05). Error bars indicate standard error.
Figure 11. Germination percentages of *Pinckneya bracteata* seeds in the full sun, full shade, and partial shade with southern and northern exposures during June, 1996. Germination in the full shade was significantly higher than in the other treatments (p<0.05). Error bars indicate standard error.
Figure 12. Germination percentages of *Pinckneya bracteata* seed at 4 temperature treatments and two dates. The 20/30 treatment was kept at 30°C during the day and 20°C at night. Higher temperatures germinated significantly better than lower temperatures (p<0.05). Error bars indicate standard error.
Figure 13. Germination percentage of *Pinckneya bracteata* seeds in 4 different treatments. Treatments 10D and 30D were maintained at 10°C and 30°C respectively for 56 days, 10-30D and 10-30E were kept at 10°C for 28 days and then at 30°C for 28 days. Treatment 10-30E was exposed to 2 minutes of sunlight at the time of the temperature change, the other treatments remained in darkness throughout. Those seed that changed temperature germinated significantly better than those that remained at a constant temperature (p<0.05). There was no significant difference between treatments within the two groups. Error bars indicate standard error.
Figure 14. Germination percentages of *Pinckneya bracteata* seeds in 4 treatments. The treatments were: rinsed and the seed coat removed, seed coat removed only, rinsed only, untreated. There was no significant difference between the treatments (p<0.05). Error bars indicate standard error.
Figure 15. Fall velocity in *Pinckneya brecteata* seeds has a linear relationship with wing area ($r^2 = 0.32$). Fall velocity decreases as wing area increases.
Figure 16. Fall velocity in *Pinckneya brackeata* seeds has a linear relationship with wingloading ($r^2 = 0.33$). Fall velocity increases with wingloading.
Figure 17. Fall velocity in *Pinckneya bracteata* seeds has a linear relationship with embryo longitude ($r^2 = 0.29$). Higher values for embryo longitude indicate a more central location of the embryo on the longest axis of the seed. A value of 0.50 is perfectly centered. Fall velocity decreases as embryo longitude values increase, or as the embryo becomes more centered.