REPRODUCTIVE BIOLOGY OF RELICT TRILLIUM

(*Trillium reliquum*)

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(*Trillium reliquum*)

Melissa Gwynne Brooks Waddell

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(*Trillium reliquum*)

Melissa Gwynne Brooks Waddell

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Date of Graduation

VITA

Melissa Gwynne (Brooks) Waddell, daughter of Robert and Elaine Brooks, graduated from the University of North Alabama in 1996 with a bachelor's degree in Geography and a minor in Biology. She graduated from Auburn University in 1998, in Horticulture and Landscape Design, and returned to Auburn University to pursue a master's of science in 1999. Married in May 2004 to Erik Waddell, she accepted a position teaching seventh grade science and environmental science in December 2005. In July 2006, she begins a master's degree in Education at the University of North Alabama.

THESIS ABSTRACT

REPRODUCTIVE BIOLOGY OF RELICT TRILLIUM

(*TRILLIUM RELIQUUM*)

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Master of Science, August 7, 2006
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Pollination and seed dispersal were studied in six populations of the endangered plant species *Trillium reliquum* in Bullock and Lee counties in Alabama and in Muscogee County, Georgia. Few populations of this species exist and most are relatively small and isolated.

Pollination was studied through field observations of floral visitors, microscopic examination of insects captured on flowers, sticky trap capture of potential floral visitors, fluorescence microscopy of styles for the examination of pollen tubes, and pollen manipulation experiments. In pollen manipulation experiments, plants were covered by insect exclusion bags constructed of no-see-um netting (0.3 mm mesh) and treated as self-pollination (no pollen transfer), hand-pollination single application, hand-pollination multiple application, and open to pollination naturally. Carrion insects including fly and beetle families dominated floral visitors. A carrion fly pollination syndrome was indicated.   In the pollen manipulation study, no visible bag effects were detected. No self-pollinated plants formed fruits, indicating self-incompatibility in spite of pollen tube formation in self-pollinated plants. Seed numbers were significantly higher (though inconsistently) in open pollination treatments than in hand-pollination treatments in the larger populations, indicating a high level of pollinator attraction. Seed numbers in open pollination treatments were consistently lower than or not significantly different from hand-pollination treatments in smaller populations, suggesting that small populations fail to attract pollinators sufficiently. Reproductive rates, as judged by %-developed seeds, were low in all populations.

Seed dispersal was studied through field observations of fruit visitors, and through collection of potential seed dispersers on sticky trap platforms supplied with fruit and/or seeds with and without elaiosomes. Ants of several species dominated collections and the larger forms were judged to be effective seed dispersers. Seed dispersal by *Vespula* wasps was also observed. This is the first report of vespicochory in *T. reliquum*.

Suggestions for preservation of this species include protection of a number of populations in order to preserve genetic diversity and maintenance of populations at sufficient size to attract pollinators.

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INTRODUCTION

The genus *Trillium* is diverse and broadly ranging, spanning two continents and three distinct regions including eastern Asia (six species), western North America (seven species) and eastern North America (35 species) (Case and Case 1997). Plants produce a single scape (stem) with a whorl of three bracts (leaves). Of the 48 species in the genus, 24 are sessile (flowers lack a pedicel) (subgenus *Phyllantherum*) and 11 species are pedicellate (subgenus *Trillium*). The presence of a scape above the leaves (pedicel) is considered the more primitive form of the genus (Case and Case 1997). Pedicellate species are found throughout the range of the genus on both continents but those with sessile flowers are found only in North America (Freeman 1975). Within North America, sessile trilliums are found predominantly in the central lowlands, Mississippi Basin and southern piedmont of the United States. *Trillium reliquum* has one of the most restricted ranges of the sessile species (Case and Case 1997).

In 1975, Freeman named and described *T. reliquum* as a new species (Freeman 1975). The species had been previously identified as *T. sessile* until Freeman described it as a distinct species after examining over 10,000 herbarium sheets and personal specimens. He coined the epithet *reliquum* (meaning relict) to reflect its geographical distribution, which he felt was a remnant of a larger range in a former geological period (Freeman 1992). The species was initially thought to exist only on four sites in Georgia and South Carolina, but it is now known to exist in Alabama as well.

The type collection was taken five miles north of Augusta, Georgia along the Savannah River in Richmond County on March 30, 1968 (Freeman 1985). Freeman also named a yellow flowered form (*Trillium reliquum* forma *luteum*) from a specimen collected in Clay County, Georgia (Freeman 1975). *Trillium reliquum* forma *luteum* coexists with the red flowered form of the species but only at certain sites in Georgia (Case and Case 1997). The species is currently known to exist at sites in Bullock, Henry and Lee counties in Alabama, in Clay, Columbia, Early, Lee, Macon, Muscogee and Talbot counties in Georgia, and in Aiken and Edgefield counties in South Carolina (United States Fish and Wildlife Service 1998).

Trillium reliquum is distinct from the rest of subgenus *Phyllantherum* by its decumbent scape, a characteristic found only in two species, *T. decumbens* and *T. reliquum*. The two species can be separated by several morphological characteristics and by their geographic ranges (Freeman 1975). *Trillium decumbens* is found in northern Alabama and northern Georgia, while *T. reliquum* is found along the Savannah River in Georgia and South Carolina and along the southern Alabama-Georgia border. *Trillium reliquum* lacks the pubescent scape found in *T. decumbens*. Specimens of *T. reliquum* are often misidentified as *T. underwoodii*, with which its range overlaps. Both species exhibit a similar color pattern on the bracts but can be distinguished by scape structure and by floral characteristics. The scape of *T. reliquum* is semi-decumbent to decumbent and forms an S-curve whereas *T. underwoodii* is upright and not curved. Introrse anther sacs (Figure 1A) occur in *T. reliquum* (Freeman 1975) as opposed to the latrorse anthers in *T. underwoodii*.

Trillium reliquum plants go through four distinct developmental stages including seedling, one-leaf juvenile, three-leaf juvenile, and flowering adult (Figure 2). A tan colored monopodial rhizome characterizes mature plants, which is cone-shaped in young plants and cylindrical in mature plants. The rhizome ranges from 3.0 to 4.0 cm in length and 1.5 to 2.0 cm in diameter. As plants mature, rhizomes begin to decay at the older end. There are typically few adventitious roots (Freeman 1985). Freeman (1985) described two to four adventitious roots near the rhizome apex; however in personal observations I have noted a few larger specimens with five adventitious roots.

Freeman (1985) noted that the sheathing bracts of each aerial shoot leave an encircling scar the following year. One scape is the norm for the species but the presence of two scapes is not uncommon. The scape is glabrous and semi-decumbent to decumbent forming an S-curve ranging from 5.5 to 24.5 cm in length and 5 to 8 mm in diameter. The scape length is 1.0 to 2.8 times the bract length (Freeman 1975). There are three bracts per scape with four occurring very rarely (personal observation). The bracts are sessile and whorled. The elliptical, elliptical-ovate, or broadly ovate bracts range from 5 to 13 cm in length. Bracts are distinctly mottled with shades of green and purple on the lamina and silvery white to pale green on the midrib (Figure 2C).

The purple flowers are solitary and sessile, bearing whorls of three sepals and three petals (Figure 2D). Flowers open between mid-March and mid-April (Figure 2). A distinct fetid odor is present upon opening and continues through flower senescence. The pistil and stamens are purple in color near the apices (Figure 1A), with the exception that *T. reliquum* forma *luteum* has yellow floral parts (Freeman 1975). Sepals are narrow and ovate reaching 18 to 52 mm in length. The sepals unfurl as the stigma becomes receptive, become recurved after pollination and finally enclose the fruit during development. Three elliptical to obovate petals are green to purple in color, range from 22 to 60 mm in length and are often twisted at the apices (Figure 2D). Six stamens are divided into two whorls of three. They are erect, incurved, and overlapping and range from 11 to 29 mm in length. The filaments are short (1 to 4 mm in length) and purple (Figure 1A). Anthers are introrse (opening inward) (Figure 1A), with creamy white anther sacs located on purple linear connectives. Connectives extend beyond the anther sacs in acute points. The three carpels are less than half the length of the stamens. The ovoid ovary is six-winged and superior, ranging from 4 to 11 mm in length. Stigmas are linear and short, from 1.5 to 4.5 mm in length, with recurved tips. The fruit is a berry-like capsule (Freeman 1985), which is ovoid, purple-green in color and approximately 2 cm long (Figure 3B). Fruits contain between 15 and 20 seeds. Seeds are ellipsoid and tan in color with a white funicular elaiosome or aril (Figure 4B), which is usually as large as the seed (Freeman 1985).

The species was federally listed in 1988. Although a few populations have been discovered since its listing, most known populations are small, isolated and demonstrate a low reproductive rate (Currie 1991). *Trillium reliquum* and *T. persistens* are the only two members of the genus on the Federal Endangered Species List and both occur in the southeastern United States (United States Fish and Wildlife Service 1998). *Trillium persistens* is found in a very restricted range along the South Carolina-Georgia border. *Trillium reliquum* occurs in mesic deciduous woodland forests of Alabama, Georgia, and

South Carolina and is dependent on mature, undisturbed forest habitats with soils ranging from rocky clays to alluvial sands (Freeman 1985). Populations are threatened by encroaching development, invasive introduced plant species and slow reproductive rates (Currie 1991). *Trillium reliquum* and other *Trillium* species have long life-spans (10 to 20 years in many and some over 40 years) with slow maturation to flowering adult (Case and Case 1997, Ohara and Kawano 2006). Long development time and slow growth magnify conservation concerns for *Trillium* species. Factors influencing pollination, seed set and seed dispersal are important to an understanding of population dynamics in *Trillium* species. Only with a thorough knowledge of reproductive biology can we hope to improve the status of declining species. Ohara et al. (2006), in an analysis of studies on *Trillium camschatcense*, stressed the importance of basic life history data to plant conservation.

Few studies of the biology of *T. reliquum* have been conducted (Folkerts et al. 1987, Boyd and Freeman 1991, Currie 1991). Various flies were observed as flower visitors by Folkerts et al. (1987). Myrmecochorous seed dispersal of *T. reliquum* was observed by Diamond (pers. comm. Diamond, A., Biologist. Troy State University, Troy, AL), but not studied in detail. Currie (1991) reported that, in addition to habitat destruction and fire, herbivory by deer is a limiting factor in plant survival and reproduction. Boyd and Freeman (1991) found that only 1 to 2% of unprotected plants survived to seed set due to heavy grazing pressure at a site in Bullock County, AL. Recent genetic studies of *T. reliquum* indicate low genetic diversity within populations and little gene flow among populations (Gonzales and Hamrick 2005). Gonzales and Hamrick (2005) speculated that isolation of populations may be an ancient condition for this species.

Pollination biology of other members of the genus *Trillium* has been relatively poorly studied (Sage et al. 2001, Steven 2003) and much of what is known has been determined through studies of Asian species (Ohara and Kawano 1987, 2005, 2006). *Trillium* vary in color, scent, and pollinators. Little is known about self-pollination. Sage et al. (2001) found that cross-pollination of *T. erectum* and *T. grandiflorum* produced higher numbers of seeds than self-pollination. Self-pollination of *T. erectum* and *T. grandiflorum* was inhibited through the failure of pollen tube growth near the bases of the stigmatic papillae. However, *T. erectum* was capable of some seed production through self-fertilization (Sage et al. 2001). In another study of *T. grandiflorum*, evidence was found for both pollinator limitation in small populations and pollinator competition in large populations (Steven 2003). Several Japanese species have been found to be primarily selfing with little insect pollination taking place (Ohara and Kawano 1987). *Trillium tschonoskii* was considered by Ohara and Kawano (2006) to be an inbreeder, mainly on the basis of pollen to ovule ratios. *Trillium camschatcense,* on the other hand, is primarily pollinated by bumblebees and beetles and was considered to be a conditional outbreeder or occasional inbreeder (Ohara and Kawano 2005). Recent genetic studies have been initiated for a few *Trillium* species (Gonzales and Hamrick 2005, Li et al. 2005).

Trillium reliquum is one of several *Trillium* species with deep red or purple flowers and a fetid or carrion-like odor, indicating that pollination by carrion-visiting insects is likely (Faegri and van der Pijl 1966). Pollination of carrion odor plants has been studied since the late nineteenth century when Hubbard (in Faegri and van der Pijl 1966) studied the pollination of *Rafflesia*. Knoll (in Faegri and van der Pijl 1966) noted the pollination of *Arum* by saprophilious flies and carrion beetles.

The presence of elaiosomes on *Trillium* seeds indicates probable dispersal by ants (Hymenoptera: Formicidae). Myrmecochores are plants with seeds bearing elaiosomes that have evolved for dispersal by ants. Elaiosomes offer a nutritional reward for ants and possibly other seed dispersers. As the elaiosome is removed, feeding ants may scrape a seed coat, but the seed typically remains unharmed and is discarded into a midden or trash pile in the ant's subterranean nest. In a study of a group of myrmecochorous plants, Beattie and Culver (1981) found that dispersal to the ants' midden provides three very important advantages over non-myrmecochorous plants: 1) diaspores (a reproductive plant part, such as a seed, fruit, or spore, that is modified for dispersal) are protected from predators foraging on the forest floor, 2) germination and seedling emergence are increased in protected and nutrient rich microsites, 3) seeds emerge in protected havens away from the competition of parent plants and non-myrmecochorous species**.** For example, *Trillium grandiflorum* benefits from ant-mediated seed dispersal because of reduced seed predation as well as dispersal of seeds to new sites and therefore increased outcrossing (Kalisz et al. 1999). In a study of mechanisms contributing to reduced recruitment of *Trillium* seedlings in forest edges, Jules (1999) concluded that ants are generally abundant and therefore reduced seed dispersal has not been important in population decline.

Most observations of seed dispersal in *Trillium* have involved species of ants. Ohara and Kawano (2005, 2006) found that both ants and beetles were attracted to elaiosomes of Japanese *Trillium* species but that only ants were effective dispersal agents. Wasps of the genus *Vespula* have also been observed as seed removers in a few *Trillium* species (Jules 1996).

The observation of *Vespula* as seed dispersers was first noted by Jules (1996) in the Pacific Northwest. *Vespula vulgaris* was observed removing seeds and elaiosomes of *Trillium ovatum*. One *Vespula,* feeding on an elaiosome before flight, showed no attempt to feed on the seed. Jules named the dispersal of seeds by *Vespula*, "vespicochory". *Vespula flavopilosa* and *Vespula maculifrons* were observed by Zettler and Spira (2001) dispersing seeds with elaiosomes of *Trillium undulatum*, *Trillium catesbaei*, and *Trillium cuneatum*. *Vespula maculifrons* excavated seeds from intact fruits of *Trillium cuneatum* by chewing through the pericarp. Removed seeds were carried off and discarded by the insects. Elaiosomes were removed from over 95% of the seeds that were recovered by the researchers, with few exhibiting damage to the seed coat. Removal of the elaiosome without seed coat damage suggests that *Vespula* is not a seed predator. Bale et al. (2003) found *Vespula* seized seeds and elaiosomes from ants excavating fruits of *Trillium discolor*.

In several studies, *Trillium* seedlings and juvenile plants were found in reduced numbers in forest fragments and along forest edges and the decline was attributed to increased seed predation, herbivory by deer and microclimate change (Ohara and Kawano1986, Augustine and Frelich 1998, Jules 1998, Tomimatsu and Ohara 2004). The few observations of *Trillium* seed predation recorded, mainly involved rodents removing seeds from experimental platforms (Jules 1999). In that study, it was observed that the number of rodents near edge populations was seven times that of populations in the forest interior (Jules 1999). Jules (1998) found that wind was the most important of abiotic changes affecting edge populations, but that soil moisture, temperature, radiation fluctuations and water fluctuations also greatly affect edge populations and populations in small forest fragments.

Herbivory adds significant pressure on reproductive rates and compounds the threat in declining *Trillium* populations. Deer preference for *Trillium* compared to other herbaceous perennials has been observed in several studies (Anderson 1994, Balgooyen and Waller 1995, Augustine and Frelich 1998, Kirschbaum and Anacker 2005, Webster et al. 2005). Kirschbaum and Anacker (2005) proposed that *Trillium* be used as phyto-indicators of deer impact. Herbivory on *Trillium* is particularly destructive due to the plant's inability to spread clonally. Deer herbivory can seriously damage an already genetically stressed population by decreasing the number of plants setting seed. Augustine and Frelich (1998) found deer herbivory of *T. sessile* reached 67% compared to more common herbaceous perennials that suffered less than 50% predation. Caged plants were compared to uncaged *Trillium* in an old growth forest. Protected plants were significantly larger than uncaged plants the second year of caging. Uncaged plants were 28% smaller than caged plants. Caged plants also flowered at a rate 19 times greater than uncaged plants (Augustine and Frelich 1998). Reproductive plants, generally taller and larger, suffered significantly higher herbivory than juvenile plants. Lepidoptera also damaged plants though defoliation and scape destruction (Augustine and Frelich 1998). Consistent grazing caused significant reduction in reproductive plant size (Anderson 1994).

In this study I provide information on the pollination biology and seed dispersal of *Trillium reliquum* that may contribute to eventual population recovery. One serious concern for this species is that existing populations are small, declining and isolated. These conditions may result in lowered reproductive rates and will be magnified by further habitat loss. Habitat fragmentation and isolation of populations have been shown to disrupt plant-pollinator interactions, and often lead to inbreeding (Steffan-Dewenter and Tscharntke 1999, Li et al. 2005). Pollinator limitation under such conditions has been shown for other *Trillium* species (Jules and Rathcke 1999, Griffin and Barrett 2002). Attraction of pollinators to plant populations has been shown to increase as population size and floral display increases in *Trillium* and other plant genera (Kunin 1992, Cane 2001, Steven et al. 2003). However, it is possible that self-fertilizing plants can avoid the limitations that result from population decline and habitat fragmentation (Jain 1976). On the other hand, obligate out-crossing plants avoid inbreeding and reduction of offspring quality (Jain 1976). The objectives of this study are to: 1) identify the potential pollinators of *Trillium reliquum*; 2) to determine if *Trillium reliquum* is able to self-pollinate; 3) to compare the effectiveness of pollination and seed production in populations of different sizes; and 4) to identify potential seed dispersers. Only with a thorough knowledge of these and other aspects of its reproductive biology can we hope to improve the status of *Trillium reliquum*.

METHODS

STUDY SITES

Research populations were selected in the states of Alabama and Georgia (Table 1). Sites in Bullock and Lee counties in Alabama were selected because of proximity to Auburn, permission of access, and lack of public use. Three sites were selected on Fort Benning, Muscogee County, Georgia. The sites on Fort Benning were selected for safety, size and accessibility. Active artillery and armored division maneuvers on the base prevented the frequent visitations necessary for pollination studies at other populations. The site characteristics are described in detail below.

Bullock A is located on the contours of a northeast-facing slope and is terraced. The area has been clear-cut above and below the *T. reliquum* population. The area below the population consists of a thicket of *Ligustrum sinense* in a low bank along the creek. This is a small population, composed of 300 to 400 plants, and is lacking in large plant clumps. (Small populations were counted and large populations were estimated.) Plants are spaced in small groups across a lower terrace, on the slope and in the upper terrace. The population is spread along the terraces and into a clear-cut area where the plants are sparsely dispersed. *Fagus grandifolia* dominates the upper canopy of the terrace hillsides. *Quercus nigra, Quercus alba, Pinus taeda,* and *Liriodendron tulipifera* comprise the rest of the upper canopy. Exotic species, *Pueraria lobata* and *Lonicera japonica* are encroaching into the population. Artificially planted *Pinus taeda* occurs above the population. As a result of logging few large trees are present. Below the population is a thicket-like growth of *Ligustrum sinense*, which is creeping up the hillside. A thicket of *Aesculus parviflorum* has covered a few *Trillium reliquum* plants. *Podophyllum peltatum*, *Viola* spp., *Toxicodendron radicans, Mitchella repens, Thalictrum thalictroides* and *Claytonia virginica* compose the majority of the herbaceous ground cover. Encroachment by invasive exotic plant species is developing into a serious problem at this site.

Bullock B is located on the east side of a wooded hillside in Bullock County, AL. The site is protected by from the creek by a steep slope. The topography of the site is naturally terraced with an eastern exposure. Four terraces form plateaus at various heights; and the majority of the plants are located on the top two terraces. The population is large, composed of over 1000 plants, and has several large clumps of flowering plants and non-flowering mature plants. Juvenile plants and seedlings make up a minority of the population. Despite large quantities of garbage dumped on the edge of the road near the population, this site is relatively undisturbed, the plants appear to be healthy and the population appears stable. Mature *Fagus grandifolia* dominates the terrace slopes. The canopy contains *Quercus nigra, Liriodendron tulipifera, Pinus glabra* and *Carya pallida*. The lower canopy is composed of *Magnolia grandiflora*, *Carpinus caroliniana*, *Cornus florida* and *Aesculus pavia*. Herbaceous perennials produce a scattered groundcover. *Sanguinaria canadensis*, *Trillium maculatum*, *Arisaema dracontium, Thalictrum thalictroides*, *Mitchella repens, Claytonia virginica*, and *Viola spp*. dominate the forest floor. (Species identifications were made using Radford et al. 1978). Bullock A and

Bullock B were probably a continuous population at one time but the clear-cut area between the sites now is very large and unsuitable for *Trillium* habitat. The remaining plants in the clear-cut area appear stressed and very few juvenile plants were found. Invasive exotic plant species are developing into a serious problem at this site also.

The Lee site is located in Lee County, AL within a residential area of mobile homes. A small stream bisects the site with *Rhapidophyllum histrix* located along the stream. Approximately 99% of the population is located on the north side of the stream with southern exposure. Plants are sparsely distributed across the road on an adjacent lot. A larger population is also located on an adjacent private wildlife preserve, surrounded by a 2.4 m deer enclosure. That area was not accessible during the study. The Lee site has the largest population of *Trillium reliquum* (over a thousand plants) and the highest diversity of *Trillium* species of any of the study sites (five species present). The population exists as relatively equal numbers of mature flowering plants, mature non-flowering plants, juvenile plants and seedlings. This seems by far to be the most stable population and the site with the least invasion of exotic species.

The 0.01 km^2 site has a mixed mesic hardwood overstory with several tree species. *Fagus grandifolia* and *Liriodendron tulipifera* have aspect dominance at the site. The upper canopy is composed of *Fagus grandifolia, Quercus alba, Liriodendron tulipifera, Juglans nigra,* and *Carya.* The lower canopy is composed of *Cornus florida, Halesia caroliniana, Ilex opaca, Acer saccharum* subsp. *floridanum, Morus rubra, Aesculus pavia*, and *Hydrangea quercifolia. Geranium maculatum, Arisaema triphyllum, Trillium catesbaei, Trillium rugellii, Trillium cuneatum, Asarum arifolium, Cynoglossum* *virginianum, Goodyera repens, Viola hastata, Polystichum acrostichoides, Toxicodendron radicans, Parthenocissus quinquefolia,* and *Carex* comprise the understory. This population was chosen for detailed study due to its condition, large size and close proximity to Auburn.

Three sites were located on Fort Benning in Muscogee County, Georgia. The Randall site is adjacent to Randall Creek. This is the smallest population in the study with about 200 to 300 plants and is located in the most interior position on the military base. This population is confined within a small area and may be prone to extirpation due to low population size and isolation. A fire on March 31, 2003 severely damaged the population and left only eight surviving plants, including only one partially scorched mature flowering plant. Data were only collected for one year at this site and were therefore excluded from statistical analysis. However, some conclusions can be made from the limited amount of information gathered at this site.

Muscogee A is located on Baker Creek and is the farthest east of the populations on the base. This population is composed of 600 to 700 plants that are spread across a large area, with the majority inside a fence protecting the plants from feral pig damage. Muscogee A has a terrace above the floodplain although a few plants are located in the floodplain. Plants at this site are more sparsely distributed than at other sites. The floodplain plants are relatively closely spaced with an average of 1.5 to 3.1m between clumps but several of the terrace plants are well over 3.1 m apart. The upper canopy is dominated by *Liriodendrion tulipifera*, *Quercus nigra*, *Fagus grandiflora, Acer rubrum, Acer saccharum* subsp. *floridanum,* and *Carya* spp. The lower canopy is dominated by

Acer saccharum subsp. *floridanum, Ulmus* spp., *Carpinus caroliniana*, and *Cornus florida*. *Lonicera japonica* and *Toxicodendron radicans* dominate the forest floor. *Zephyranthes atamasco, Asarum arifolium*, *Polystichum acrostichoides, Mitchella repens* and *Parthenocissus quinquefolia* are also prominent on the forest floor. The slope is east facing and shaded. Muscogee A was burned during the winter of 2002 while plants were in a dormant condition and the population did not incur major damage. Flowering plants and non-flowering adult plants comprise the majority of the floodplain population and upper terrace population. Another small population purportedly exists upstream but could not be located during this study.

Muscogee B is located on Randall Creek upstream from the Randall site. This is the largest and densest of the Fort Benning study populations with over 1000 plants. The stream divides the population and the smaller group of plants on the east side is threatened by trash dumping and trampling from trespassers. For that reason the eastern portion of the population was not utilized in this study. The banks of the stream are much steeper than at the other sites and plants are located high above the creek. The tree canopy is dominated by *Fagus grandifolia*, *Quercus nigra*, *Liriodendron tulipifera* and *Carya* spp. The lower canopy composed of *Cornus florida*, *Aesculus pavia,* and *Acer saccharinum* subsp. *floridanum*. Groundcover is dominated by *Toxicodendron radicans* and *Parenthenocissus quinquefolia.* Non-flowering mature plants and juvenile plants comprise the majority of the population.

CONTROL OF DEER HERBIVORY

All study sites are potentially under high herbivore pressure by deer. Deer herbivory has been shown to produce extensive loss of flowering and juvenile plants in this and other species of *Trillium* (Augustine and Frelich 1998, Rockwood and Lobstein 1994, Boyd and Freeman 1991). In order to prevent the loss of experimental plants, measures were taken to protect them from deer herbivory. Deer resistant cages were placed over adult plants as they began to emerge and remained in place until fruits were harvested. Cages consisted of approximately 30 cm^3 wire boxes pinned to the ground with landscaping pins and were constructed of galvanized 2.5 cm 20 gauge hardware cloth. The cages were strong enough to withstand a weight of at least 65.8 kg. Large populations were initially protected with 2.4 m plastic deer fencing, however the large caged areas were difficult to work within so 2.4 m fencing was not used in the second year of this study, but were replaced with individual cages.

POLLINATION STUDY

Techniques used in the pollination study included field observation and collection of floral visitors, microscopic examination of pollen on floral visitors, examination by fluorescence microscopy of pistils to detect pollen tube formation, application of fluorescent powder on flowers to determine potential dispersal distance, and pollen manipulation of bagged flowers to determine the influence on seed production. In order to sample floral visitors, flowers were visually examined for insects during each visit to a population. When possible, insects were collected with a hand net and returned to the lab for observation and identification. Intact specimens were stored in a freezer and later examined for the presence of *Trillium reliquum* pollen.

Sticky traps were also used to collect insects visiting plants or in the vicinity of the plants at the Lee site. A sticky trap material (Tanglefoot® or Vasoline®) was placed on five artificial petals constructed of purple card stock and placed between the petal and pistil of an open flower. In ten other flowers, stigmas were coated with sticky trap material. Sticky traps were also constructed of small Petri dishes containing filter paper thinly coated with sticky trap material, and supported by 16 penny galvanized nails at the same height as mature flowering plants. Five traps were supplied with small pieces of canned tuna in an attempt to simulate the scent of *T. reliquum* flowers. During the flowering period, traps were observed every other day. When traps contained several insect specimens, they were removed and replaced with new traps. All traps were removed after flower senescence. In the laboratory, insects were removed from traps with an ethanol solvent and identified with the aid of a dissecting scope. These specimens were altered by the trap material and solvent and were not suitable to examine for the presence of pollen. References used for insect identification included Borror et al. (1989), McAlpine et al. (1987), McAlpine et al. (1981), Hall and Townsend (1977), Oldroyd, (1965), and James (1947).

In order to determine the influence of pollination on seed production and to compare pollination levels among study populations, experimental plants were bagged to exclude pollinator activity. Bags were 30cm by 25cm, sewn from no-see-um netting (0.3mm mesh), and tied with cotton drawstrings. Bags were placed over the flower bud and bracts of each bagged plant and secured by drawstrings tied around the stem. Pieces of cotton batting were used to cushion the stems and to prevent insects from entering at the point where bags were tied. Ten juvenile plants and ten mature plants on Lee site were also covered with bags that were punched with a hole-punch in order to determine if damage occurs to plants due to shading by the bag material. Bags were placed on plants before flower opening and remained until fruit began to develop (approximately 6 weeks). Each experimental plant was arbitrarily assigned to one of several treatments: self-pollinated, outcrossed single hand-pollination, outcrossed multiple hand-pollination, and open to pollination by the pollinators present at the site (unbagged). The self-pollination treatment consisted of five replicates in each of the smaller sites, Bullock A and Randall, and 10 in each of the larger sites, Bullock B, Muscogee A, Muscogee B and Lee in 2002, for a total of 50 replicates in 2002. In 2003 this treatment consisted only of 10 replicates in each of Bullock B and Lee sites, and was discontinued in 2004. Pollen was transferred, using an extra-fine camelhair paintbrush, from anther to stigma of the same flower in plants of the self-pollinated treatment in 2002. In 2003 plants were simply bagged with no pollen transfer. This treatment was discontinued in the third year of the study since plants of this treatment in the first and second years produced no seeds. In the outcrossed single hand-pollination treatment, bags were opened when stigmas were receptive and pollen from plants located on the opposite side of the population was applied to stigmas with a paintbrush. In multiple hand-pollination treatments, stigmas were treated with pollen from flowers located on the opposite side of the population once each day for three days. Plants in the open pollination treatment were selected as similar in age to the plants placed in other treatments and were marked with wire flags, but were not treated with bags or hand-pollination. Treatments consisted of five or ten replicates depending on population size.

Fruits were collected in plastic collection jars, labeled, and removed from each experimental plant at the end of the season and returned to the lab for measurement and seed counts. Fruits were measured with calipers and weighed on a Mettler balance before seeds were removed. Seeds and elaiosomes were extracted, counted, measured for length and width with an ocular micrometer on a dissecting microscope and weighed with a Mettler balance. In order to determine the percent weight of elaiosomes, seeds were weighed before and after elaiosome removal. Undeveloped ovules were also counted in each fruit collected. Comparisons of measurements and seed counts among treatments were made by two-way ANOVA using Statview software. Sample sizes were small because of the limited number of flowering plants at each site. In many cases, sample sizes were diminished due to loss of bagged plants to fungal disease, herbivory, drought or fire before fruits had matured.

Five pistils from each of the self, single, multiple and open pollination treatments were collected from the Lee site, stained, and observed for pollen tube formation. Pistils were removed after stigmas senesced then returned to the lab in a cooler to prevent heat damage. The styles were fixed with a solution of 1 part formalin: 8 parts 80% ethyl alcohol: 1 part acetic acid for a minimum of 24 hours. They were then rinsed in tap water and placed in a solution of 8N sodium hydroxide until the tissue became soft and clear (8 to 24 hours). The styles were then rinsed in tap water for two hours to remove excess sodium hydroxide. A 0.1% solution of aniline blue dye dissolved in 0.1 N K_3PO_4 was used to stain the styles for four hours (Martin 1959). Styles were then sliced thinly with a razor blade, placed on a microscope slide and viewed with fluorescence microscopy.

In the first year of the study, fluorescent powder was used in an attempt to determine the potential distance of pollen dispersal (Townsend and Levey 2005). In the Lee population, anthers of 15 open flowers were dusted with three different colors of fluorescent powder to determine potential distance of pollinator transport. The powder was visually searched for one week later on other plants with the expectation that insect visitors would remove and transport the powder in a fashion similar to pollen transfer. However, no powder was detectable on any other plants in the population. It is likely that any dispersed powder was removed by the excessive rain and windy conditions that occurred during that time.

SEED DISPERSAL STUDY

The seed dispersal study consisted of field observation, collection and identification of seed dispersers and seed predators and capture of potential dispersers/predators on sticky traps supplied with fruits and/or seeds to determine to which part of the seed they are attracted. Plants in fruit were visually examined for visitors during each visit to the study sites. In order to sample the diversity of fruit visitors, Tanglefoot® sticky trap material was applied to leaves and/or fruits of five plants when the fruits were ripe. Potential dispersers and predators at the study site were also sampled with sticky traps supplied with fruits and/or seeds. Sticky traps consisted of

100 mm diameter plastic Petri dishes coated with a thin film of Tanglefoot® sticky trap material and containing one of six treatments: a fruit containing seeds, a fruit only (seeds removed), ten seeds with elaisomes intact, ten seeds with elaiosome removed, ten elaiosomes glued to 3.0 mm glass beads, or seeds with elaiosomes glued to beads. A number of sticky trap replicates were lost due to rain and wind. Only 3 treatments, fruit with seeds, fruit only, and seeds with elaiosomes removed, yielded sufficient data for analysis. After 3 weeks, when plants at the study sites were senescing, seed trays were returned to the lab for insect removal and identification. Insects were removed from trays with an ethanol solvent and identified using a dissecting scope. Insect identifications were made using Borror et al. (1989), McAlpine et al. (1987), McAlpine et al. (1981), Hall and Townsend (1977), Oldroyd (1965), and James (1947).

RESULTS

POLLINATION STUDY

Floral visitors from three fly families; Calliphoridae, Dolichopodidae, and Phoridae were often observed. Members of all three families were seen in high numbers swarming above and around flowers. Calliphorids and dolichopodids were observed to land on flowers. Microscopic examination of calliphorid specimens collected from flowers on Lee site revealed *T. reliquum* pollen on their bodies in 2002 and 2003. Tiny, unidentified scarab beetles were observed on *T. reliquum* plants near open flowers. Calliphorids were abundant and were seen on flowers at all study sites. The remaining insect families were observed at the larger sites, Bullock B, Muscogee A, Muscogee B and Lee.

Three orders of insects (Diptera, Coleoptera and Hymenoptera) constitute the majority of insects collected on sticky traps (Table 2). Diptera were the most diverse and abundant, constituting approximately 32% of the insects collected. Of the 11 families of Diptera collected, Phoridae was the most common. Calliphoridae were also abundant and specimens collected on sticky traps placed in flowers consisted of three calliphorid genera: *Lucilia, Phaenicia,* and *Bellardia.* Although a great variety of arthropods, including members of nine different orders, were collected on sticky traps on and near the plants, only two orders, Diptera (seven families) and Coleoptera (two families), were represented in the collections on sticky traps placed inside flowers. Ants (Hymenoptera), although abundant on sticky traps outside flowers, were not collected on sticky traps inside flowers.

Seed number varied from 6 to 49 seeds in a single fruit. Most fruits contained undeveloped ovules in addition to developed seeds. Although most fruits varied within a maximum of 76 total ovules (developed and undeveloped), one variant fruit contained a total of 113 ovules.

No seeds or fruits were formed by any of the plants placed in any of the self-pollination treatments. In all 70 replicates (2002 and 2003) the flowers senesced normally but no fruit developed.

Seed numbers in the open pollination treatment were significantly higher ($P<0.05$) than either hand pollination treatment (single or multiple) at the Lee site in 2002 (Figure 5). At all other sites in 2002 the numbers of seeds in the open pollination treatment were less than or not significantly different from hand pollination treatments.

In 2003 seed numbers were significantly higher in the open pollination treatment than in either hand pollination treatment (single or multiple) at Bullock B and Muscogee A. Plants in the open pollination treatment at Bullock B produced seeds in 71% of ovules. This was significantly higher than at other sites. In two sites of this study (Bullock A and Muscogee B), seed counts in the open pollination treatments were consistently lower than or not significantly different from hand pollination treatments. Single application of pollen, by hand, produced significantly higher numbers of seeds than multiple application or open pollination at Muscogee A in 2002 and 2003.

Seeds varied from 20.17 to 45.70 mg with the elaiosome forming from 26.27 % to 48.03 % of the total weight (Table 3). Percent weight of the elaiosome varied significantly among sites with elaiosomes smaller at Bullock A in 2002. Fruits varied from 0.848 to 3.143g. Fruit weight varied significantly among sites in 2002 and 2003 (Table 4).

All stigmas and styles observed in the pollen tube study displayed pollen tube formation (Figure 7). There were no observable differences among treatments in the number of tubes formed. Self-pollinated plants fluoresced similar amounts of pollen as cross-pollinated plants and those that were open to natural pollination. Large quantities of pollen were collected on the stigmas of plants in each treatment.

SEED DISPERSAL STUDY

Insects of several families were collected on fruits or seeds on sticky trap platforms (Table 5). The insects collected on sticky traps treated with fruit and seeds were significantly dominated by Phoridae, Anthomyiidae and Formicidae (Figure 8). Formicidae (ants) were the third largest group with three genera and six species identified. Platforms containing seeds with elaiosomes removed did not attract as many potential dispersers as those with fruit or fruit and seeds with elaiosomes intact (Table 5).

Paratrechina faisonensis, Camponotus chromaiodes, Crematogaster ashmeadi, Aphaenogaster spp., and *Trachymyrmex septentrionalis* (Formicidae) were observed removing fruit and seeds from plants. The smallest ants (*Crematogaster ashmeadi* and *Paratrechina faisonensis)* removed flesh from fruits but were incapable of carrying a seed even when two ants worked together. These ants often removed elaiosomes, allowing seeds to drop to the forest floor below the plant. However, larger ants (*Camponotus chromaiodes*, *Aphaenogaster spp.*, *and Trachymyrmex septentrionalis*) often removed sections of fruit and seeds with elaiosomes. The larger ants could easily remove seeds with elaiosomes intact and carry them down the scape and away from the plant. Ants continued to remove pieces of fruits, elaiosomes and seeds until the capsule was devoid of food.

On July 6, 2001 fruits were removed from the Lee County site to photograph fruits, seeds and elaiosomes in better light for greater detail. The fruits were transported to another location approximately 26.1 km west northwest of the plant population. While photographing the fruit, a wasp, *Vespula maculifrons,* visited the fruit. The wasp cut away sections of fruit and carried them away. The first visiting *Vespula* removed a seed with elaiosome intact and carried it away (out of sight) into the trees along the edge of the site, approximately 10 meters away. Within a few minutes another wasp (or the same wasp returning) appeared and began excavating fruit. An additional *Vespula* appeared while that one was still excavating and began to excavate from the opposite side of the fruit. Excavating behavior continued until a seed was reached. Two *Vespula* were observed fighting over a seed and elaiosome. One *Vespula* attempted to remove a seed and elaiosome that were extremely large. After several failed attempts at flying (after 2.5 minutes), the *Vespula* removed the elaiosome from the seed and flew away with the elaiosome, leaving the seed behind. The seed was dropped approximately 17 cm from the fruit. Another wasp, after working with a seed for 2.5 minutes, removed the elaiosome and dropped the seed near the fruit. The seeds that were left behind after elaiosome removal were taken to the lab for measurement. They were unusually large (46.9 and 48.1 mg). A total of 21 *Vespula* visits were observed. In 13 visits, seeds were removed with elaiosomes intact. In two visits, elaiosomes were removed and seeds were left behind. In six visits only fruit pulp was removed. Two *Vespula* were collected for identification.

DISCUSSION

Great variation was observed in this study in characteristics of *Trillium reliquum* such as size of fruits, seeds and elaiosomes and numbers of seeds and ovules. Although a portion of this variation may be influenced by environmental factors, it is reflective of genetic differences among populations as well. Fruit size varied significantly among populations but not among pollination treatments. Gonzales and Hamrick (2005) detected large amounts of genetic variation among populations and suggested that there is currently little gene flow between populations, that population isolation is likely an ancient condition for this species, and that perhaps Georgia and Alabama populations represent different historical lineages.

The most frequent and abundant among the floral visitors observed during this study were flies and beetles, mostly very small species, in several families known to be attracted to carrion. I propose that *Trillium reliquum* fits the characteristics of a carrion-fly pollination syndrome, sapromyophily, in which dark red or purple coloration and carrion-like odor attract carrion-visiting insects (Faegri and van der Pijl 1966). This pollination strategy has been previously suggested by others studying this plant (Folkerts et al. 1987, Patrick et al. 1995).

Phoridae, the most common floral visitors, are known to inhabit damp areas often around decomposed animal and plant matter, near fungi and flowers (Borror et al. 1989).

Phorid flies have been suggested as possible pollinators for the carrion flower, *Aristolochia* sp. and several other angiosperms (Hall and Brown 1993, Elberling and Olesen 1999). In this study they were captured in sticky traps placed in flowers and so are definitely floral visitors. However, because of the sticky trap material and the solvent used to remove them, I was not able to examine them for *T. reliquum* pollen on their bodies. The diminutive size of the flies makes observation difficult but further study may provide direct observation of pollination in *T. reliquum.*

Blowflies of the family Calliphoridae were the most often observed flies in *T. reliquum* flowers and are known to be attracted to the smell of carrion and to the purplish red color of flowers of many species (Erzinclioglu 1996). Calliphoridae were also observed as *T. reliquum* floral visitors by Folkerts et al. (1987). In this study, calliphorids of three species were observed to have *Trillium reliquum* pollen on their bodies. *Lucilia illustris*, the most common species of Calliphoridae collected in this study, is a carrion-feeding species native to the United States (Kano and Shinonaga 1968). Members of *Phaenicia*, also collected in this study, are associated with carrion and decaying matter and along with *Lucilia* species are known to pollinate flowers of other genera including *Rafflesia*, *Metrodorea, Gouania,* and *Allium* (James 1947, Kano and Shinonaga 1968, Currah and Ockendon 1984, Hidayati 2000, Pombal et al. 2000, Souza-Silva et al. 2001). *Bellardia*, native to Europe, was introduced to the eastern United States as a parasite of the European earthworm (McAlpine et al. 1987). Little is known of the biology of these flies in the United States.

Tiny scarab beetles were also collected on *T. reliquum* flowers. Beetle mediated pollination of *Trillium camschatcens*e, a white-flowering pedicellate species, was documented by Ohara and Kawano(2005). An association with carrion beetles may be similar to that of carrion flies (Faegri and van Der Pijl 1966). Scarabaeidae have been noted as pollinators in studies since the late nineteenth century (Faegri and van der Pijl 1966).

I detected obvious evidence of pollination self-incompatibility in this species. Despite the fact that pollen tubes were formed and grew into the style in self-pollinated plants, no self-pollinated plants formed fruit. These data suggest that the mechanism for self-incompatibility lies not within the stigma or style but adjacent to or within the ovary. Consistent with my findings, Gonzales and Hamrick (2005) found genetic patterns in populations of *Trillium reliquum* indicative of self-incompatibility. My results are in direct contradiction, however, with the recent findings of Heckel and Leege (2006). In their Macon County, Georgia population they claim to have seen not only self-compatibility or autogamy (seed production resulting from intrafloral pollen transfer) but also apomixis (seed development without pollination). Apomixis is apparently only rare in the genus *Trillium*, and its occurrence has been debated by a number of authors (Jeffrey and Haertl 1939a, 1939b, Swamy 1948, Nesom and Duke 1985, Barrett and Helenurm 1987). It is possible that populations differ in the degree to which self-pollination occurs, showing the "leaky self-incompatible system" referred to by Gonzales and Hamrick (2005). However, I propose that the findings of Heckel and Leege (2006) may be due to their failure to exclude tiny floral visitors from their experimental plants. They warned that their results should be interpreted with caution, as they did not manually transfer pollen in their self-pollination treatments. In addition, they used exclusion bags constructed of bridal veil which typically has a much larger mesh size than the no-see-um netting used in this study. Although bridal veil has been used in a number of pollination studies and is sufficient to exclude relatively large floral visitors such as bees, it is not sufficient to exclude tiny floral visitors such as the phorids, dolichopodids and scarabeids that were frequent visitors of *T. reliquum* flowers in this study. These small flies and beetles are not typical pollinators and may have been overlooked by the authors of that study. However, the fact that Heckel and Leege (2006) observed fruit production in plants in their population of *Trillium reliquum* when all but the smallest of floral visitors were excluded reinforces my conclusion that small flies and beetles are principal pollinators of *Trillium reliquum*.

Although Heckel and Leege (2006) suggested that there was no pollination limitation in their very large population (several thousand plants), my evidence suggests there is reduced pollination success in smaller populations. Because seed numbers in the open pollination treatment were significantly higher than hand-pollination treatments in the largest population and at the most pristine site in my study (well over 1000 plants, Lee site, 2002), it seems that insect visitors are plentiful and function as efficient pollinators when populations of flowering *T. reliquum* plants are large. Similar results were recorded in 2003 at Bullock B and Muscogee A, which also harbor large populations. In the smaller populations, however (Bullock A and Muscogee B), the numbers of seeds in the open pollination treatment were consistently smaller than or not significantly different from hand pollination treatments, indicating that floral visitation was limited. These data suggest that insect visitors at large sites are plentiful and function to achieve pollination levels greater than what could be achieved by artificial means; but that at small sites floral visitation and/or insect transport of pollen is low.

This is consistent with other studies that have shown that attraction of pollinators to plant populations in *Trillium* and other plant genera is influenced by population size and floral display (Kunin 1992, Jules and Rathcke 1999, Cane and Tependino 2001, Spira 2001, Griffin and Barrett 2002, and Steven et al. 2003). Surprisingly, single application of pollen by hand produced significantly higher numbers of seeds than multiple application or open pollination at Muscogee A in 2002 and 2003. This anomalous result may be at least partially explained by a failure to be consistent in artificial application of pollen. Small sample size may also have been a cause.

Although plants in large populations fare better in seed production than plants in small populations, *T. reliquum* rarely if ever meet their maximum potential of seed production. The highest ratio of developed seeds to potential ovules (71%) was observed in plants in the open pollination treatment at Muscogee A. This was significantly higher than at the other sites. Low fruiting success was observed in the large population studied by Heckel and Leege (2006) in Macon County, Georgia. Such a low reproductive rate may have serious consequences for a plant with so few remaining populations (U.S. Fish and Wildlife Service 1988, Currie 1991).

Ants are undoubtedly important seed dispersers for *T. reliquum* as has been seen in a number of other *Trillium* species. *Paratrechina faisonensis, Camponotus chromaiodes, Crematogaster ashmeadi, Aphaenogaster* spp., and *Trachymyrmex septentrionalis* (Formicidae) are all candidate disperser species and were observed removing fruits and seeds from plants in this study. The smallest ants (*Crematogaster ashmeadi* and *Paratrechina faisonensis)* however, are likely not as efficient dispersers as the larger species because, being too small to carry the seeds, they remove elaiosomes and drop the seeds very near to the parent plant. However, larger ants (*Camponotus chromaiodes*, *Aphaenogaster* spp., and *Trachymyrmex septentrionalis*) are likely to achieve greater dispersal distances.

This study also provides the first description of vespicochory in *T. reliquum*. *Vespula maculifrons* were observed removing fruits and intact seeds and were able to fly with all but the largest seeds. *Vespula* are potentially able to achieve even greater dispersal distances than the largest ants and are more likely to disperse seeds to areas outside of existing populations. More research is needed to determine the frequency of vespicochory, actual seed dispersal distances, and the influence of seed and elaiosome weights.

In this study, I find evidence to strengthen concern for this species in that existing populations are small, declining and isolated. These conditions apparently result in lowered reproductive rates, which can only be magnified by further habitat loss as, has been shown in other studies (Steffan-Dewenter and Tscharntke 1999, Li et al. 2005). Many of the sites used in this study are threatened by encroachment of non-native plants, clear-cutting, herbivory and human traffic. Only half of the sites used in this study are protected by public ownership and all of those are located on a military base in Georgia. I suggest that there is a need for conservation efforts that strive to protect a number of populations in order to maintain genetic diversity and that each population should be managed to maintain sufficient size for efficient pollinator activity.

Further research is warranted to precisely determine the minimum population size needed to sustain a healthy population with high pollinator activity. Habitat loss and urbanization threaten the species in the immediate future.

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Figure1: Developmental Stages of Trillium *reliquum*: anthers A. Introrse anthers sacs and pistil, B. Anther sacs at anthesis,
C. Anther sacs post-anthesis.

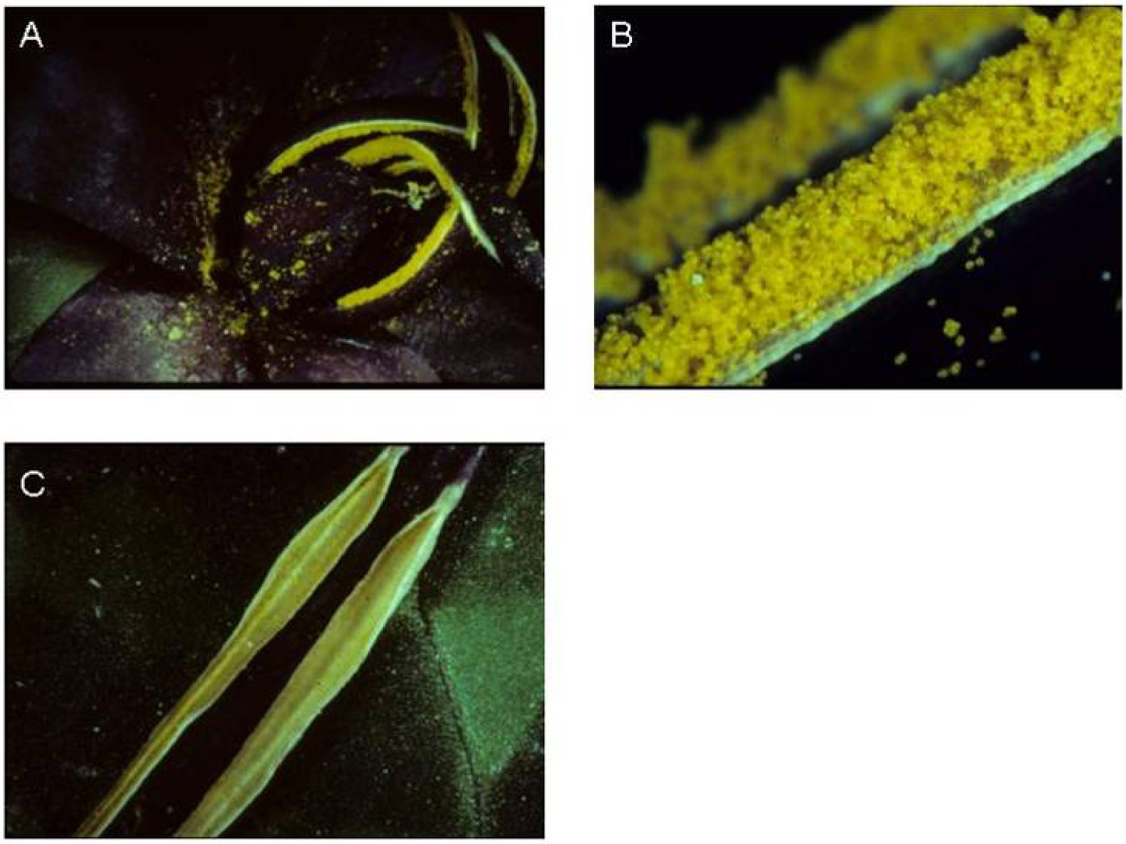

Figure 2. Developmental Stages of *Trillium Reliquum*: A. One-leaf juvenile, B. Three-leaf juvenile, C. Adult, D. Open Flower with purple anthers and pistils

Figure 3. Developmental stages of *Trillium reliquum*. A. Flower post-anthesis, B. Fruit

Figure 4. Developmental stages of *Trillium reliquum*: seed and elaiosome. A. Variation in Seed and elaiosome, B. Seed with elaiosome, C. Seed with elaiosome removed, D. Seed cut open

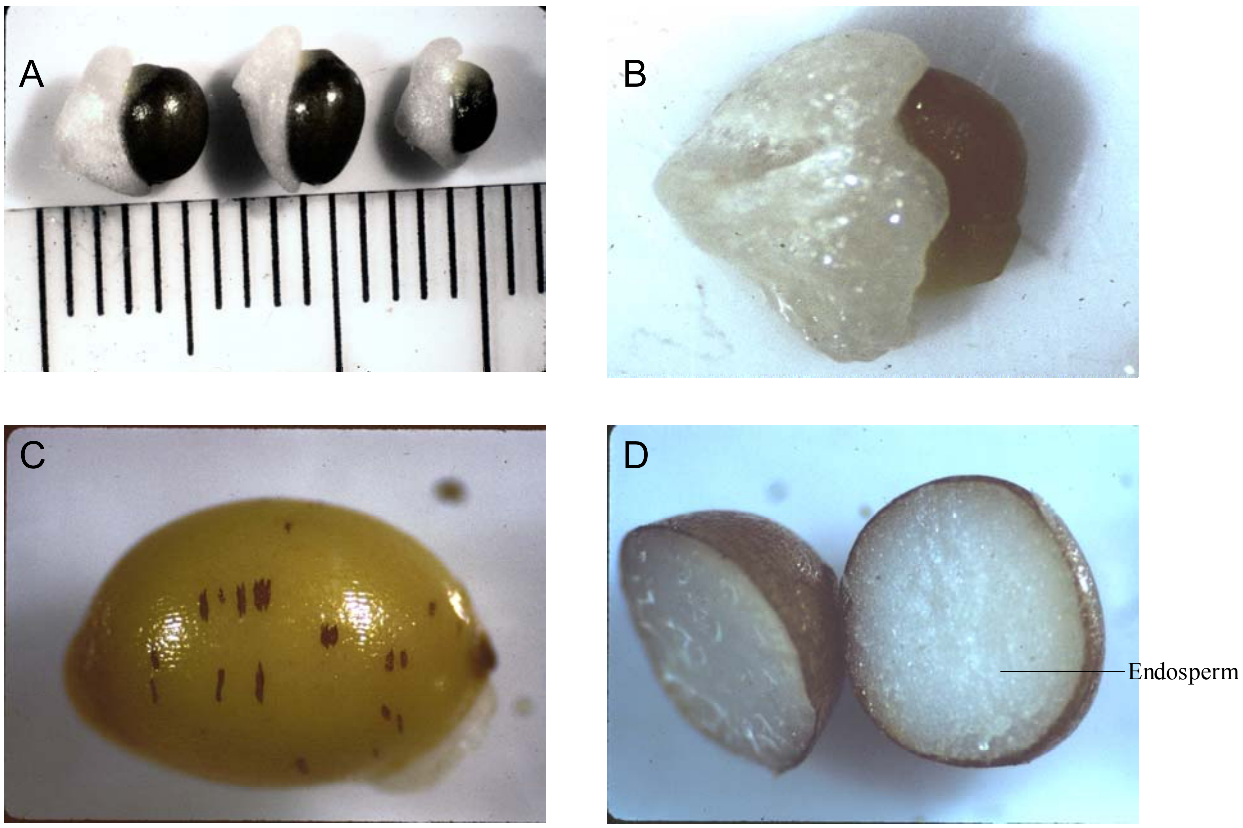

Figure 5. Number of seeds per fruit in *Trillium reliquum* as influenced by pollination treatment at five sites in Alabama and Georgia 2002. S=single pollen application, M=multiple pollen application, O=open pollen application

n=3 Significant Difference $p < 0.05$

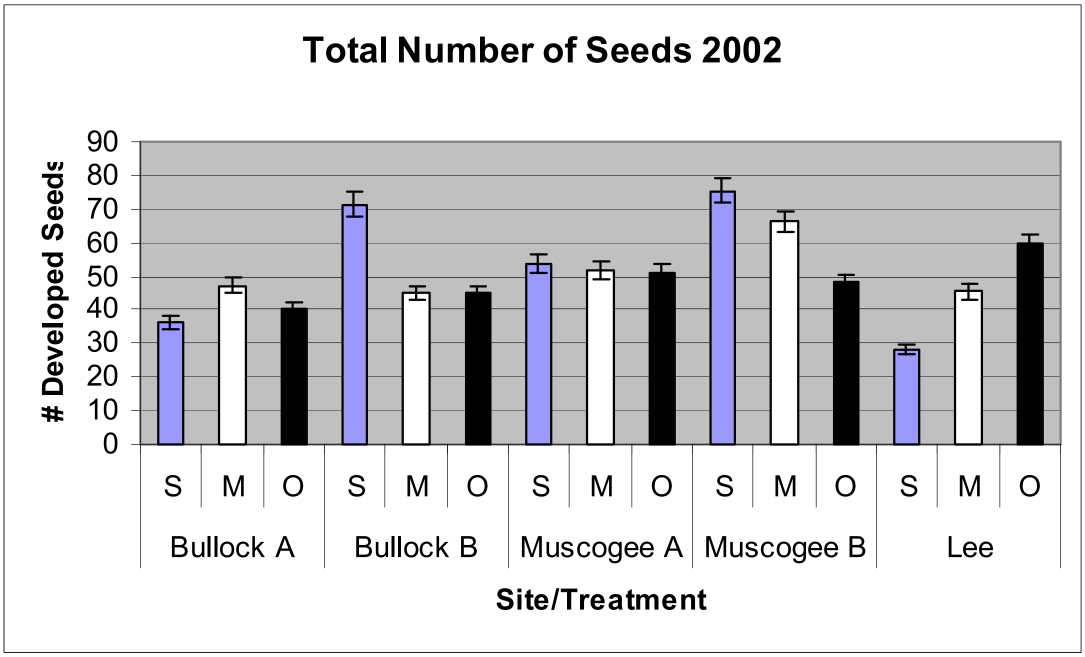

Figure 6. Number of seeds per fruit in *Trillium reliquum* as influenced by pollination treatment at five sites in Alabama and Georgia 2003. S= single pollen application, M=multiple pollen application, O=open pollination application.

n=3 Significant difference $p < 0.05$

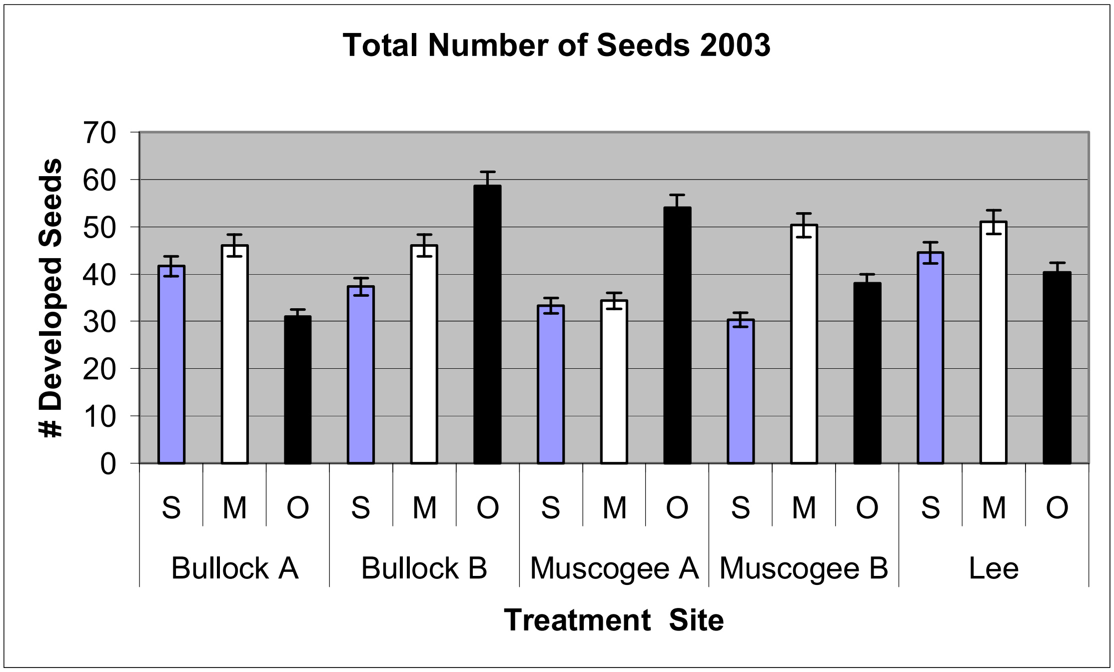

Figure 7. Pollen tubes of *Trillium reliquum* viewed with fluorescence microscopy. A. Open pollination treatment, B. Self-pollination treatment, C. Growth of pollen tubes in self-pollination treatment.

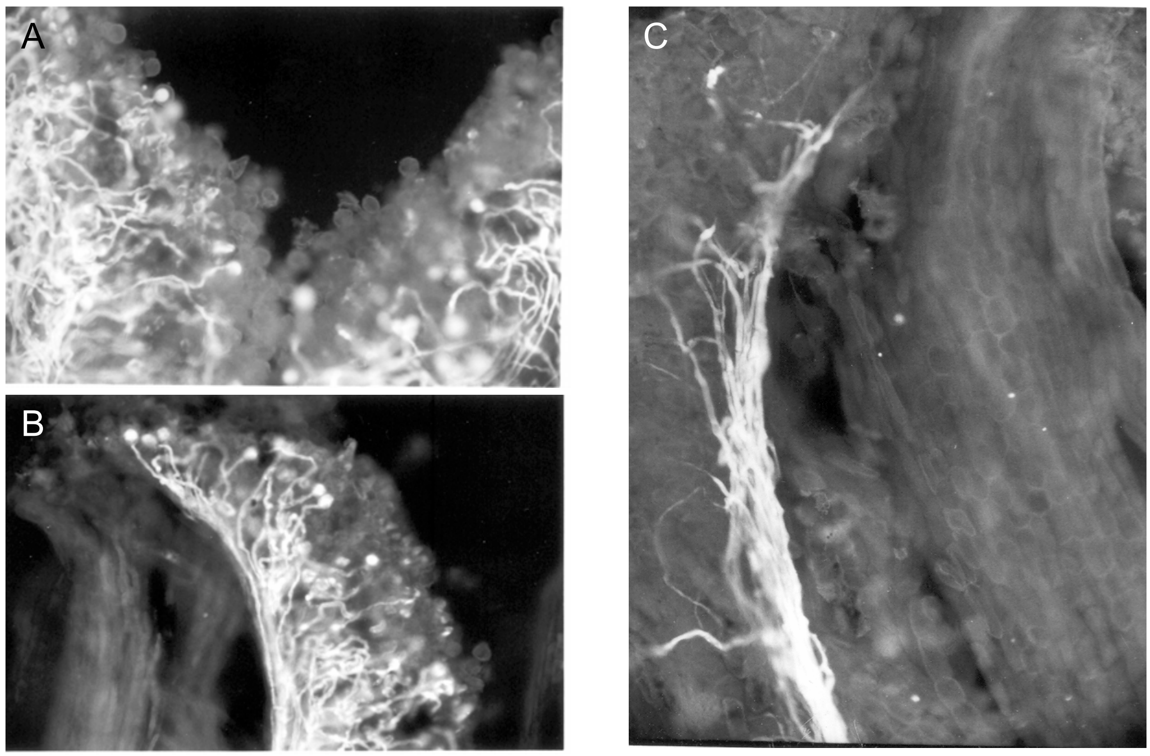

Figure 8. Number and identification of insects collected on sticky traps with *Trillium reliquum* seeds or fruit at a site in Lee County, AL in 2003.

n= 5 trays. $p < 0.05$.

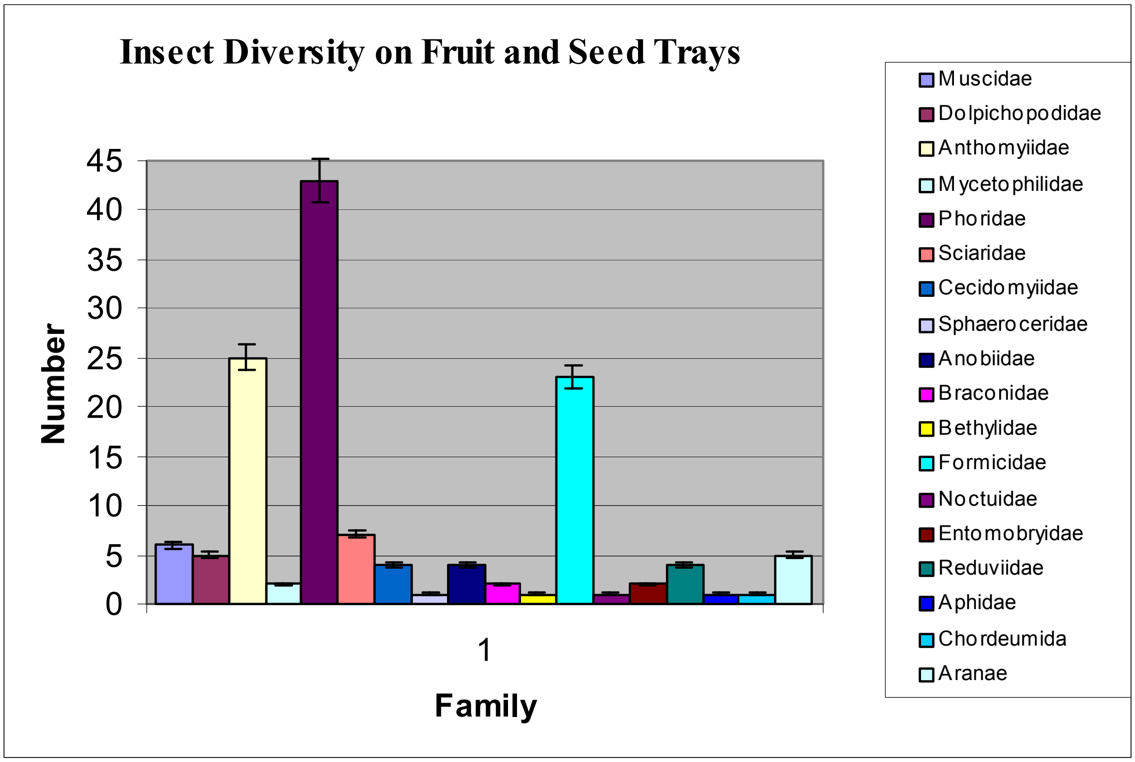

Table 1. Characteristics of study sites in Bullock and Lee counties, AL and Muscogee County, GA.

Site	**Population size**	**Population Composition**	**Plant Density**	**Exposure**	**Disturbance**
Bullock A	300-400	Mostly Juvenile plants	Sparse	Northeast	High-adjacent clear-cut
Bullock B	>1000	Adult juvenile and seedling	Dense	East	Low to Medium
Lee	>1000	Adult juvenile and seedling	Dense	South	Low
Muscogee A	600-700	Mostly adult and juvenile; with few seedlings	Sparse	East	Very Low
Muscogee B	~1000	Juvenile and few adult	Sparse to Medium	East	Medium
Randall	200-300	Mostly Juveniles	Sparse to Medium	Southeast	High-adjacent clear cut and deer plot

Table 2. Floral visitors and potential floral visitors of *Trillium reliquum*, collected on sticky traps at sites in Bullock and Lee counties, AL and Muscogee County, GA in 2002 and 2003.

Order	Family	Genus	Species	Number	On Flower
Diptera	Muscidae	*Fannia*		7	X
	Calliphoridae	*Lucilia*	*L. illustris*	5	X
	Calliphoridae	*Phaenicia*		3	X
	Calliphoridae	*Bellardia*		2	X
	Calliphoridae	*Stomorhina*	*S. lunata*	1	
	Chloropidae	*Stenoscinis*	*S. longipes*	1	X
	Sciomỳzidae	*Pherbellia*		1	
	Phoridae			41	X
	Sciaridae			7	
	Mycetophilidae			3	
	Tachinidae			1	
	Sepsidae	*Sepsini*	*S. sepsis*	1	X
	Dolichopodidae			2	X
	Bibionidae			12	X
Coleoptera	Scarabaeidae			9	X
	Mordellidae			6	
	Elateridae			1	
	Phalacridae			4	
	Staphylinidae			1	
	Anobiidae			1	
	Nitidulidae			5	
	Tenebrionidae			2	
Hymenoptera	Braconidae			2	
	Sphecidae			1	
	Vespidae			1	
	Ichneumonidae			1	
	Formicidae			23	
	Diapriidae			1	
	Pteromalidae			1	
	Myrmicinae			23	
Orthoptera	Tettigoniidae			2	
Blattaria	Blatellidae			2	
Collembola	Isotomidae			1	
	Delphacidae			1	
	Cercopidae			1	
Lepidoptera	Noctuidae			1	
Microcoryphia	Meinertellidae			1	
Aranae				1	

Table 3. Mean seed weight per fruit and percent elaiosome weight of *Trillium reliquum* as influenced by pollination treatment at five sites in Alabama and Georgia in 2002 and 2003.

S=single pollen application, M=multiple pollen application, O=Open pollination.
n=5 seeds per fruit.

Site	Treatment	Seed Weight 2002 (mg)	% Elaiosome Weight 2002	Seed Weight 2003 (mg)	%Elaiosome Weight 2003
Bullock A	S	31.87	30.24	32.54	31.62
	M	32.21	30.38	33.99	25.08
	N	36.03	35.11	33.97	35.35
Bullock B	S	31.40	34.05	33.13	30.66
	M	33.37	48.03	33.88	26.53
	N	36.63	39.82	34.39	27.15
Muscogee A	S	32.80	33.07	20.17	33.29
	M	45.70	35.39	37.40	36.71
	N	33.97	39.46	31.19	30.61
Muscogee B	S	34.10	28.35	32.78	27.19
	M	37.40	41.28	30.25	41.35
	N	36.40	35.18	28.17	26.27
Lee	S	24.76	35.43	38.07	28.58
	M	39.62	31.95	39.62	29.44
	N	29.67	31.01	29.00	37.55

Table 4. Fruit weight of *Trillium reliquum* as influenced by site and pollination treatment at five sites in Alabama and Georgia in 2002 and 2003. Mean= 1.4531g

S=single pollen application, M=multiple pollen application, O=Open pollination. n=3 fruits per site.

Site	Treatment	Fruit Weight (g)	Fruit Weight (g)
Bullock A	S	1.679	3.143
	M	0.952	1.701
	N	1.385	2.418
Bullock B	S	1.141	1.073
	M	1.100	1.703
	N	1.437	2.038
Muscogee A	S	1.190	1.280
	M	1.099	1.149
	N	1.986	1.907
Muscogee B	S	1.147	1.128
	M	2.996	2.565
	N	1.811	2.045
Lee	S	1.876	1.871
	M	0.848	1.229
	N	1.999	2.226
Overall Mean		1.453	1.687

Table 5. Number and identification of potential seed dispersers collected on sticky traps with *Trillium reliquum* fruit or seeds at a site in Lee County, AL in 2003.

ORDER	FAMILY	FRUIT ONLY	SEEDS ONLY
Diptera	Tephritidae	3	
	Culicidae	1	
	Phoridae		5
	Sciaridae		1
Coleoptera	Nitidulidae	3	1
	Anabiidae		2
	Scarabaeidae	1	
	Histeridae	2	
	Phalacridae	3	
	Erotylidae	1	
Hymenoptera	Encyrtidae	1	
Orthoptera	Tephritidae	4	
	Tettigoniidae	1	
Collembola	Entomobryiidae	1	
Homoptera	Aphidae	1	1
Hemiptera	Lygaeidae		1
	Aradidae	2	
	Tingidae	1	
	Cicadellidae	3	
	Miridae	3	
Diplopoda			1
Isopodia			1
Aranae		1	