INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION IN THE MELASTOMACEAE AND FABACEAE

by

SUSAN M. HAWKINS

(Under the Direction of John M. Ruter and Carol D. Robacker)

ABSTRACT

Interspecific and intergeneric crosses were made between species in the families Melastomaceae and Fabaceae in an attempt to produce hybrids that might be of ornamental value. Species from the genera *Dissotis* and *Tibouchina* in the Melastomaceae family were used as parents. In the Fabaceae family, species from *Baptisia* and *Thermopsis* were used as parents. Crosses between the Melastomaceae species resulted in low seed set with poor germination rate. Only 31 putative hybrids were obtained from 1,036 pollinations. Crosses between the Fabaceae species resulted in a similar rate of seed set as the Melastomaceae crosses, but germination rate was much higher. From the 2,544 pollinations made, 3,783 putative hybrid seedlings were obtained; 245 seedlings were kept for evaluation and future breeding. The Fabaceae project should be continued as production of both intergeneric and interspecific hybrids is feasible. The Melastomaceae project is not a viable breeding project.

INDEX WORDS: Melastomaceae, Fabaceae, *Tibouchina*, *Dissotis*, *Baptisia*, *Thermopsis*, interspecific, intergeneric, ornamental plant breeding, hybridization

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DEDICATION

I dedicate this thesis to my son, Brandon Z. Pitman, the light of my life and my inspiration. I couldn't have asked for a better kid. I also dedicate it to my mother, Marian F. Hawkins, who loved being in a garden and seeing what was in bloom until the very end of her life.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

Introduction and Literature Review

Interspecific and Intergeneric Crosses

Hybrids between different species and genera have long been used in horticulture. Some of the advantages of hybridization are an increased vigor of the progeny, the production of a plant with the best characteristics of both parents, and a possible increase in pest or disease resistance. Whether the objective of hybridization is to produce an ornamental plant, or a plant used for food, fiber, phytoremediation, or medicine, it is important to choose parent species with desirable qualities.

Interspecific and intergeneric crosses have been made to confer disease or pest resistance. A cross between chrysanthemum (*Dendranthema morifolium* (Ramat.) 'Zhongshanjingui') and *Artemesia vulgaris* L. 'Variegata' conferred resistance to chrysanthemum aphids on the resulting hybrid (DENG et al., 2010). A cross between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. produced hybrids that were highly resistant to late blight (SARKAR et al., 2011). Intergeneric crosses have also been made to increase the genetic variability of a cultivated species and incorporate desirable genetic traits from a wild species, as was done in a breeding project crossing a wild with a cultivated Brassicaceae (*Erucastrum carminoides* x *Brassica olearaceae* var. alboglabra) (APARAJITA et al., 2009). Cold tolerance in chrysanthemum was enhanced by an intergeneric cross between *Chrysanthemum* spp. and *Ajania przewalskii* Poljak. (DENG et al., 2011). Intergeneric crosses of Genista and Cystisus (Fabaceae family) had higher fertility, as measured by pollen viability and pollen tube growth, than interspecific crosses within the two

genera (BELLENOT-KAPUSTA et al., 2006). An intergeneric cross between chrysanthemum (*Chrysanthemum grandiflorum* (Ramat.) Kitamura) var. 'Zhongshanjingui' and *A. vulgaris* produced a hybrid that rooted more quickly and produced more lateral roots than its chrysanthemum parent (DENG et al., 2012).

Incompatibilities between parents in an interspecific or intergeneric cross can lead to poor seed set. Techniques exist to overcome this challenge. In a breeding project to develop hybrids resistant to papaya ringspot virus from a cross between *Carica papaya* and *Vasconcellea cauliflora* J., treating the stigmas before pollination with a nutrient solution of either sucrose, sucrose plus boron, or sucrose with calcium chloride proved effective in increasing fruit set (JAYAVALLI et al., 2011). Embryo rescue was used to overcome intergeneric barriers to successful reproduction due to seed abortion in an intergeneric cross between *Ascocenda* and *Phalaenopsis* (TSAI et al., 2009). Both embryo rescue and ovule culture were successful in producing seedlings in interspecific crosses of *Tulipa* L. (CUSTERS et al., 1995).

Rate of seed set for intergeneric crosses would be expected to be lower than that of interspecific crosses due to greater incompatibilities between parents. In crosses among species of *Portulaca* and *Calandrinia*, no seed set occurred in any intergeneric cross due to pollen-pistil incompatibility (WICKRAMASINGHE et al., 2009). Prezygotic barriers to fertilization also led to lack of fertilization and seed set in crosses between *Gossypium hirsutum* L. and the species *Hibiscus cannabinus* L. and *Abelmoschus esculentus* (L.) Moench (KANTARTZI and ROUPAKIAS, 2008).

Seed set for interspecific crosses may vary widely, even among crosses with the same female or male parents. Crosses between *Dendranthema grandiflorum* (Ramat.) Kitamura and *D. indicum* (L.) Des Moul. had a seed set rate of 59%, while crosses between *D. grandiflorum* and

D. nankingense (Nakai) Tzvel produced virtually no seed (SUN et al., 2010). Crosses of *Potamogeton wrightii* Morong x *P. perfoliatus* L. produced seed at a rate of 41.37% while crosses of *P. wrightii* x *P.* x *intortusifolius* had a seed set rate of 18.16%. The reciprocal cross *P.* x *intortusifolius* x *P. wrightii* set seed at a rate of 5.08% (ZHANG et al., 2011). Thus fertility of interspecific crosses may not only depend on the species used, but on which species are used as the male or female parent.

Melastomaceae Background

The Melastomaceae family comprises between 185 to 190 genera containing 5000 species (ALMEDA and CHUANG, 1992). The distribution of species in the Melastomaceae is diverse. Melastomaceae species are found in Asia, Africa, North and South America, and Australia; South America contains the majority of species (MICHELANGELI et al., 2013; RENNER and MEYER, 2001). Almost all Melastomaceae genera are endemic to tropical or semitropical climates; the genus *Rhexia* in eastern North America is the exception (RENNER et al., 2001). Some species, such as *Dissotis rotundifolia* (Sm.) Triana, have naturalized in places with tropical climates such as Indonesia, Brazil, Puerto Rico, and Hawaii (LIOGIER and MARTORELL, 1982; RENNER and MEYER, 2001). Melastomaceae species inhabit a wide variety of habitats, both forested and open, as well as on roadsides and land that has been disturbed (MICHELANGELI et al., 2013; POREMBSKI et al., 1996). Growth habits are as diverse as distribution; the family contains shrubs, herbaceous plants, and small trees (RENNER, 1993).

The two genera from the Melastomaceae family that were chosen for this project are *Dissotis* and *Tibouchina*. Both are part of the tribe Melastomeae (CLAUSING and RENNER, 2001). The Melastomeae consists of 48 genera containing approximately 550 species that are distributed worldwide (RENNER and MEYER, 2001). *Dissotis* is native to Africa and *Tibouchina* to South

America (RENNER and MEYER, 2001). *Tibouchina* contains species of horticultural importance while *Dissotis* species have not been widely used as ornamentals. Many *Dissotis* species have been used in traditional medicine in their countries of origin (ODIMEGWU et al., 2008; SYIEM and KHUP, 2006).

Melastomaceae Genetic Information

Tibouchina chromosome numbers are based on x=9, ranging from n=9 to n=63 (ALMEDA, 1997; ALMEDA and CHUANG, 1992). Many species of *Tibouchina* are polyploid. Although tetraploidy is the most common polyploid level, ploidy level varies (ALMEDA, 1997). *Tibouchina urvilleana* (DC.) Cogn., is reported to be octoploid by Almeda with a count of n=36, while Raven reports a count of n=28 for the species (ALMEDA, 1997; SOLT and WURDACK, 1980). *T. semidecandra* DC., with n=28, is reported to be hexaploid (BIR et al., 1988). T. *laxa* (Desr.) Cogn., *T. mutabilis* (Vell.) Cogn., *T. organensis* Cogn., *T. candolleana* (DC.) Cogn., *T. clavata* (Pers.) Wurdack, *T. breedlovei* Wurdack, *T. naudiniana* (Dec.) Cogn., and *T. granulosa* (Desr.) Cogn. have a count of n=18 and are reported to be tetraploid (ALMEDA, 1997; ALMEDA and CHUANG, 1992; SOLT and WURDACK, 1980). *T. lepidota* (Bonpl.) Baillon is reported to possibly be 14-ploid, with counts of n=ca. 62 according to Hunzinger and n=ca. 61according to Raven (HUNZIKER et al., 1985; SOLT and WURDACK, 1980).

Ploidy count may vary within the same species. *T. aristiguetae* Wurdack in Trujillo, Venezuela has a count of n=63 and is 14-ploid (ALMEDA, 1997). However, another population of the species in Merida, Venezuela, is reported by to be hexaploid, at n=ca. 27 (ALMEDA, 1997; SOLT and WURDACK, 1980).

Dissotis has been variously reported by Solt and Wurdack (SOLT and WURDACK) as having a chromosome count of n=15 and by Almeda (ALMEDA) as having a chromosome count

of n=10 (ALMEDA, 1997; SOLT and WURDACK, 1980). *D. rotundifolia* is a diploid and is reported to be n=15 (SOLT and WURDACK, 1980). Variable numbers of chromosomes have been recorded even within one species of *Dissotis*, *D. canescens* Hook, which has 2n=28 to 32 (HANSON et al., 2001). *D. canescens* has been reported to have a 4C value of 0.74 pg (HANSON et al., 2001). Melastomaceae chromosomes are reported to be extremely small (0.5 – 1.0 micron) (SOLT and WURDACK, 1980), so low amounts of DNA in diploid species may be the norm for this family, making estimation of genome size via flow cytometry difficult.

Melastomaceae Floral Morphology, Pollination Ecology, and Fertility

Most Melastomaceae species have poricidal anthers and exhibit herkogamy (RENNER, 1989). Herkogamy and poricidal anthers promote outcrossing in Melastomaceae. Automatic selfing is rare, but may occur if the anthers or style bend so that they contact each other, as in the species *Monolena trichopoda* R.H. Warner (RENNER, 1989). Spontaneous self-pollination has also been observed in *Tibouchina papyrus* (Pohl) Toledo and *Tibouchina stenocarpa* (DC.) Cogn., and rarely in *Tibouchina heteromalla* Cogn. (CAMPOS et al.; DOS SANTOS et al., 2012; GOLDENBERG and SHEPHERD, 1998). The poricidal anthers require manipulation for the pollen to disperse. Pollen disperses when the pollinator vibrates the anthers with its flight muscles, a process called buzz pollination (LUO et al., 2008; RENNER, 1989). Although some researchers have reported pollen dehiscing due to the effects of wind or rain, this is rare (RENNER, 1989). In plant breeding or in pollen collection in the field, buzz pollination is mimicked by using a tuning fork in the key of E to get pollen to dehisce (RENNER, 1989). Most species of Melastomaceae offer only pollen as a reward to the pollinator and are pollinated by bees (RENNER, 1989). The nectariferous Melastomaceae species are predominantly pollinated by hummingbirds, although some species are pollinated by bats, rats, or mice (RENNER, 1989).

Many Melastomaceae species exhibit stamen dimorphism (RENNER, 1989). The two sets of stamens may differ in color and morphology as well as position (LUO et al., 2008). The position of the types of stamens is such that one set provides the pollinator with pollen for food and the other provides pollen for pollination, or "functional pollen". The "functional pollen" is deposited on the body of the pollinator in a location that the pollinator cannot reach while grooming itself. Instead of being removed, the "functional pollen" may be deposited onto the stigma of next flower the pollinator visits (LUO et al., 2008; RENNER, 1989). No difference in viability or morphology has been found to exist between either type of pollen in most species (CAMPOS et al.; RENNER, 1989). An exception is *Melastoma malabathricum* L. where pollen from each set of stamens differed in morphology. Pollination stamens also contained more pollen than feeding stamens and a higher percentage of viable pollen (LUO et al., 2008).

In a few Melastomaceae species, flower color changes with pollination or anthesis. In *T. heteromalla*, the stamens and base of the petals changes from white to reddish-purple as anthesis progresses over a period of six to eight days (CAMPOS et al.). Stamens of *T. heteromalla* also changed in color from white to purple during anthesis (CAMPOS et al.). In *Tibouchina sellowiana* (Cham.) Cogn. and *T. pulchura*, flower petals change from pink-edged white to purple over the course of anthesis. Stamens and styles change color as well, from white to pink. (PEREIRA et al., 2011). Although the color change normally takes place by the third day of anthesis, it is delayed for one additional day in unpollinated flowers (PEREIRA et al., 2011). In pollinated flowers, the breeding system also influenced timing of the color change. Apomictic or autonomously selfing flowers remained a paler shade of pink on the second day than did flowers that were self- or cross-pollinated (PEREIRA et al., 2011).

Melastomaceae pollen is binucleate (TOBE and RAVEN, 1984). Pollen is triporate and circular, although it may appears triangular in outline (EDEOGA and EBOKA, 2006; RENNER, 1993).

The pollen-only Melastomaceae species are homogamous (RENNER, 1989). In the few nectariferous species, protandary has been reported (RENNER, 1989). Although most Melastomaceae species have hermaphroditic flowers, the genus *Miconia* also contains dioecious species (RENNER, 1989).

Apomixis in the form of polyembryony has been reported in nine Melastomaceae genera (DOS SANTOS et al., 2012; MENDES-RODRIGUES and OLIVEIRA, 2012; RENNER, 1989). A study of Melastomaceae species in the Brazilian Cerrado, or savannah, region found that 43 of the 120 species studied were apomictic (DOS SANTOS et al., 2012). Another study in the same region found apomixis in 11 out of 20 species; most were in the genus *Miconia* (GOLDENBERG and SHEPHERD, 1998). (GOLDENBERG and SHEPHERD, 1998; MENDES-RODRIGUES and OLIVEIRA, 2012)

Most non-apomictic Melastomaceae species studied have been found to be self-compatible (GOLDENBERG and SHEPHERD, 1998; RENNER, 1989). In a study of Brazilian Cerrado species, 53 of 77 non-apomictic species were self-compatible (DOS SANTOS et al., 2012). In the tribe Melatomeae, 72.2% of species studied were found to be self-compatible (GOLDENBERG and SHEPHERD, 1998). Not every method for testing self-compatibility may distinguish between apomictic and sexually reproducing species; it is possible some species which have been recorded to be self-compatible may in fact be apomicts (DOS SANTOS et al., 2012; RENNER, 1989). Molecular and cytological studies of self-compatible species are necessary to definitively rule out apomixy (DOS SANTOS et al., 2012).

Melastomaceae Seed Germination

Melastomaceae seeds are small and require sowing on top of potting substrate to germinate (SOLT and WURDACK, 1980). The substrate should be peat-based, as the majority of Melastomaceae species are acidophiles (SOLT and WURDACK, 1980). Seeds of Melastomaceae species require light to germinate (ALMASI, 2000; SIMÃO and TAKAKI, 2008). A study of *Tibouchina mutabilis* found that seeds required light at the wavelengths of the visible spectrum for germination (SIMÃO and TAKAKI, 2008). *Tibouchina herbacea* (DC.) Cogn. seeds planted in an area without a canopy cover germinated best (ALMASI, 2000). Seedling survival was better without canopy cover also (ALMASI, 2000). In a study in Costa Rica, seedlings of *Miconia gracilis* Triana and *Miconia nervosa* (Sm.) Triana were grown in two types of open patches: one with all low-growing vegetation removed and one with low-growing vegetation intact. Not only did seedlings in bare areas germinate at a higher percentage, only seedlings in areas without vegetation survived more than one year (ELLISON and DENSLOW, 1993). Seedlings grown in forest understory had a lower rate of germination than seedlings in either of the open patches and none survived past one year (ELLISON and DENSLOW, 1993). Temperature has less influence than light on germination. Fluctuations in temperature between 20° and 30° C had no effect on percentage of *T. mutabilis* seeds germinating (SIMÃO and TAKAKI, 2008). Germination percentage for *T. mutabilis* was less when temperatures were below or above that range (SIMÃO and TAKAKI, 2008).

Germination of seeds of capsular-fruited species has been reported to take one to two weeks (SOLT and WURDACK, 1980). Seed from berry-producing species has been variously reported as germinating in one to two weeks or in one to two months (ELLISON and DENSLOW, 1993; SOLT and WURDACK, 1980). Melastomaceae seedlings grown in cultivation remain small

for several months then begin to grow rapidly (SOLT and WURDACK, 1980). In contrast, field grown Melastomaceae seedlings were found to remain less than 2 cm tall a year after germination (ELLISON and DENSLOW, 1993).

Advantages and Disadvantages of Polyploidy

Polyploidy has several advantages. A possible advantage is heterosis. Diploid hybrids also evince heterosis. However, the effect may be stronger in polyploids, as was shown by a study of diploid and triploid hybrids of maize (AUGER et al., 2005). The effect of polyploidy on heterosis depends on the species, as hybrid vigor is not always more evident in polyploids. In a study of diploid and autotetraploid *Brassicas*, the polyploid plants produced up to 64% less biomass than the diploid plants (ABEL and BECKER, 2007). Homozygous recessives can be masked, increasing the instance of heterozygosity in polyploids (STADLER, 1929). Soltis and Soltis postulated that the proportion of heterozygotes to homozygotes when autotetraploids are selfed can be as high as 1:34:1 (SOLTIS and SOLTIS, 2000). Heterozygosity is an advantage if the crop is being bred to improve a quantitative trait, such as number of flowers, rate of growth, size of flowers, and some types of disease resistance. Heterosis was found to cause significantly faster stem elongation in Lisianthus (*Eustoma grandiflorum* Shinn.) (ECKER, 1993), In a study of red foliage color in flowering dogwood (*Cornus florida* L.), plants with alleles inherited from both parents showed a marked increase in red foliage (WADL et al., 2011). Heterozygosity in *Alstroemeria* influenced both leaf length and width (HAN et al., 2002). A possible disadvantage of polyploidy is that it can alter the expression of genes in the plant. A considerable number of the genes in hexaploid wheat failed to express or exhibited reduced expression (HE et al., 2003). A study of *Arabidopsis suecica* (Fries) Norrlin, Meddel suggested that polyploidy can also cause the deletion of genes and rearrangements of loci due to irregular meiosis (OLGA et al., 2004). The

effects of altered gene expression and epigenetic instability on the progeny of the proposed cross would be unpredictable. Plants exhibiting polyploidy may also be sterile. Evidence of difficulty in completing normal meiosis was found in a study of allohexaploid wheat by Sears (SEARS, 1976). However, inducing polyploidy may restore fertility to sterile diploid hybrids. Treatment of diploid and triploid roses with oryzalin, inducing polyploidy, was shown to increase pollen viability, increasing the fertility of the species (KERMANI et al., 2003).

Fabaceae

The Fabaceae family contains between 670 to 750 genera and 18,000 to 19,000 species (POLHILL et al., 1981). The two genera selected for this project, *Baptisia* and *Thermopsis*, are found mainly in temperate climates such as that of the Southeastern United States. Many species of *Baptisia*, some of them interspecific hybrids, are widely available in nurseries. *Thermopsis* species are not as widely grown as *Baptisia,* especially in the Southeast.

Baptisia and *Thermopsis* belong to the tribe Thermopsidae, which comprises 46 species in six genera (CHEN et al., 1994). Flowers of species in Thermopsidae have 10 non-attached stamens; leaves have petioles and are trifoliate (CHEN et al., 1994). Except for *Thermopsis barbata* Benth. ex Royle, which produces reddish-purple flowers, all species in *Thermopsis* have yellow flowers (CHEN et al., 1994; WU and RAVEN, 1994). Flower color in *Baptisia* ranges from white to yellow to blue (LARISEY, 1940b). In both *Baptisia* and *Thermopsis,* perfect flowers with superior ovaries are borne on racemes; some *Baptisia* species produce flowers that occur individually in leaf axils (CHEN et al., 1994; LARISEY, 1940). Seed pods of *Baptisia* are inflated while those of *Thermopsis* are compressed (CHEN et al., 1994).

Seed of species in both genera are hard and require scarification by either acid or mechanical means for best germination (BOYLE and HLADUN, 2005; CENKCI et al., 2007; VOSS et

al., 1994). *Baptisia* seeds also contain phenolic compounds in the seed coat that inhibit germination of the seeds themselves as well as seeds of other species (VOSS et al., 1994). A study by Bratcher (BRATCHER et al., 1993) on *Baptisia australis* (L.) R. Br. found that stratification for two to four weeks caused seed to germinate at a lower rate than untreated seed, but stratification for periods longer than four weeks caused a higher rate of germination than that of untreated seed (BRATCHER et al., 1993).

Species in *Thermopsis* and *Baptisia* are all herbaceous; they are the only two genera in Thermopsidae that do not consist of species with woody plants (CHEN et al., 1994). *Thermopsis* contains 23 species and has a wide-spread distribution with species endemic to Asia and much of temperate North America (CHEN et al., 1994). Three *Thermopsis* species, *T. fraxinifolia* Nutt. ex M.A. Curtis, *T. mollis* (Michx.) M.A. Curtis ex A. Gray, and *T. villosa* Fernald & B.G. Schub. are native to the Southeastern United States (CHEN et al., 1994). *Baptisia* contains 15 to 17 species and is endemic to the Southeastern and Midwestern United States (AULT, 2003; CHEN et al., 1994; LARISEY, 1940).

Although polyploidy is wide-spread in the Fabaceae family, no polyploids have been found to exist in *Baptisia*. Only two polyploids have been found in *Thermopsis*, *T. gracillis* Howell and *T. divicarpa* A. Nelson (CHEN et al., 1994). The chromosome numbers of both *Baptisia* and *Thermopsis* are based on x=9, with 2n=18 for all species, except for *T. gracillis* and *T. divicarpa*, which are 2n=36 (CHEN et al., 1994; COOPER, 1936).

Biochemical evidence suggests that *Baptisia* evolved from *Thermopsis* in the Southeastern United States (DEMENT and MABRY, 1975). One phylogeny of Thermopsidae places *T. chinensis* Benth. ex S. Moore and *T. villosa* in the same clade as several species of *Baptisia*, including *B. australis*, based on internal transcribed spacer (ITS) sequences (WANG et

al., 2006). Most taxonomists do not recognize interspecific crosses in *Thermopsis*, although Isley postulated that the western *Thermopsis* species had hybridized (DEMENT and MABRY, 1975; ISELY, 1981). However, hybridization readily occurs between species of *Baptisia* (ALSTON and TURNER, 1963; BAETCKE and ALSTON, 1968; DEMENT and MABRY, 1975; LARISEY, 1940a; LEEBENS-MACK and MILLIGAN, 1998).

In the genus *Baptisia*, interspecific crosses have been used to create many novel cultivars (AULT, 2003; AVENT, 2002; CULLINA, 2000). *Thermopsis* has not been used to create hybrids to our knowledge and is also not much known or used in the ornamental plant industry. Yet several species of *Thermopsis* have good horticultural qualities. Many species of *Thermopsis*, particularly *T. villosa*, are very tolerant of both drought and heat, making *Thermopsis* a good substitute for lupines in the Southeastern United States (ARMITAGE, 1989). *Baptisia* has proven difficult to propagate vegetatively through cuttings (AULT, 2003; CULLINA, 2000). *Thermopsis* roots more easily than *Baptisia* (HAWKINS et al., 2013) and will bloom two years after germination, whereas *Baptisia* requires three years to bloom from seed (Personal communication, Heather Alley, State Botanical Garden of Georgia, 2013).

Melastomaceae Research Objectives

No intergeneric crosses exist between genera in the Melastomaceae family. To our knowledge such a cross has not been attempted between the genera *Dissotis* and *Tibouchina*. The overall goals of this project as it relates to the Melastomaceae were to create interspecific and intergeneric hybrids and to investigate the feasibility of hybridization between the two genera and between species within each genus. Morphological differences between the hybrids and the parents could be studied. Such a cross might also result in a plant with improved ornamental qualities. Some of the species in these genera have flowers that are attractive but

small. A hybrid with the larger flowers that may result from the cross between a species with small flowers but a desirable growth habit and a large flowered species would probably be more desirable in the horticulture industry than the smaller-flowered parent.

A secondary goal was to attempt to improve the growth habit of selected species. A temporary improvement of growth habit would be induced by application of a plant growth regulator (PGR). A more permanent improvement would be accomplished by the application of oryzalin to induce polyploidy.

Fabaceae Research Objectives

Although *Baptisia* and *Thermopsis* are closely related, there is no known intergeneric hybrid resulting from a cross between them. Morphological differences between the parent species and the hybrid progeny could be studied.

Baptisia has proven difficult to propagate vegetatively through cuttings (CULLINA, 2000). A *Baptisia-Thermopsis* hybrid that retained all the ornamental qualities of its parents and is able to be reproduced vegetatively more easily than any of the original *Baptisia* species, a possible side-effect of introgression of *Thermopsis* genes, could be of great interest to the horticulture industry. *Thermopsis* has proven to be a very drought-tolerant plant (ARMITAGE, 1989). Drought-tolerance has become an important quality in ornamental plants for the Southeastern United States, since many parts of this region have experienced much lower rainfall than average in recent years. A species that exhibited drought-tolerance as well as the best ornamental qualities of both its parents would be of horticultural importance.

Literature Cited

Abel, S. and Becker, H.C., 2007. The effect of autopolyploidy on biomass production in homozygous lines of *Brassica rapa* and *Brassica oleracea*. *Plant Breeding* 126:642-643.

Almasi, K.N., 2000. A non-native perennial invades a native forest. *Biological Invasions* 2:219-230.

Almeda, F., 1997. Chromosome numbers and their evolutionary significance in some neotropical and paleotropical Melastomataceae. *BioLlania*:167-190.

Almeda, F. and Chuang, T.I., 1992. Chromosome-Numbers and Their Systematic Significance in Some Mexican Melastomataceae. *Syst Bot* 17:583-593.

Alston, R.E. and Turner, B.L., 1963. Natural Hybridization Among Four Species of *Baptisia* (Leguminosae). *Am J Bot* 50:159-173.

Armitage, A.M., 1989. *Herbaceous perennial plants : a treatise on their identification, culture, and garden attributes /* Allan M. Armitage. Athens, Ga. : Varsity Press, 1989.

Auger, D.L., Coe, E.H., Jr., Birchler, J.A., Kato, A., Gray, A.D., and Ream, T.S., 2005. Nonadditive gene expression in diploid and triploid hybrids of maize. *Genetics* 169:389-397.

Ault, J., 2003. Breeding and development of new ornamental plants from North American native taxa. *Acta Horticulturae*:37-42.

Avent, T., 2002. Revenge of the 'Redneck Lupines'. *Horticulture* 99:70.

Baetcke, K.P. and Alston, R.E., 1968. The Composition of a Hybridizing Population of *Baptisia sphaerocarpa* and *Baptisia leucophaea*. *Evolution* 22:157-165.

Bir, S.S., Cheema, P., and Sidhu, M.K., 1988. SOCGI plant chromosome number reports-VII, p. 219-228.

Boyle, T.H. and Hladun, K., 2005. Influence of seed size, testa color, scarification method, and immersion in cool or hot water on germination of *Baptisia australis* (L.) R. Br. seeds. *HortScience : a publication of the American Society for Horticultural Science* 40:1846-1849.

Bratcher, C.B., Dole, J.M., and Cole, J.C., 1993. Stratification improves seed germination of five native wildflower species. *HortScience* 28:899-901.

Cenkci, S., Kargioglu, M., Dayan, S., and Konuk, M., 2007. Endangered status and propagation of an endemic plant species, Thermopsis turcica (Fabaceae). *Asian Journal of Plant Sciences* 6:288-293.

Chen, C.J., Mendenhall, M.G., and Turner, B.L., 1994. Taxonomy of *Thermopsis* (Fabaceae) in North America. *Ann Mo Bot Gard* 81:714-742.

Cooper, D.C., 1936. Chromosome numbers in the Leguminosae. *Am J Bot* 23:231-233.

Cullina, W., 2000. A Guide to Growing and Propagating Native Flowers of North America. 1st ed.:322.

Custers, J.B.M., Eikelboom, W., Bergervoet, J.H.W., and Eijk, J.P.v., 1995. Embryo-rescue in the genus *Tulipa* L.; successful direct transfer of *T. kaufmanniana* Regel germplasm into *T. gesneriana* L. *Euphytica* 82:253-261.

Dement, W.A. and Mabry, T.J., 1975. Biological implications of flavonoid chemistry in *Baptisia* and *Thermopsis*. *Biochemical Systematics and Ecology* 3:91-94.

Ecker, R.B.A., 1993. Quantitative Genetic Analysis of Growth Rate in Lisianthus. *Plant Breeding* 111:253.

Edeoga, H.O. and Eboka, A.U., 2006. Pollen morphology and systematics in some Nigerian species of *Dissotis* Benth. (Melastomataceae). *Journal of Economic and Taxonomic Botany* 30:413-419.

Ellison, A.M. and Denslow, J.S., 1993. Seed and seedling ecology of neotropical Melastomataceae. *Ecology* 74:1733.

Goldenberg, R. and Shepherd, G.J., 1998. Studies on the reproductive biology of Melastomataceae in "cerrado" vegetation. *Plant Systematics and Evolution* 211:13-29.

Han, T.H., Jacobsen, E., Jeu, M.J.d., and Eck, H.J.v., 2002. Mapping of quantitative trait loci involved in ornamental traits in *Alstroemeria*. *HortScience : a publication of the American Society for Horticultural Science* 37:585-592.

Hanson, L., McMahon, K.A., Johnson, M.A.T., and Bennett, M.D., 2001. Regular Article: First Nuclear DNA C-values for 25 Angiosperm Families. *Annals of Botany* 87:251-258.

Hawkins, S.M., Chappell, M., and Martin Jr, M.T., 2013. Defining a Protocol for Vegetative Propagation of *Baptisia*, *Eupatorium* and *Thermopsis*. *Journal of Environmental Horticulture* 31:162-168.

He, P., Zhou, J.M., Gill, B.S., and Friebe, B.R., 2003. Allopolyploidy alters gene expression in the highly stable hexaploid wheat. *Plant molecular biology* 52:401-414.

Isely, D., 1981. Leguminosae of the United States. III. Subfamily Papilionoideae: tribes Sophoreae, Podalyrieae, and Loteae, p. 181.

Kantartzi, S. and Roupakias, D.G., 2008. Breeding barriers between *Gossypium* spp. and species of the Malvaceae family. *Australian Journal of Botany* 56:241-245.

Kermani, M.J., Sarasan, V., Roberts, A.V., Yokoya, K., Wentworth, J., and Sieber, V.K., 2003. Oryzalin-induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. *TAG. Theoretical And Applied Genetics. Theoretische Und Angewandte Genetik* 107:1195-1200.

Larisey, M.M., 1940a. A Monograph of the Genus *Baptisia*. *Ann Mo Bot Gard* 27:119-244.

Larisey, M.M., 1940b. A revision of the North American species of the genus *Thermopsis*. *Ann. Mo. Bot. Gdn.* 27:245-258.

Leebens-Mack, J. and Milligan, B.G., 1998. Pollination Biology in Hybridizing *Baptisia* (Fabaceae) Populations. *Am J Bot* 85:500-507.

Mendes-Rodrigues, C. and Oliveira, P.E., 2012. Polyembryony in Melastomataceae from Brazilian Cerrado: multiple embryos in a small world. *Plant Biology* 14:845-853.

Michelangeli, F.A., Guimaraes, P.J.F., Penneys, D.S., Almeda, F., and Kriebel, R., 2013. Phylogenetic relationships and distribution of New World Melastomeae (Melastomataceae). *Bot J Linn Soc* 171:38-60.

Odimegwu, D.C., Ibezim, E.C., Esimone, C.O., Nworu, C.S., and Okoye, F.B.C., 2008. Wound healing and antibacterial activities of the extract of *Dissotis theifolia* (Melastomataceae) stem formulated in a simple ointment base. *Journal of Medicinal Plants Research* 2:11-16.

Olga, P., Nuno, N., Manuela, S., Michelle, S.L., Andreas, M., Luca, C., Wanda, V., Craig, S.P., and Susan, R.W., 2004. Chromosomal Locus Rearrangements Are a Rapid Response to Formation of the Allotetraploid Arabidopsis suecica Genome. *Proceedings of the National Academy of Sciences of the United States of America* 101:18240-18245.

Polhill, R.M., Raven, P.H., and Stirton, C.H., 1981. Evolution and systematics of the Leguminosae, p. 1-26, Advances in legume systematics. Part 1 [Polhill, R.M.; Raven, P.H. (Editors)]. Royal Botanic Gardens., Kew, Richmond; UK.

Porembski, S., Szarzynski, J., Mund, J.P., and Barthlott, W., 1996. Biodiversity and vegetation of small-sized inselbergs in a West African rain forest (Taï, Ivory Coast). *Journal of Biogeography* 23:47-55.

Renner, S.S., 1989. A Survey of Reproductive Biology in Neotropical Melastomataceae and Memecylaceae. *Ann Mo Bot Gard* 76:496-518.

Renner, S.S., 1993. Phylogeny and classification of the Melastomataceae and Memecylaceae. *Nordic Journal of Botany* 13:519-540.

Renner, S.S., Clausing, G., and Meyer, K., 2001. Historical Biogeography of Melastomataceae: The Roles of Tertiary Migration and Long-Distance Dispersal. *Am J Bot*:1290.

Sarkar, D., Tiwari, J.K., Sushruti, S., Poonam, Sanjeev, S., Gopal, J., Singh, B.P., Luthra, S.K., Pandey, S.K., and Pattanayak, D., 2011. Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. *Plant Cell, Tissue and Organ Culture* 107:427-440.

Sears, E.R., 1976. GENETIC CONTROL OF CHROMOSOME PAIRING IN WHEAT. *Annual Review of Genetics* 10:31-51.

Simão, E. and Takaki, M., 2008. Effect of light and temperature on seed germination in *Tibouchina mutabilis* (Vell.) Cogn. (Melastomataceae). *Biota Neotropica* 8:63-68.

Solt, M.L. and Wurdack, J.J., 1980. Chromosome numbers in the Melastomataceae. *Phytologia* 47:199-220.

Soltis, P.S. and Soltis, D.E., 2000. The role of genetic and genomic attributes in the success of polyploids. In: Ayala, F. J., Fitch, W. M., and Clegg, M. T. (eds.), Variation and evolution in plants and microorganisms: toward a new synthesis 50 years after Stebbins. Proceedings of a colloquium, Irvine, California, USA, 27-29 January, 2000. National Academy Press, Washington; USA.

Stadler, L.J., 1929. Chromosome number and the mutation rate in avena and triticum. *Proceedings of the National Academy of Sciences of the United States of America* 15:876-881.

Syiem, D. and Khup, P.Z., 2006. Study of the traditionally used medicinal plant *Osbeckia chinensis* for hypoglycemic and anti-hyperglycemic effects in mice. *Pharmaceutical Biology* 44:613-618.

Voss, K., Harnischfeger, G., Lieberei, R., and Mevenkamp, G., 1994. Seed germination behaviour of *Baptisia tinctoria* (L.) R. Br. *Angewandte Botanik* 68:53-59.

Wadl, P.A., Saxton, A.M., Wang, X.W., Pantalone, V.R., Rinehart, T.A., and Trigiano, R.N., 2011. Quantitative trait loci associated with red foliage in *Cornus florida* L. *Molecular Breeding* 27:409-416.

Wang, H.C., Yang, J.B., Compton, J.A., and Sun, H., 2006. A phylogeny of Thermopsideae (Leguminosae: Papilionoideae) inferred from nuclear ribosomal internal transcribed spacer (ITS) sequences. *Bot J Linn Soc* 151:365-373.

Wu, Z. and Raven, P.H., 1994. *Flora of China / Wu Zheng-yi and Peter H. Raven, co-chairs of the editorial committee*. Beijing : Science Press ; St. Louis : Missouri Botanical Garden, c1994-.

CHAPTER 2

Interspecific and Intergeneric Hybridization in *Dissotis and Tibouchina*[1]

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Abstract

Intergeneric and interspecific crosses are often used to create novel cultivars. The Melastomaceae family, a large family of mainly tropical and subtropical plants, contains several species already used in the ornamental trade. Species in two genera of the Melastomaceae family, *Dissotis* and *Tibouchina*, were used in an attempt to create interspecific and intergeneric hybrids. Intergeneric crosses set seed at a rate of 17.9% and interspecific crosses had a 28.7% rate of seed set. Germination was extremely poor, as only 0.31% of intergeneric crosses and 0.66% of interspecific crosses germinated. Crosses produced 31 seedlings. Three of the seedlings were from intergeneric crosses and 28 were from interspecific crosses. The prognosis for conventional breeding for species in *Dissotis* and *Tibouchina* is poor due to low seed set, poor germination, and slow growth of progeny.

Introduction

Hybrids between different species and genera have long been used in horticulture. Some of the advantages of hybridization are an increased vigor of the progeny, the production of a plant with the best characteristics of both parents, and a possible increase in pest or disease resistance. Whether the objective of hybridization is to produce an ornamental plant, or a plant used for food, fiber, phytoremediation, or medicine, it is important to choose parent species with desirable qualities.

Interspecific and intergeneric crosses have been made to confer resistance to pests and diseases or increased adaptation to environmental conditions. A cross between chrysanthemum (*Dendranthema morifolium* (Ramat.) ‘Zhongshanjingui’) and *Artemesia vulgaris* L. ‘Variegata’ conferred resistance to chrysanthemum aphids on the resulting hybrid (DENG et al., 2010a). A

cross between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. produced hybrids that were highly resistant to late blight (SARKAR et al., 2011). Cold tolerance in chrysanthemum was enhanced by an intergeneric cross between *Chrysanthemum* spp. and *Ajania przewalskii* Poljak. (DENG et al., 2011).

Incompatibilities between parents in an interspecific or intergeneric cross can lead to poor seed set. Techniques exist to overcome this challenge. In a breeding project to develop hybrids resistant to papaya ringspot virus from a cross between *Carica papaya* and *Vasconcellea cauliflora* J., treating the stigmas before pollination with a nutrient solution of either sucrose, sucrose plus boron, or sucrose with calcium chloride proved effective in increasing fruit set (JAYAVALLI et al., 2011). Both embryo rescue and ovule culture were successful in producing seedlings in interspecific crosses of *Tulipa* L. (CUSTERS et al., 1995)

The Melastomaceae family comprises between 185 to 190 genera containing 5000 species (ALMEDA and CHUANG, 1992). The distribution of species in the Melastomaceae is diverse. Melastomaceae species are found in Asia, Africa, North and South America, and Australia; South America contains the majority of species (MICHELANGELI et al., 2013; RENNER and MEYER, 2001). Some species, such as *Dissotis rotundifolia* (Sm.) Triana, have naturalized in places with tropical climates such as Indonesia, Brazil, Puerto Rico, and Hawaii (LIOGIER and MARTORELL, 1982; RENNER and MEYER, 2001). The two genera from the Melastomaceae family that were chosen for this project are *Dissotis* and *Tibouchina*. *Dissotis* is native to Africa and *Tibouchina* to South America (RENNER and MEYER, 2001). *Tibouchina* contains species of horticultural importance while *Dissotis* species have not been widely used as ornamentals.

Tibouchina chromosome numbers are based on x=9, ranging from n=9 to n=63 (ALMEDA, 1997; ALMEDA and CHUANG, 1992). Many species of *Tibouchina* are polyploid. Although tetraploidy is the most common polyploid level, ploidy level varies (ALMEDA, 1997). *Dissotis* has been variously reported by Solt and Wurdack as having a chromosome count of n=15 and by Almeda as having a chromosome count of n=10 (ALMEDA, 1997; SOLT and WURDACK, 1980). *D. rotundifolia* is a diploid and is reported to be n=15 (SOLT and WURDACK, 1980).

Most Melastomaceae species have poricidal anthers and exhibit herkogamy (RENNER, 1989). Herkogamy and poricidal anthers promote outcrossing in Melastomaceae. The poricidal anthers require manipulation for the pollen to disperse. Pollen disperses when the pollinator vibrates the anthers with its flight muscles, a process called buzz pollination (LUO et al., 2008; RENNER, 1989). Although some researchers have reported pollen dispersing due to the effects of wind or rain, this is rare (RENNER, 1989). In plant breeding or in pollen collection in the field, buzz pollination is mimicked by using a tuning fork in the key of E to get pollen to dehisce (RENNER, 1989). Melastomaceae seeds are small and require sowing on top of potting substrate to germinate (SOLT and WURDACK, 1980). The substrate should be peat-based, as the majority of Melastomaceae species are acidophiles (SOLT and WURDACK, 1980). Seeds of Melastomaceae species require light to germinate (ALMASI, 2000; SIMÃO and TAKAKI, 2008). Germination of seeds of capsular-fruited species, such as those in *Dissotis* and *Tibouchina*, has been reported to take one to two weeks (SOLT and WURDACK, 1980). Melastomaceae seedlings grown in cultivation remain small for several months then begin to grow rapidly (SOLT and WURDACK, 1980). The overall goals of this project were to investigate the feasibility of hybridization within

and between various species of *Dissotis* and *Tibouchina* create interspecific and intergeneric hybrids of these genera.

Materials and Methods

Various species of *Dissotis* and *Tibouchina* species were placed in a greenhouse in Athens, Georgia in February 2012 (Table 2.1). Two sets of *D. rotundifolia* and of *Tibouchina fothergillae x pilosa* were acquired. One set of each species was obtained from the Trial Gardens at the University of Georgia Athens campus and the other was from the University of Georgia (UGA) Tifton campus. The plants in the Trial Garden had originally come from the Tifton campus. *D. rotundifolia* plants were originally seedlings from the same seed lot. *T. fothergillae* x *pilosa* plants had been clonally propagated from the same stock plant. Cuttings of each species were taken, treated with a 5s dip of K-IBA at 1000 $mg \cdot l^{-1}$, and stuck in Fafard Germination Mix containing processed pine bark (40%), Canadian sphagnum peat, perlite, and vermiculite (Sun Grow Horticulture, Agawam, MA), and placed under intermittent mist. After 6 weeks cuttings were potted up in Fafard 3B Mix consisting of Canadian sphagnum peat (45%), processed pine bark, perlite, and vermiculite in 2.8 liter trade containers.

Dissotis species started blooming in July 2012. *Tibouchina* species started blooming in August 2012. Interspecific and intergeneric crosses were made as species came into bloom beginning July 2012 and ending in March 2013. Female flowers were emasculated to prevent self-pollination. Emasculations were carried out before anthers had completely unfolded when possible. Pollen was extracted from flowers of male parents by vibrating anthers with a tuning fork (key of E). Pollen was captured in glass containers and applied to the stigmas of flowers of the female parents using either a Q-tip or a small paintbrush. A total of 1,036 crosses were made; 588 crosses were interspecific and 448 crosses were intergeneric.

Fruits were harvested when ripe. Since all species used produced capsular fruit, fruits were determined to be ripe when they were light to medium brown and hard. Seed was extracted from the ripe fruits and examined under a Stereomaster dissecting microscope to assess probable viability. Seed that was shriveled and flattened was deemed to be non-viable. Seed determined to be of probable viability was sown in 0.56 liter pots on top of Fafard Germination Mix.

Two different methods of germination were used. The first method, used from September to November 2012, was to use a humidity tent. Seeds were sown on top of media and kept under high humidity in the laboratory. Beginning in January 2013, harvested seeds were sown on top of media and placed under mist in a greenhouse.

Seedlings that germinated were transplanted into separate 0.6 liter pots containing Fafard Germination Mix and placed in a greenhouse under 50% shade. Once seedlings had grown to at least two to three inches tall, they were transplanted into 2.8 liter trade containers filled with a pine bark substrate with micronutrients and removed from shade.

Seed set from initial crosses was low, so pollen germination and pollen tube growth through the styles were evaluated to determine whether barriers to fertilization existed between the species used as parents in the study. Intergeneric and interspecific crosses (Table 2.2) were performed during January and February 2013. The number of repetitions of each cross was dependent on the number of flowers available. Some species were less floriferous in the winter, such as *D. princeps, T. granulosa* 'Gibraltar', and *T. lepidota,* so fewer crosses were performed using those species. Flowers were emasculated, and pollen was gathered from the male parent by striking the anthers with a tuning fork causing the pollen to dehisce into a glass container. Once collected, the pollen was applied to the stigma of the female parent. Styles were harvested 24 hours after pollination and placed in ethanol:acetic acid (1:2 w/v) for 1 to 24 hours. They were

transferred to 65% ethanol for 20 minutes, autoclaved in 0.8 mol/L NaOH at 120°C for 20 minutes, and stained with 0.1% aniline blue in 0.1M K_3PO_4 (BO et al., 2009; LEDESMA and SUGIYAMA, 2005). The styles were kept in the aniline blue for 4 to 24 hours. The styles were then mounted on slides and examined under an Olympus BX51 fluorescent microscope. Pictures were taken with an Olympus D70 microscope camera and Olympus DP Controller software.

Data was taken on number of crosses producing fruit with seed of probable viability and number of crosses germinating. Percentages of seed-producing crosses and germinating crosses were calculated from this data. Percentages were arcsine transformed before analysis and then transformed back before reporting. Data was analyzed using SAS 9.3 and PROC GLM (SAS Institute, Inc., Cary, NC). Means separation was performed using Tukey's LSD where $p < 0.05$.

Pollen tube data was taken on pollen tube germinations performed and the number of tests in which pollen tubes reached the end of the style.

Results

Pollen tube germination tests showed that prezygotic barriers to fertilization due to pollen and pistil incompatibility did not exist in the crosses tested. Pollen tubes were able to grow to the end of the style in every cross tested (Table 2.2) (Figures 2.1 and 2.2). Intergeneric crosses and interspecific crosses showed the same pattern.

Seed set from both intergeneric and interspecific crosses was low. Intergeneric crosses resulted in 17.9% seed set while interspecific crosses set seed at a rate of 28.7% (Table 2.3). No difference was found in the percentage of seed set between intergeneric or interspecific crosses made ($p = 0.1598$).

The first method of propagation, germinating seed under high humidity, produced no seed germination after 90 days. All germinating seed was propagated by the second method,

placing seeds under intermittent mist in the greenhouse. Germination time ranged from two weeks to nine weeks.

Germination was low for seeds of both intergeneric and interspecific crosses, and germination percentages were not found to be different ($p = 0.5106$) between the two types of crosses. Overall germination percentage of interspecific crosses was 0.66% while the percentage of intergeneric crosses with germinating seed was 0.31% (Table 2.3).

Seeds from only three crosses germinated; one of the crosses was intergeneric and two were interspecific. (Table 2.4). *D. canescens* by *D. princeps* produced only three seedlings while *D. princeps* by *D. rotundifolia* produced 25 seedlings. Three seedlings were obtained from the cross of *D. canescens* by *T. lepidota*. Morphological examination of the seedlings showed that seedlings resembled female parents in growth habit, leaf shape, and leaf size (Figures 2.3 and 2.4). Although several of the *D. princeps* x *D. rotundifolia* putative hybrids eventually bloomed, all plants except one had lavender-colored flowers like the female parent. One *D. princeps* x *D. rotundifolia* putative hybrid produced some pink flowers (Figure 2.4a) as well as lavender flowers, but did not survive. Leaf morphology of the *D. princeps* x *D. rotundifolia* putative hybrids was like that of the female parent (Figures 2.4b and 2.4c). One seedling of the *D. canescens* x *T. lepidota* cross bloomed and the flower was exactly like that of the female parent in size, shape, petal number, and color (Figure 2.4e). Leaf morphology of the *D. canescens* x *T. lepidota* putative hybrid was also exactly like that of the female parent (Figure 2.4e). Although seedlings strongly resembled the female parent, they were weak and did not grow vigorously. Seedling performance was poorer than that of either parent, suggesting that they could be hybrids. Seedling mortality was high. Of the 31 seedlings produced, 9 survived as of 7 April 2014. All the surviving seedlings were from the *D. princeps* x *D. rotundifolia* cross.

Interspecific and intergeneric crosses were analyzed to determine which species might be better male or female parents. In interspecific crosses, a difference in percentage of crosses setting seed was found among female parents ($p = 0.0154$) (Table 2.5). Both populations of *D. rotundifolia* as well as *D. princeps* had higher percentages of seed set than any of the *Tibouchina* species used as female parents. No plants of either population of *T. fothergillae x pilosa* set seed when used as female parents. No difference in percentage of crosses with germinating seed was found among female parents in interspecific crosses ($p = 0.8946$) (Table 2.5). No difference in percentage of crosses setting seed ($p = 0.084$) or in percentage of crosses with germinating seed was found among male parents used in interspecific crosses (Table 2.6).

In intergeneric crosses, no difference was found among female parents in percentage of crosses setting seed ($p = 0.3345$) (Table 2.7), nor was there a difference among female parents in percentage of crosses with germinating seed ($p = 0.3464$) (Table 2.7). Male parents showed no difference in percentage of crosses setting seed ($p = 0.485$) or in percentage of crosses with germinating seed ($p = 0.8693$) (Table 2.8).

Detailed data and results for crosses are presented in Table 2.9.

Discussion

Seed set was obtained for both intergeneric and interspecific crosses, although rate of seed set was low. Fertility of crosses may not only depend on the species used, but on which species are used as the male or female parent. In this study, although there was a difference in seed set between some of the male and female parents, there was no statistical difference in percentage of germination. Statistically, the species used as either male or female parent did not influence cross fertility. However, there was a clear biological difference. The only female

parents with germinating seed were *D. canescens* and *D. princeps.* The only male parents with germinating seed were *D. princeps, D. rotundifolia,* and *T. lepidota*.

Pollen-pistil incompatibility was not found for the crosses tested so was likely not a cause of low fertility. Ovules of Melastomaceae species were too small to examine for the pollen tube entering the ovule. Even though pollen tubes were shown to grow to the end of the flower styles, it was unclear whether fertilization occurred in every case. Low rates of seed set could have been at least partly due to lack of fertilization.

Post-zygotic factors leading to embryo abortion are more likely to be the reason for low seed set. Many of the fruits examined only produced flat, undeveloped seeds. Possibly the embryo and the endosperm were incompatible, leading to embryo abortion soon after fertilization. Embryo rescue is often used when seeds abort due to post-fertilization reasons. But seeds obtained from the intergeneric and interspecific crosses in this study were too small (approximately 1 mm in diameter) to feasibly use embryo rescue. If further crosses are made, ovule culture is a possibility for increasing the number of progeny from crosses. Ovule culture may be performed earlier than embryo rescue, and does not require the isolation of the embryo from the ovule. In a study of embryo rescue in tulips, ovule culture had a higher rate of producing viable seedlings than embryo culture and could be successfully performed four weeks earlier (CUSTERS et al., 1995). Experiments would need to be performed to determine if ovule culture would produce more viable seedlings than conventional germination and the optimum timing for the procedure.

Of most concern is the extremely low rate of seed germination. Visual examination of the seed was used to determine probably viability, as a more definitive method such as a tetrazolium test would be destructive to the seeds. Seed that was flat instead of rounded was deemed to be

non-viable. Flattish, collapsed seed was found to be a sign of aborted embryos in *Epilobium angustifolium* L. (WIENS et al., 1987); it is probable that flat seed in this study also contained aborted embryos. Even though the Melastomaceae seed used for germination appeared to have fully developed embryos and endosperm judging by size and conformation, germination of both interspecific and intergeneric crosses was extremely poor. Incompatibility between the embryos and the endosperm could account for poor germination (WIENS et al., 1987).

Another factor to consider in the low germination rate is the propagation protocol used. No germination resulted when seeds were kept under a humidity tent, although this method had worked for other researchers (SOLT and WURDACK, 1980). Even seed of *D. rotundifolia* that was obtained from B & T World Seeds failed to germinate under the humidity tent, but did germinate once placed on the mist bench. Probably the mist mimicked the rainy season of the regions where the species in this study originated. Conditions in the humidity tent were likely too different from the normal environment of the Melastomaceae species to produce good germination. Seeds in the humidity tent might not have received the amount of moisture they needed to germinate. Possibly the seed coats contained phenolic compounds that hindered germination until the compounds were leached away. Since all germination of seed of the intergeneric and interspecific crosses occurred on the mist bench, the recommended protocol for germination is to sow seed on top of media and place under intermittent mist until seed germinates. After germination, seedlings should be carefully monitored and transplanted once true leaves have emerged. Continuing to keep seedlings on the mist bench beyond that point seemed to retard their growth, which is extremely slow in any case.

Low germination of seed from crosses and subsequent slow growth of the seedlings make the species used in this study poor prospects for a conventional breeding program. Seedlings

took several months from germination to grow more than two or three inches tall. Once their roots filled the 0.6 liter pots into which they were initially transplanted, the seedlings grew rapidly and commenced flowering. At this point, further crosses could have been made to produce an F_2 generation. Segregation of maternal and paternal traits might have been apparent in progeny of the F_2 generation. But low seed set, poor germination, and slow progeny growth from the initial crosses were the main factors in the decision not to continue the study past the first generation of crosses.

Species in *Dissotis* and *Tibouchina* have high ornamental value; some species have valuable practical uses as well. Methods such as inducing mutations through EMS or radiation might create novel cultivars for the ornamental industry. But creating interspecific and intergeneric hybrids through conventional breeding would take too long to be economically or practically feasible. Small size of the chromosomes would also lead to less recombination, making it more difficult to create novel cultivars. If conventional breeding is carried out on Melastomaceae species, the species to be used should be examined for pollen viability and compatibility of parents.

Literature Cited

Almasi, K.N., 2000. A non-native perennial invades a native forest. *Biological invasions* 2:219-230.

Almeda, F., 1997. Chromosome numbers and their evolutionary significance in some neotropical and paleotropical Melastomataceae. *BioLlania*:167-190.

Almeda, F. and Chuang, T.I., 1992. Chromosome-Numbers and Their Systematic Significance in Some Mexican Melastomataceae. *Syst Bot* 17:583-593.

Bo, J., Zonggen, S., Jinbo, S., Da, Y., Xianyong, S., and Hongfei, L., 2009. Germination and growth of sponge gourd (*Luffa cylindrica*) pollen tubes and FTIR analysis of the pollen tube wall. *Scientia Horticulturae* 122:638-644.

Custers, J.B.M., Eikelboom, W., Bergervoet, J.H.W., and Eijk, J.P.v., 1995. Embryo-rescue in the genus *Tulipa* L.; successful direct transfer of *T. kaufmanniana* Regel germplasm into T. gesneriana L. *Euphytica* 82:253-261.

Deng, Y., Chen, S., Chen, F., Cheng, X., and Zhang, F., 2011. The embryo rescue derived intergeneric hybrid between chrysanthemum and *Ajania przewalskii* shows enhanced cold tolerance. *Plant Cell Reports* 30:2177-2186.

Deng, Y., Chen, S., Lu, A., Chen, F., Tang, F., Guan, Z., and Teng, N., 2010. Production and characterisation of the intergeneric hybrids between *Dendranthema morifolium* and *Artemisia vulgaris* exhibiting enhanced resistance to chrysanthemum aphid (Macrosiphoniellasanbourni). *Planta* 231:693-703.

Jayavalli, R., Balamohan, T.N., Manivannan, N., and Govindaraj, M., 2011. Breaking the intergeneric hybridization barrier in *Carica papaya* and *Vasconcellea cauliflora*. *Scientia Horticulturae* 130:787-794.

Ledesma, N. and Sugiyama, N., 2005. Pollen Quality and Performance in Strawberry Plants Exposed to High-temperature Stress. *Journal of the American Society for Horticultural Science* 130:341-347.

Liogier, A.H. and Martorell, L.F., 1982. *Flora of Puerto Rico and adjacent islands : a systematic synopsis / Henri Alain Liogier, Luis F. Martorell*. Río Piedras, P.R. : Editorial de la Universidad de Puerto Rico, 1982. 1a ed.

Luo, Z., Zhang, D., and Renner, S.S., 2008. Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. *Functional Ecology* 22:794-800.

Michelangeli, F.A., Guimaraes, P.J.F., Penneys, D.S., Almeda, F., and Kriebel, R., 2013. Phylogenetic relationships and distribution of New World Melastomeae (Melastomataceae). *Bot J Linn Soc* 171:38-60.

Renner, S.S., 1989. A Survey of Reproductive Biology in Neotropical Melastomataceae and Memecylaceae. *Ann Mo Bot Gard* 76:496-518.

Renner, S.S. and Meyer, K., 2001. Melastomeae come full circle: biogeographic reconstruction and molecular clock dating. *Evolution* 55:1315-1324.

Sarkar, D., Tiwari, J.K., Sushruti, S., Poonam, Sanjeev, S., Gopal, J., Singh, B.P., Luthra, S.K., Pandey, S.K., and Pattanayak, D., 2011. Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. *Plant Cell, Tissue and Organ Culture* 107:427-440.

Simão, E. and Takaki, M., 2008. Effect of light and temperature on seed germination in *Tibouchina mutabilis* (Vell.) Cogn. (Melastomataceae). *Biota Neotropica* 8:63-68.
Solt, M.L. and Wurdack, J.J., 1980. Chromosome numbers in the Melastomataceae. *Phytologia* 47:199-220.
Wiens, D., Calvin, C., Wilson, C., Davern, C., Frank, D., and Seavey, S., 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* 71:501.

Tables and Figures

Table 2.1. Melastomaceae species used as parents in breeding program.

Species
Dissotis canescens
Dissotis debilis
Dissotis princeps
Dissotis rotundifolia
Tibouchina fothergillae x pilosa
Tibouchina granulosa 'Gibraltar'
Tibouchina lepidota

Table 2.2. Interspecific and intergeneric crosses in *Dissotis* and *Tibouchina* performed to assess pollen tube germination.

Female Parent	**Male Parent**	**Repetitions**
D. debilis	*D. rotundifolia*	11
D. debilis	*T. lepidota*	5
D. princeps	*D. rotundifolia*	2
D. princeps	*T. granulosa 'Gibraltar*	5
D. princeps	*T. lepidota*	8
D. rotundifolia	*D. debilis*	11
D. rotundifolia	*D. princeps*	14
D. rotundifolia	*T. granulosa'Gibraltar'*	14
D. rotundifolia	*T. lepidota*	10
T. granulosa'Gibraltar'	*D. princeps*	1
T. lepidota	*D. princeps*	3

Table 2.3. Summary of results for intergeneric and interspecific Melastomaceae crosses.

Type of Cross	No. of Crosses	No. of Crosses with Seed	% Crosses with Seed	No. Crosses Germinating	% Crosses Germinating
Intergeneric	448	81	17.9	1	0.31
Interspecific	588	190	28.7	3	0.66
Significance			NS		NS

* - significant at P <= 0.05, NS – not significant.

Table 2.4. Number of seedlings produced for Melastomaceae crosses with germinating seed.

Female Parent	Male Parent	No. of Crosses	Crosses with Seed	Crosses with Germinating Seed	No. of Seedlings
D. canescens	*D. princeps*	68	26	2	3
D. canescens	*T. lepidota*	46	12	1	3
D. princeps	*D. rotundifolia*	61	29	1	25

Table 2.5. Percentage of crosses with seed set and germinating seed for female parents in interspecfic crosses.

Female Parent	**% Crosses with Seed Set**	**% Crosses with Germinating Seed**
D. canescens	33.4abc	1.9
D. debilis	14.3bcd	0.0
D. princeps	35.6ab	1.6
D. rotundifolia (TG)	44.7a	0.0
D. rotundifolia (Tifton)	41.9ab	0.0
T. fothergillae x pilosa (TG)	0.0d	0.0
T. fothergillae x pilosa (Tifton)	0.0d	0.0
T. lepidota	4.3cd	0.0
Significance	*	NS

* - significant at P <= 0.05, NS – not significant.

Table 2.6. Percentage of crosses with seed set and germinating seed for male parents in interspecfic crosses.

Male Parent	**% Crosses with Seed Set**	**% Crosses with Germinating Seed**
D. canescens	23.9	0.0
D. debilis	43.5	0.0
D. princeps	35.7	1.9
D. rotundifolia (TG)	28.0	2.1
D. rotundifolia (Tifton)	32.6	0.0
T. fothergillae x pilosa (TG)	4.3	0.0
T. lepidota	0.0	0.0
Significance	NS	NS

* - significant at P <= 0.05, NS – not significant.

Table 2.7. Percentage of crosses with seed set and germinating seed for female parents in intergeneric crosses.

Female Parent	**% Crosses with Seed Set**	**% Crosses with Germinating Seed**
D. canescens	31.0	2.8
D. debilis	14.6	0.0
D. princeps	14.3	0.0
D. rotundifolia (TG)	34.4	0.0
D. rotundifolia (Tifton)	51.0	0.0
T. fothergillae x pilosa (TG)	2.9	0.0
T. fothergillae x pilosa (Tifton)	2.2	0.0
T. lepidota	1.5	0.0
Significance	NS	NS

* - significant at P <= 0.05, NS – not significant.

Table 2.8. Percentage of crosses with seed set and germinating seed for male parents in intergeneric crosses.

Male Parent	**% Crosses with Seed Set**	**% Crosses with Germinating Seed**
D. canescens	0.0	0.0
D. debilis	0.0	0.0
D. princeps	8.1	0.0
D. rotundifolia (TG)	0.0	0.0
D. rotundifolia (Tifton)	0.0	0.0
T. fothergillae x pilosa (TG)	20.9	0.0
T. fothergillae x pilosa (Tifton)	46.4	0.0
T. granulosa 'Gibraltar'	33.3	0.0
T. lepidota	16.1	1.7
Significance	NS	NS

* - significant at P <= 0.05, NS – not significant.

Table 2.9. Detailed data for Melastomaceae crosses.

Female Parent	Male Parent	No. of Crosses	No. of Crosses with Seed	% Crosses with Seed	No. Crosses with Germinating Seed	% Crosses with Germinating Seed
D. canescens	*D. debilis*	52	25	48.1%	0	0.0%
D. canescens	*D. princeps*	68	26	38.2%	2	7.7%
D. canescens	*D. rotundifolia (TG)*	88	20	22.7%	0	0.0%
D. canescens	*D. rotundifolia (Tifton)*	17	4	23.5%	0	0.0%
D. canescens	*T. fothergillae x pilosa (TG)*	49	20	40.8%	0	0.0%
D. canescens	*T. fothergillae x pilosa (Tifton)*	35	9	25.7%	0	0.0%
D. canescens	*T. lepidota*	46	12	26.1%	1	8.3%
D. debilis	*D. canescens*	19	1	5.3%	0	0.0%
D. debilis	*D. princeps*	42	6	14.3%	0	0.0%
D. debilis	*D. rotundifolia (TG)*	26	1	3.8%	0	0.0%
D. debilis	*D. rotundifolia (Tifton)*	6	2	33.3%	0	0.0%
D. debilis	*T. fothergillae x pilosa (TG)*	6	2	33.3%	0	0.0%
D. debilis	*T. fothergillae x pilosa (Tifton)*	10	1	10.0%	0	0.0%
D. debilis	*T. lepidota*	16	0	0.0%	0	0.0%
D. princeps	*D. canescens*	10	2	20.0%	0	0.0%
D. princeps	*D. debilis*	4	1	25.0%	0	0.0%
D. princeps	*D. rotundifolia (TG)*	29	16	55.2%	1	6.3%
D. princeps	*D. rotundifolia (Tifton)*	32	13	40.6%	0	0.0%
D. princeps	*T. fothergillae x pilosa (TG)*	12	0	0.0%	0	0.0%
D. princeps	*T. fothergillae x pilosa (Tifton)*	7	1	14.3%	0	0.0%
D. princeps	*T. granulosa 'Gibraltar'*	6	2	33.3%	0	0.0%
D. princeps	*T. lepidota*	45	4	8.9%	0	0.0%
D. rotundifolia (TG)	*D. canescens*	29	10	34.5%	0	0.0%
D. rotundifolia (TG)	*D. debilis*	14	7	50.0%	0	0.0%
D. rotundifolia (TG)	*D. princeps*	57	28	49.1%	0	0.0%
D. rotundifolia (TG)	*T. fothergillae x pilosa (TG)*	31	9	29.0%	0	0.0%
D. rotundifolia (TG)	*T. fothergillae x pilosa (Tifton)*	12	4	33.3%	0	0.0%
D. rotundifolia (TG)	*T. lepidota*	27	11	40.7%	0	0.0%
D. rotundifolia (Tifton)	*D. canescens*	17	6	35.3%	0	0.0%

D. rotundifolia (Tifton)	*D. debilis*	6	3	50.0%	0	0.0%
D. rotundifolia (Tifton)	*D. princeps*	45	18	40.0%	0	0.0%
D. rotundifolia (Tifton)	*T. fothergillae x pilosa (TG)*	6	0	0.0%	0	0.0%
D. rotundifolia (Tifton)	*T. fothergillae x pilosa (Tifton)*	1	1	100.0%	0	0.0%
D. rotundifolia (Tifton)	*T. lepidota*	29	1	3.4%	0	0.0%
T. fothergillae x pilosa (TG)	*D. debilis*	2	0	0.0%	0	0.0%
T. fothergillae x pilosa (TG)	*D. princeps*	17	2	11.8%	0	0.0%
T. fothergillae x pilosa (TG)	*D. rotundifolia (TG)*	16	0	0.0%	0	0.0%
T. fothergillae x pilosa (TG)	*D. rotundifolia (Tifton)*	10	0	0.0%	0	0.0%
T. fothergillae x pilosa (TG)	*T. lepidota*	3	0	0.0%	0	0.0%
T. fothergillae x pilosa (Tifton)	*D. princeps*	15	1	6.7%	0	0.0%
T. fothergillae x pilosa (Tifton)	*D. rotundifolia (TG)*	20	0	0.0%	0	0.0%
T. fothergillae x pilosa (Tifton)	*D. rotundifolia (Tifton)*	5	0	0.0%	0	0.0%
T. fothergillae x pilosa (Tifton)	*T. lepidota*	1	0	0.0%	0	0.0%
T. lepidota	*D. canescens*	1	0	0.0%	0	0.0%
T. lepidota	*D. princeps*	17	1	5.9%	0	0.0%
T. lepidota	*D. rotundifolia (TG)*	6	0	0.0%	0	0.0%
T. lepidota	*D. rotundifolia (Tifton)*	1	0	0.0%	0	0.0%
T. lepidota	*T. fothergillae x pilosa (TG)*	23	1	4.3%	0	0.0%

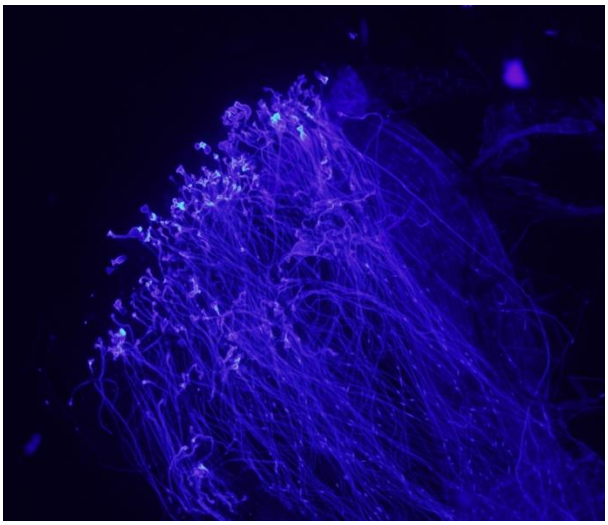

Figure 2.1a. Pollen germinating on end of stigma and beginning of pollen tubes in *D. debilis x T. lepidota* cross.

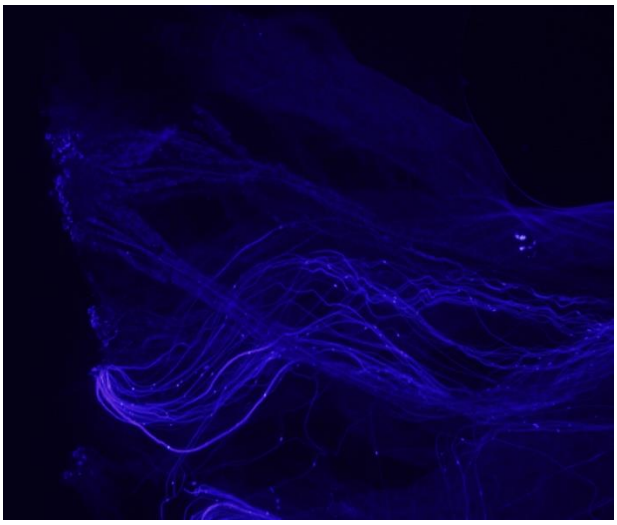

Figure 2.1b. Pollen tubes growing to end of style in *D. debilis x T. lepidota* cross.

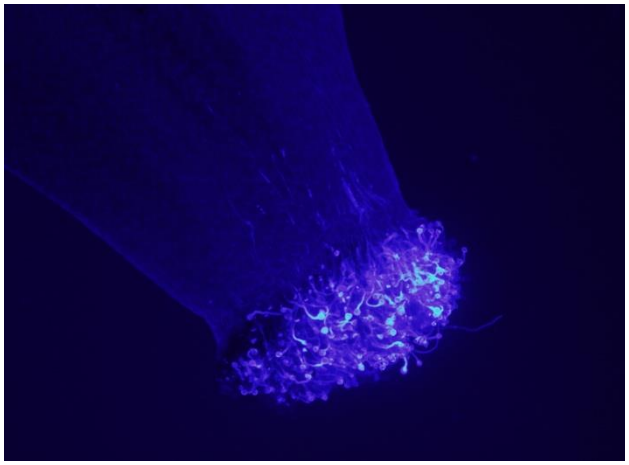

Figure 2.2a. Pollen germinating on end of stigma and beginning of pollen tubes in *D. rotundifolia* x *D. debilis* cross.

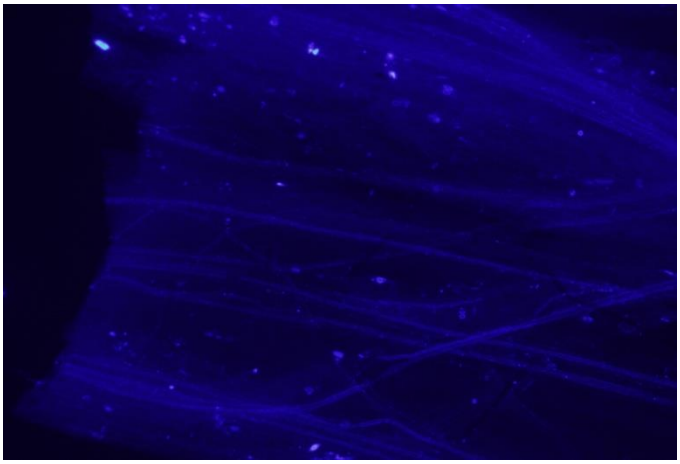

Figure 2.2b. Pollen tubes growing to end of style in *D. rotundifolia* x *D. debilis* cross.

Figure 2.3a. *D. canescens.*

Figure 2.3b. *D. princeps*

Figure 2.3c. *D. rotundifolia*

Figure 2.3d. *T. lepidota*

Figure 2.3. Parents of Melastomaceae hybrids attained from breeding program.

Figure 2.4a. *D. princeps* x *D. rotundifolia* putative hybrid

Figure 2.4b. *D. princeps* x *D. rotundifolia* putative hybrid

Figure 2.4c. *D. princeps* (left) with *D. princeps* x *D. rotundifolia* putative hybrid (right)

Figure 2.4d. *D. princeps* (left) with *D. princeps* x *D. rotundifolia* putative hybrid (right)

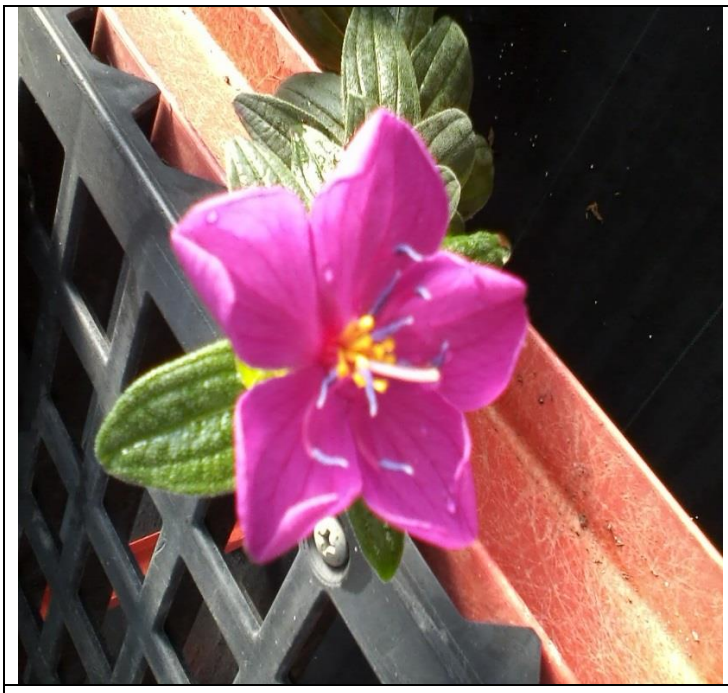

Figure 2.4e. *D. canescens* x *T. lepidota* putative hybrid

Figure 2.4f. Putative hybrids of *D. canescens* x *T. lepidota* and *D. canescens x D. princeps*

Figure 2.4. Melastomaceae hybrids attained from breeding program.

CHAPTER 3

Effects of Spray and Drench Treatments of Paclobutrazol on *Dissotis* and *Tibouchina*[2]

[2] Hawkins, S.M., J. Ruter and C. Robacker. To be submitted to *HortTechnology*.

A**bstract**

Dissotis rotundifolia (Sm.) Triana and *Tibouchina fothergillae x pilosa* are species in the Melastomaceae family with high ornamental potential. The growth habits of these species are not ideal for nursery production or shipping. *D. rotundifolia* grows rapidly and needs frequent pruning. *T. fothergillae* x *pilosa* has a very open growth habit and could benefit from a more compact form. In this study the effect of the plant growth regulator paclobutrazol on *D. rotundifolia* and *T. fothergillae x pilosa* was assessed to determine whether it could produce plants with a more compact growth habit. Paclobutrazol was applied as a drench and a spray. Drench application was more effective in reducing the growth of both species. Spray application was effective in reducing the growth of *D. rotundifolia* but was not effective on *T. fothergillae x pilosa.* Neither drench nor spray application delayed or reduced flowering in *D. rotundifolia.* *T. fothergillae x pilosa* did not flower during the study. For both *D. rotundifolia* and *T. fothergillae x pilosa*, neither drench nor spray application had an effect on root dry weight. Low to medium dosages were effective at controlling plant growth in *D. rotundifolia* and *T. fothergillae x pilosa* without adverse effects on plants. Drench treatments have more persistent effects on plant growth than spray treatments, but are usually more expensive per application due to increased labor needed for this method. The increased labor cost is justified if plants must be held in the nursery for several months before sale or shipping.

Introduction

Growth habit impacts the aesthetic value of an ornamental and the economics of the crop. Ornamental plants that grow too rampantly in the nursery often need to be pruned back before sale or shipment, causing additional labor costs. An ornamental that has a weak and open growth

habit is often damaged during shipping. Although long-term breeding goals for a species or hybrid may include shorter stature and more compact growth, nurseries need interim solutions.

Plant growth regulators (PGRs) are often used to control plant height and growth habit to produce a more marketable plant. PGRs may also be used to promote or retard the onset of flowering. Application of paclobutrazol and chloromequat to pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) was found to reduce plant height, but also caused plants to produce fewer flowers (HAQUE et al., 2007). Treatment of azaleas (*Rhododendron simsii* Planch.) with both chloromequat and paclobutrazol induced earlier flower bud formation (CHRISTIAENS et al., 2012). Treatment of *Dissotis plumosa* (a synonym for *Dissotis rotundifolia*) with chloromequat, daminozide, and ancymidol reduced growth in both greenhouse and field-grown plants (CRILEY, 1976). Paclobutrazol applied as a spray delayed flowering time in mini-carnation (*Dianthus caryophyllus f. Spray* Hort.) from 15 to 23 days but increased the number of blooms per flowering shoot (ATANASSOVA et al., 2004). In *Hibiscus rosa-sinensis* L., paclobutrazol delayed flowering by 22 days compared to the control treatment (NAZARUDIN, 2012).

Effects on plant growth and flowering may differ among cultivars. When paclobutrazol was applied to *Lilium* L.A. hybrids, flowering in 'Royal Respect' was reduced at the highest concentration applied while flowering in 'Ercolano' was unaffected at any dosage. Plant height was also more reduced in 'Royal Respect' than in 'Ercolano' at every concentration of paclobutrazol (FRANCESCANGELI et al., 2007).

Effects of PGRs are often strongly dependent on treatment level. Pyrethrum treated at lower doses of ethrel decreased both plant height and flower yield. However, in the same study, application of ethrel at higher levels also decreased plant height but increased flower yield (HAQUE et al., 2007). China aster (*Callistephus chinensis* L. Nees) was found to produce fewer

flowers at higher dosages of paclobutrazol (MISHRA et al., 2005). In *Lupinus varius* L., days to flowering and number of flowers were negatively impacted at higher dosages of paclobutrazol by drench while time to flowering was positively impacted at the highest dosage by spray (KARAGUZEL et al., 2004). High dosages of PGRs may cause plants to cease growing entirely (JOHANSEN et al., 1999).

Dissotis and *Tibouchina* are two genera in the Melastomaceae family that contain species of ornamental value. However, the growth habit of certain species within these genera is not ideal for shipment or for economic nursery production. *D. rotundifolia*, a species from Africa, is a trailing plant that flowers profusely. The species roots easily and is extremely drought tolerant. *D. rotundifolia* grows vigorously and requires pruning to maintain it in a saleable state. Severe pruning prevents the species from blooming for several weeks afterwards. *Tibouchina fothergillae x pilosa*, a hybrid between two South American species, has purple flowers and attractive fuzzy foliage. The branches of *T. fothergillae x pilosa* have a very open growth habit, making it difficult to ship. Paclobutrazol is a PGR that acts by inhibiting the biosynthesis of gibberellin (LEVER, 1986). The compound has low solubility and has been found to persist in soil as long as 12 months after application (LEVER, 1986). Paclobutrazol was chosen to test for its potential to control growth in *D. rotundifolia* and in *T. fothergillae x pilosa*, as it has been used to control growth in other Melastomaceae species (ABDULLAH et al., 1998; JOHANSEN et al., 1999; ROBERTS and EATON, 1988). The objective of the experiment was to determine the effectiveness of paclobutrazol applied as a drench or as a foliar spray on controlling the growth and flowering of *D. rotundifolia* and *T. fothergillae x pilosa*.

Materials and Methods

Cuttings of *D. rotundifolia* and *T. fothergillae x pilosa* from container-grown plants of the same clone growing in the greenhouse were taken in early March 2013. Stock plants of *D. rotundifolia* were plants obtained from the Trial Gardens at the University of Georgia, Athens, GA. Stock plants of *T. fothergillae x pilosa* were plants obtained from the Tifton campus of UGA. Cuttings were treated with a 5-second dip of K-IBA, and placed under mist until rooted. Cuttings rooted within 28 days and were transplanted into 2.8 liter trade pots with Fafard 3B potting media (Sun Grow Horticulture, Agawam, MA) consisting of Canadian sphagnum peat (45%), processed pine bark, perlite, and vermiculite. Paclobutrazol (Bonzi, Uniroyal Chemical Company), was applied as a drench or a spray on 9 April 2013. Although method of treatment was different, the levels of treatment were equivalent in a.i. applied per plant. Treatments were applied at the rate of 0, 1, 2, or 4 ($mg \cdot l^{-1}$) for drench and 0, 25, 50, and 100 ($mg \cdot l^{-1}$) for spray. Treatments were applied to ten *D. rotundifolia* and seven *T. fothergillae x pilosa* plants per treatment. Plants treated by spray application were sprayed with paclobutrazol to minimal run-off; control plants were sprayed with plain water. Plants treated by drench application were watered with 100 mL paclobutrazol solution. Control plants for drench treatment were watered with the same volume of plain water instead of the paclobutrazol solution.

Plants were arranged in a completely randomized design by taxon. Height and width measurements were taken weekly for six weeks for *D. rotundifolia* and eight weeks for *T. fothergillae x pilosa*. The growth index was computed as height x width. The height, width, and growth index difference were calculated as the difference between the measurements at the beginning and end of the study. Number of flowers per week was also recorded for each *D. rotundifolia* plant. None of the *T. fothergillae x pilosa* plants bloomed during the period of the

study. The flower index (flowers per square centimeter) for each week was computed from the number of flowers divided by the growth index. At the end of the experiments, plants were harvested and separated into roots and shoots. Roots were washed in a large container with frequent changes of water. Plants were oven dried for 24 hours at 60° C. Roots and shoots were weighed to the nearest 0.01 gram. The root-shoot ratio was calculated as root dry weight divided by shoot dry weight from the weight of the dried roots and shoots for each sample.

Data was analyzed using SAS 9.3 (SAS Institute, Inc., Cary, NC) with proc glm. Means separation was done using Tukey's LSD ($p < 0.05$) for differences within treatment method.

Results

Drench was more effective than spray at controlling the growth of *D. rotundifolia* for every measure of growth except height difference and root dry weight (Table 3.1). Rate of treatment had an effect on every measure of growth except shoot dry weight and root dry weight (Table 3.2). The effect of both spray and drench treatments on growth index was most evident five weeks after treatment (Figure 3.1). Neither drench nor spray treatment had any effect on flower index over the study period (data not shown).

For *T. fothergillae x pilosa,* an interaction between treatment method and rate of application was found for all measures of growth except root dry weight and root:shoot ratio (Table 3.3).The clearest interactions were found in final width, width difference, and shoot dry weight (Figures 3.to 3.4). The interactions indicated that although there was no difference in the effect of drench and spray at the control or the lowest level of treatment with paclobutrazol, drench had a greater effect than spray on reducing the growth of *T. fothergillae x pilosa* at the medium and highest rates. The difference in effects was not evident until the third week after treatment, and was most marked after the fourth week (Figure 3.5).

Discussion

Paclobutrazol was able to reduce the growth of *T. fothergillae x pilosa* whether applied as a drench or a spray, although treatments by drench at the two highest rates were more effective than spray treatments at the same rate level. Treatment by drench at the highest rate level resulted in a plant that was not of marketable size or growth habit. Plants treated at four $mg \cdot l^{-1}$ were 83% shorter than control plants and exhibited a rosette-like growth habit instead of the normal shrub habit of the hybrid. The greater effect of drench treatment on *T. fothergillae x pilosa* at higher rates most probably resulted from the persistence of paclobutrazol in the potting substrate and from the morphology of the plant. When applied as a spray, paclobutrazol must be applied to the shoot apex (LEVER, 1986). *T. fothergillae x pilosa* has pubescent leaves; leaves at the apical meristem are densely pubescent. The trichomes on the hybrid's leaves and stems very likely prevented the paclobutrazol from penetrating the epidermis of the leaves at the apical meristem as effectively as it would in a non-pubescent species such as *D. rotundifolia*.

Plant growth regulators may slow growth of roots as well as shoots. Treatment of *Tibouchina urvilleana* (Dc.) and *Melastoma decemfidum* Roxb. with high concentrations of paclobutrazol decreased root dry weight (ABDULLAH et al., 1998). Treatment of butterfly bush (*Buddleia davidii* Franch. 'Dubonnet') by granular and liquid formulations of paclobutrazol reduced root dry weight at most levels of treatment (RUTER, 1992). Paclobutrazol did not decrease root growth of *D. rotundifolia* or *T. fothergillae x pilosa* when applied by either drench or spray. The effect on roots may be highly species dependent. Paclobutrazol was found to have no effect on root length of *Hibiscus rosa-sinensis* L. (NAZARUDIN, 2012). Density of fine roots was actually stimulated in pin oak (*Quercus palustris*) and white oak (*Quercus alba*) by application of paclobutrazol to the soil at their roots (WATSON, 1996).

Low to medium rates of paclobutrazol applied as either drench or spray were effective in reducing the growth of *D. rotundifolia.* Paclobutrazol applied as a drench at the medium rate of two $mg \cdot l^{-1}$ was most effective at reducing the growth of *T. fothergillae* x *pilosa* without causing an adverse effect on plant morphology. More labor is involved in applying drench treatments to large numbers of plants than in spraying them, which increases the cost of a single application. Spray applications may need to be repeated to maintain an effective level of paclobutrazol in the plant. Because paclobutrazol persists in the substrate, a single drench application will provide a sufficient amount to reduce growth for a longer period of time. Even though drench treatments are usually more expensive per application to apply than spray treatments, the persistence of the effect of drenches justifies the increased labor cost if plants must be held in the nursery for several months before sale or shipping.

Literature Cited

Abdullah, T., Malek, A.A., and Ahmad, S.H., 1998. Chemical manipulation of growth and flowering in potted *Melastoma decemfidum* and *Tibouchina semidecandra*. *Acta Horticulturae*:297-301.

Atanassova, B., Filipova, I., and Alexieva, V., 2004. Effect of plant growth regulators Alar, MEIA and paclobutrazol on the phenophase of flowering and some ornamental parameters of mini-carnation (*D. caryophyllus f. spray* Hort.). *Bulgarian Journal of Agricultural Science* 10:305-309.

Christiaens, A., Van Labeke, M.C., Pauwels, E., Gobin, B., De Keyser, E., and De Riek, J., 2012. Flowering Quality of Azalea (*Rhododendron simsii*.) Following Treatments with Plant Growth Regulators. *ACTA HORTICULTURAE* 1:219-226.

Criley, R.A., 1976. Growth retardants for control of ground covers, p. 8-8, Departmental Paper, Hawaii Agricultural Experiment Station.

Francescangeli, N., Marinangeli, P., and Curvetto, N., 2007. Paclobutrazol for height control of two *Lilium* L.A. hybrids grown in pots, p. 425-430, Spanish Journal of Agricultural Research. Spanish National Institute for Agricultural and Food Research and Technology (INIA), Madrid; Spain.

Haque, S., Farooqi, A.H.A., Gupta, M.M., Sangwan, R.S., and Khan, A., 2007. Effect of ethrel, chlormequat chloride and paclobutrazol on growth and pyrethrins accumulation in *Chrysanthemum cinerariaefolium* Vis. *Plant Growth Regulation* 51:263-269.

Johansen, I.E., Findsen, S., and Andersen, A.S., 1999. Propagation, growth, retardation, flower induction, and postproduction performance of *Tibouchina urvilleana* (DC.) Cogn. *Gartenbauwissenschaft* 64:200-205.

Karaguzel, O., Baktir, I., Cakmakci, S., and Ortacesme, V., 2004. Growth and flowering responses of *Lupinus varius* L. to paclobutrazol. *HortScience* 39:1659-1663.

Lever, B.G., 1986. 'Cultar' - a technical overview. *Acta Horticulturae*:459-466.

Mishra, D.K., Mishra, H.R., and Yadava, L.P., 2005. Influence of paclobutrazol on early flowering and aesthetic value of China aster (*Callistephus chinensis* L. Nees). *Journal of Applied Horticulture (Lucknow)* 7:34-37.

Nazarudin, A., 2012. Plant Growth Retardants Effect on Growth and Flowering of Potted *Hibiscus rosa-sinensis* L. *J. Trop. Plant Physiol.* 4:12.

Roberts, C.M. and Eaton, G.W., 1988. Response of *Tibouchina* to chlormequat, paclobutrazol, and fertilizer. *HortScience* 23:1082-1082.

Ruter, J.M., 1992. Growth and flowering response of butterfly-bush to paclobutrazol formulation and rate of application. *HortScience* 27:929-929.

Watson, G.W., 1996. Tree root system enhancement with paclobutrazol. *Journal of Arboriculture* 22:211-217.

Tables and Figures

Table 3.1. Comparison of the effect of drench and spray paclobutrazol application at all rates on growth of *D. rotundifolia.*

Treatment	Final Height (cm)	Height Diff. (cm)	Final Width (cm)	Width Diff. (cm)	Final GI (cm^2)	GI Diff. (cm^2)	Shoot Dry Weight (gm)	Root Dry Weight (gm)	Root:Shoot Ratio
Drench	8.4b	2.4	33.2b	17.1b	287.3b	192.4b	8.3b	5.5	0.8a
Spray	9.2a	2.4	43.4a	25.1a	394.1a	267.6a	10.7a	5.1	0.5b
Significance	*	NS	**	*	**	*	*	NS	**

**,*,NS - Significant (n = 10) at *P* <= 0.01 or 0.05, or not significant at *P* > 0.05, respectively.

Table 3.2 Comparison of the effect of all rates of paclobutrazol application for both drench and spray application on growth of *D. rotundifolia.*

Rate Level	Final Height (cm)	Height Diff. (cm)	Final Width (cm)	Width Diff. (cm)	Final GI (cm^2)	GI Diff. (cm^2)	Shoot Dry Weight (gm)	Root Dry Weight (gm)	Root:Shoot Ratio
1	9.3ab	3.5a	54.1a	37.3a	493.3a	393.2a	11.6	5.1	0.5b
2	8.4ab	1.6b	39.1b	21.5b	333.1b	217.1b	9.6	5.3	0.7ab
3	9.4ab	2.8ab	32.8bc	15.1b	313.3bc	194.6b	8.6	5.5	0.7ab
4	8.1b	1.7b	27.2c	10.5b	223.0c	115.4b	8.2	5.2	0.7a
Significance	*	*	**	**	**	**	NS	NS	**

Rate Levels – 1 = control treatments, 2 = 1 mg·l^{-}drench or 25 mg·l^{-}spray, 3 = 2 mg·l^{-}drench or 50 mg·l^{-}spray, 4 = 4 mg·l^{-}drench or 100 mg·l^{-}spray. **,*,NS - Significant (eight replications) at $P <= 0.01$ or 0.05, or not significant at $P > 0.05$, respectively.

Table 3.3. Influence of paclobutrazol application (drench or spray) and rate of application on growth of *T. fothergillae* x *pilosa.*

Treatment	Rate ((mg/l $^{-1}$))	Final Height (cm)	Height Diff. (cm)	Final Width (cm)	Width Diff. (cm)	Final GI (cm^2)	GI Diff. (cm^2)	Shoot Dry Weight (gm)	Root Dry Weight (gm)	Root:Shoot Ratio
Drench	0	46.3	40.4	48.8	38.0	2,265.5	2,201.1	18.6	13.0	0.7
	1	32.1	25.7	42.7	32.1	1,376.5	1,307.0	14.3	12.2	0.9
	2	26.6	20.8	36.7	25.6	1,022.9	958.2	8.1	7.5	0.9
	4	12.4	7.2	31.2	20.5	394.7	336.8	9.7	7.5	1.3
Spray	0	42.6	35.8	42.8	32.0	1,827.9	1,752.6	15.9	11.8	0.8
	25	40.3	33.5	43.9	33.2	1,773.1	1,698.8	12.6	9.8	0.8
	50	36.6	31.1	48.7	39.2	1,795.4	1,744.1	14.9	12.7	0.9
	100	37.8	30.7	48.1	37.5	1,820.5	1,744.3	16.5	15.0	0.9
Significance										
Treatment		**	**	**	**	**	**	**	NS	NS
Rate		**	**	NS	NS	**	**	**	NS	**
Treatment x Rate		**	**	**	**	**	**	**	NS	NS

**,*,NS - Significant (n = 8) at $P <= 0.01$ or 0.05, or not significant at $P > 0.05$, respectively.

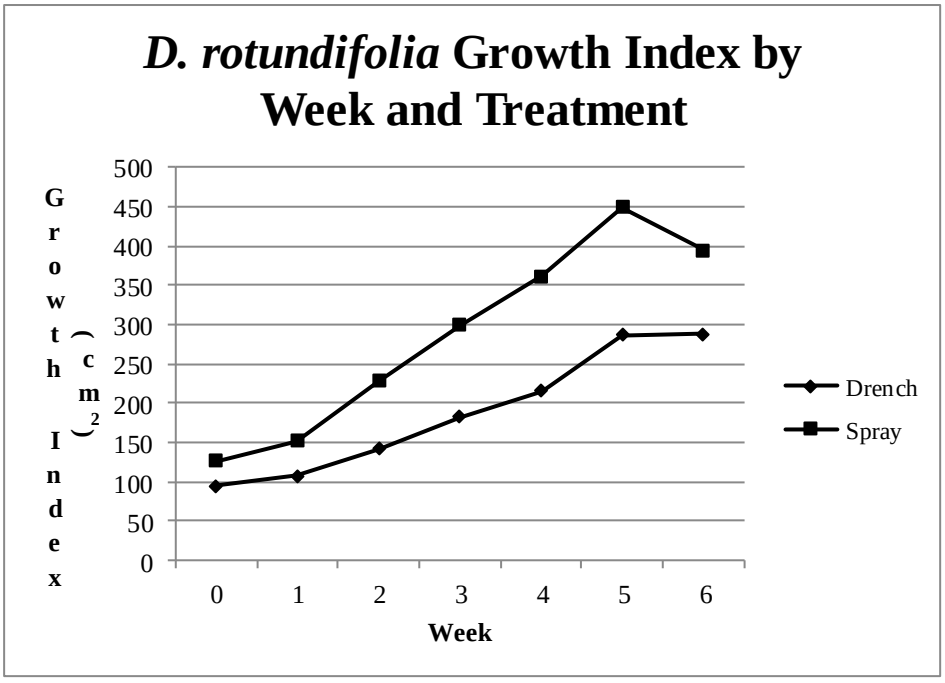

Figure 3.1. Influence of paclobutrazol application (drench or spray) averaged over treatment levels on growth of *D. rotundifolia* over time.

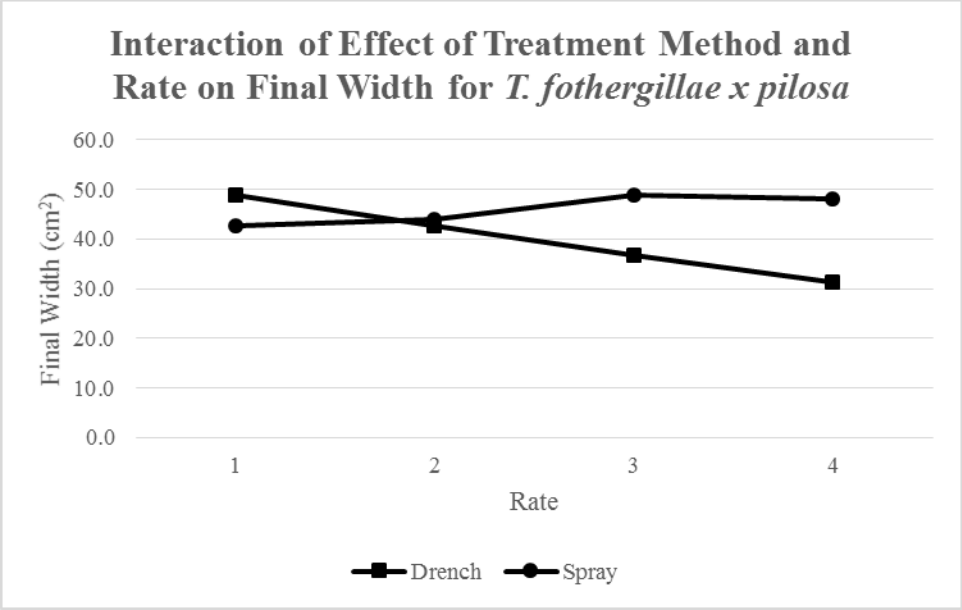

Figure 3.2. Interaction of Effect of Treatment method and Rate Level on Final Width of *T. fothergillae* x *pilosa*. Rate Levels – 1 = control treatments, 2 = mg·l^{-} drench or 25 mg·l^{-} spray, 3 = 2 mg·l^{-}drench or 50 mg·l^{-} spray, 4 = 4 mg·l^{-}drench or 100 mg·l^{-}spray.

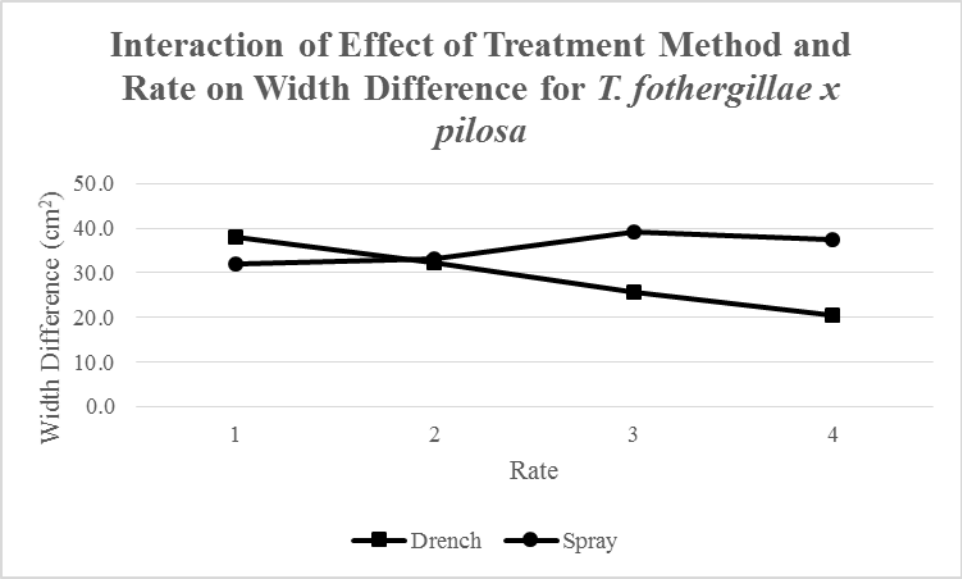

Figure 3.3. Interaction of Effect of Treatment method and Rate Level on Width Difference of *T. fothergillae* x *pilosa*. Rate Levels – 1 = control treatments, 2 = 1 mg·l^{-}drench or 25 mg·l^{-}spray, 3 = 2 mg·l^{-}drench or 50 mg·l^{-}spray, 4 = 4 mg·l^{-}drench or 100 mg·l^{-}spray.

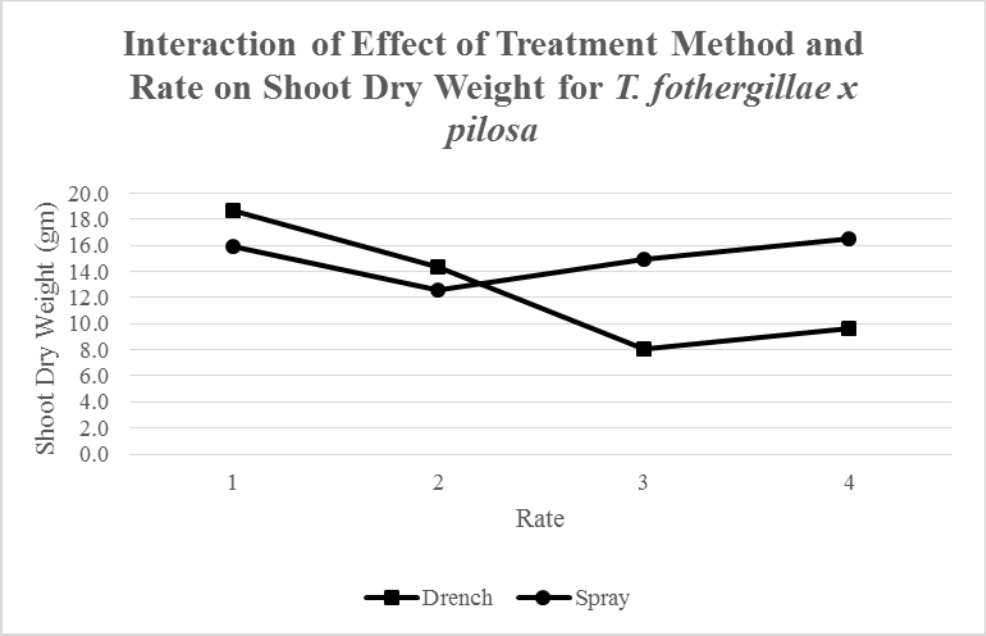

Figure 3.4. Interaction of Effect of Treatment method and Rate Level on Shoot Dry Weight of *T. fothergillae* x *pilosa*. Rate Levels – 1 = control treatments, 2 = 1 mg·l^{-}drench or 25 mg·l^{-}spray, 3 = 2 mg·l^{-}drench or 50 mg·l^{-}spray, 4 = 4 mg·l^{-}drench or 100 mg·l^{-}spray.

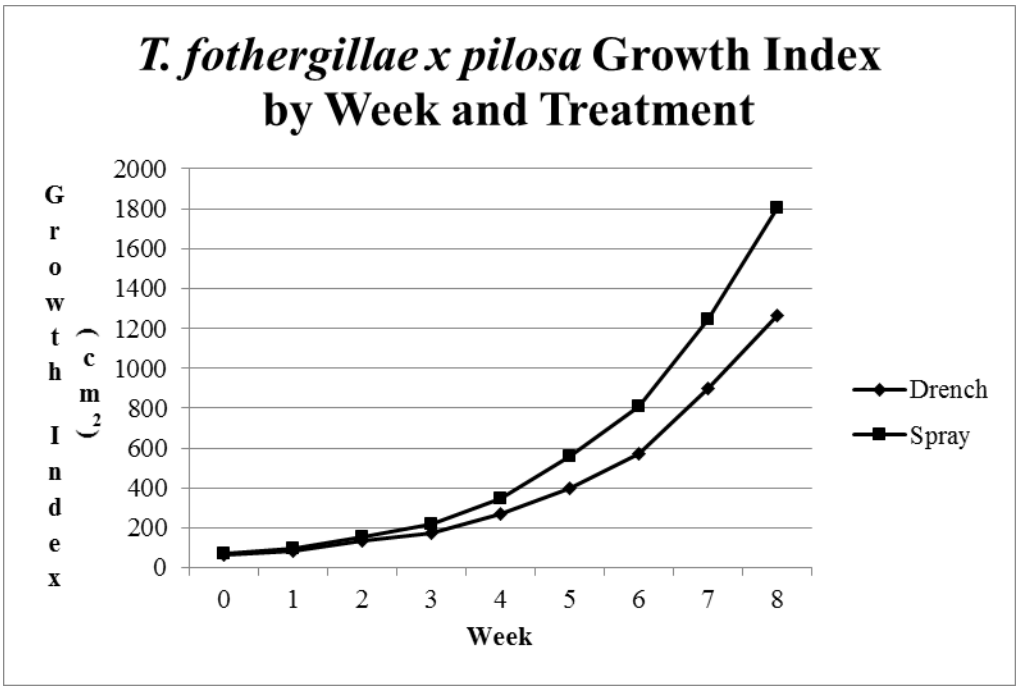

Figure 3.5. Influence of paclobutrazol application (drench or spray) averaged over treatment levels on growth of *T. fothergillae* x *pilosa* over time.

CHAPTER 4

Effect of Oryzalin on Inducing Polyploidy in *Dissotis rotundifolia*

43

[3] Hawkins, S.M., J. Ruter and C. Robacker. To be submitted to SNA as a proceedings paper.

Abstract

Dissotis rotundifolia (Sm.) Triana is a species in the Melastomaceae family with good ornamental potential. Nursery production can be difficult due to the aggressive growth habit of the species. To help reach the goal of obtaining a more compact *D. rotundifolia*, rooted cuttings were treated with oryzalin. Flow cytometry was performed to determine the ploidy level of the treated cuttings. One tetraploid was obtained. Growth of the tetraploid is being monitored to determine if its growth will be more compact than that of diploid plants.

Introduction

Dissotis rotundifolia (Sm.) Triana is a species in the Melastomaceae family. The Melastomaceae family consists of 185 to 190 genera that contain 5,000 species (RENNER and MEYER, 2001). Most Melastomaceae species are tropical or sub-tropical species (RENNER et al., 2001). The genus *Dissotis* is from Africa. *D. rotundifolia* is found in waste places and along roadsides, as well as in small granite outcroppings in the middle of rain forests (POREMBSKI et al., 1996). *D. rotundifolia* has naturalized in tropical and subtropical areas such as Puerto Rico and Hawaii (LIOGIER and MARTORELL, 1982; RENNER and MEYER, 2001). *D. rotundifolia* is a diploid, with 2n = 30 (SOLT and WURDACK, 1980).

D. rotundifolia is currently in use as an ornamental. The trailing species flowers abundantly and has attractive dark green foliage. *D. rotundifolia* roots easily and is extremely drought tolerant. The species grows so vigorously that it must be pruned back every few weeks to prevent it from overrunning the greenhouse bench. Pruning the plants will cause flowering to cease for several weeks. Vigorous growth and the need for frequent pruning make nursery production of the plant problematic.

Induction of polyploidy will sometimes cause a plant to become smaller and more compact than its diploid form (CONTRERAS et al., 2009). Autotetraploid *Brassicas* produced 64% less biomass than their diploid counterparts (ABEL and BECKER, 2007). Induced autotetraploids of *Helianthus annuus* L. var. *morden* were reduced in both height and width by one-third compared to the diploid control plants (SRIVASTAVA and SRIVASTAVA, 2002).

Induction of polyploidy in ornamental species has been accomplished by application of chemicals such as colchicine, an alkaloid extracted from *Colchicum autumnale*, or oryzalin [4-(dipropylamino)-3,5-dinitrobenzenesulphonamides]. Both substances double chromosomes by inhibiting the formation of mitotic spindles (HARTEN, 1998). Colchicine is more toxic to humans than oryzalin and must usually be applied at higher rates to induce polyploidy (HARTEN, 1998). Oryzalin has successfully been used to induce polyploidy in many ornamental species, such as Japanese barberry (*Berberis thunbergii* var. *atropurpurea*), *Hibiscus acetosella* Welw. ex Hiern., and ornamental ginger (*Hedychium muluense* R. M. Smith) (CONTRERAS et al., 2009; LEHRER et al., 2008; SAKHANOKHO et al., 2009). The purpose of this study was to induce polyploidy in *D. rotundifolia* through treatment with oryzalin in an attempt to produce a more compact version of the species.

Materials and Methods

Cuttings of *D. rotundifolia* were taken in December 2013, stuck in Fafard Germination Mix containing processed pine bark (40%), Canadian sphagnum peat, perlite, and vermiculite (Sun Grow Horticulture, Agawam, MA), and placed under intermittent mist for seven weeks in a greenhouse in Athens, GA. In February 2014 apical meristems were treated with 50 µm oryzalin solution (supplied as Surflan® A.S.; United Phosphorus, King of Prussia, PA) solidified with 0.4% agar. Treatments were applied as 1, 2, or 3 drops of oryzalin applied one day apart. A

control treatment of one drop of agar only was also used. The solution was heated in a microwave oven to melt the agar before application. Approximately 25 µl of oryzalin or agar solution was applied to the apical meristem and then the meristem was covered with a plastic cap for 24 hours. Then, the cap was removed and meristems rinsed with tap water. A total of 64 cuttings were treated, with 16 cuttings per treatment. Treated cuttings were allowed to grow in the greenhouse for three weeks after treatment before being tested for ploidy level.

Flow cytometry was performed to determine the ploidy level of the treated cuttings. Three samples were prepared for each treated cutting. Approximately 1 cm^2 of newly expanded leaf tissue was chopped in a petri dish with 500 µl of nuclei extraction buffer (CyStain PI® Absolute P Nuclei Extraction Buffer; Partec GmbH, Münster, Germany). Tissue was incubated in the solution for 30s. Solution was filtered through Partec CellTrics® disposable filters with a pore size of 30 µm to remove leaf material. Nuclei were stained with a solution of 2.0 ml of CyStain® PI absolute P, 12 µl propidium iodide stock solution, and 6 µl Rnase stock solution. Samples were incubated for at least 60m on ice. Samples were analyzed with a CyAn™ ADP Analyzer (Beckman Coulter, Brea, CA) to determine mean relative DNA fluorescence. Ploidy was determined by comparing the mean DNA fluorescence of each sample with the 2C peak of a diploid.

Results and Discussion

One tetraploid (4x) and three mixaploids (2x + 4X) were identified (Figure 1). No mortality occurred in any of the treated cuttings. The tetraploid plant was potted up in a pine bark based substrate with added nutrients in a 2.8 liter trade container and placed in a greenhouse at the University of Georgia Horticulture Farm, Watkinsville, GA. On 28 March 2014, a flower bud was observed on the tetraploid plant.

Induction of polyploidy did not delay flowering in *D. rotundifolia*. In contrast, flowering in induced polyploids of *Helianthus annuus* var. *morden* was delayed by almost two weeks (SRIVASTAVA and SRIVASTAVA, 2002). Induction of polyploidy in *Lilium* L. cultivars by either oryzalin or colchicine caused the polyploid plants to flower from four to ten days later than the diploid plant of the same cultivar (BALODE, 2008).

Since tetraploidy can be induced in *D. rotundifolia* by use of oryzalin, the possibility exists that the growth habit of the plant may be improved if the tetraploid plant proves to be more compact than its diploid counterpart. A cutting should be taken of the plant derived from the tetraploid cutting and rooted so that the entire plant is tetraploid. The tetraploid plant must be monitored and compared to the growth of diploid specimens. The study should be repeated with a larger number of cuttings to determine the dose of oryzalin that is optimum for inducing polyploidy and to increase the likelihood of obtaining at least one tetraploid plant that is more compact than its diploid counterpart.

Literature Cited

Abel, S. and Becker, H.C., 2007. The effect of autopolyploidy on biomass production in homozygous lines of *Brassica rapa* and *Brassica oleracea*. *Plant Breeding* 126:642-643.

Balode, A., 2008. Applying colchicine and oryzalin in *Lilium* L. polyploidisation. *Agronomijas Vestis*:22-28.

Contreras, R.N., Ruter, J.M., and Hanna, W.W., 2009. An oryzalin-induced autoallooctoploid of *Hibiscus acetosella* 'Panama Red'. *Journal of the American Society for Horticultural Science* 134:553-559.

Harten, A.M.v., 1998. *Mutation breeding : theory and practical applications / A.M. van Harten*. Cambridge, U.K. : New York : Cambridge University Press, 1998.

Lehrer, J.M., Brand, M.H., and Lubell, J.D., 2008. Induction of tetraploidy in meristematically active seeds of Japanese barberry (*Berberis thunbergii* var. *atropurpurea*) through exposure to colchicine and oryzalin. *Scientia Horticulturae* 119:67-71.

Liogier, A.H. and Martorell, L.F., 1982. *Flora of Puerto Rico and adjacent islands : a systematic synopsis / Henri Alain Liogier, Luis F. Martorell*. Río Piedras, P.R. : Editorial de la Universidad de Puerto Rico, 1982. 1a ed.

Porembski, S., Szarzynski, J., Mund, J.P., and Barthlott, W., 1996. Biodiversity and vegetation of small-sized inselbergs in a West African rain forest (Taï, Ivory Coast). *Journal of Biogeography* 23:47-55.

Renner, S.S., Clausing, G., and Meyer, K., 2001. Historical Biogeography of Melastomataceae: The Roles of Tertiary Migration and Long-Distance Dispersal. *Am J Bot*:1290.

Renner, S.S. and Meyer, K., 2001. Melastomeae come full circle: biogeographic reconstruction and molecular clock dating. *Evolution* 55:1315-1324.

Sakhanokho, H.F., Islam-Faridi, N., Kelley, R.Y., and Rajasekaran, K., 2009. Induced Polyploidy in Diploid Ornamental Ginger (*Hedychium muluense* R. M. Smith) Using Colchicine and Oryzalin. *HortScience : a publication of the American Society for Horticultural Science* 44:1809-1814.

Solt, M.L. and Wurdack, J.J., 1980. Chromosome numbers in the Melastomataceae. *Phytologia* 47:199-220.

Srivastava, R. and Srivastava, G.K., 2002. Autopolyploids of *Helianthus annuus* L. var. morden. *CYTOLOGIA* 67:213-220.

Figures

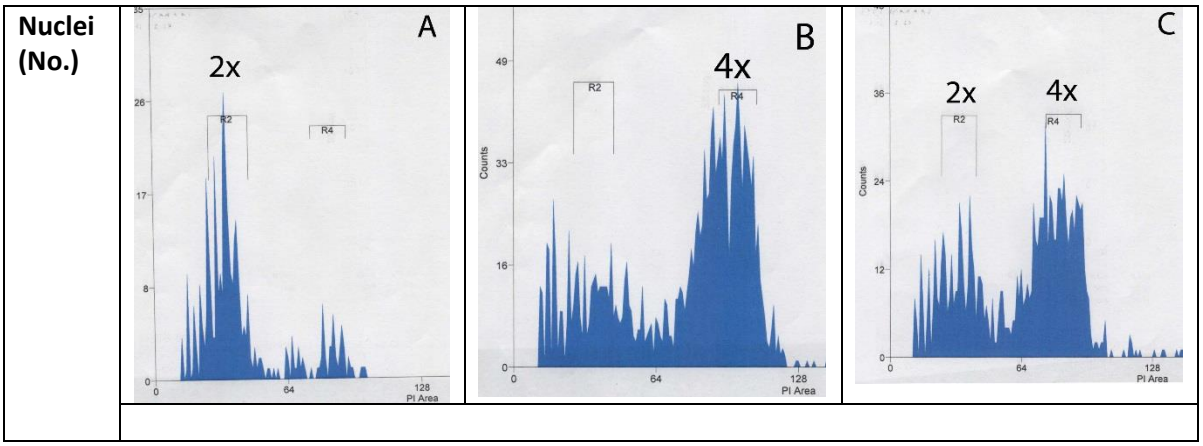

Figure 4.1. Histograms generated using flow cytometry from leaves of (A) diploid *D. rotundifolia*, (B) induced tetraploid of *D. rotundifolia*, and (C) induced cytochimera of *D. rotundifolia*.

CHAPTER 5

Interspecific and Intergeneric Hybridization in *Baptisia* and *Thermopsis*[4]

[4] Hawkins, S.M., J. Ruter and C. Robacker. To be submitted to *HortScience.*

Abstract

Interspecific and intergeneric crosses were performed between species in the genera *Baptisia* and *Thermopsis* with the goal of creating hybrids with the best qualities of both parents. *Baptisia australis* (L.) R. Br. was used as both male and female parent in intergeneric crosses. *Thermopsis chinensis* Benth. ex S. Moore, *T. lupinoides* (L.) Link, and *T. villosa* Fernald & B.G. Schub. were used as male and female parents in both interspecific and intergeneric crosses. Pollen was collected from *B. alba* (L.) Vent., *B. bracteata* Muhl. ex Elliott, and *B. lanceolata* (Walt.) Ell. and used to make interspecific and intergeneric crosses. Putative hybrids were obtained from both interspecific and intergeneric crosses. Interspecific crosses produced a higher percentage of fertile crosses and number of seeds per fertile cross than intergeneric crosses. Germination rate was not different between interspecific and intergeneric crosses. *B. australis* consistently produced fewer seed per cross when used as either male or female parent than *Thermopsis* species in both interspecific and intergeneric crosses. When comparing species in both interspecific and intergeneric crosses to determine the best female parents, we found no difference among female parents for percentage of fertile crosses or germination rate. When comparing species to determine the best male parents, no difference was found for percentage of fertile crosses or germination rate in either interspecific or intergeneric crosses. Since seedlings could be obtained from both interspecific and intergeneric crosses, production of a *Baptisia-Thermopsis* hybrid is feasible. Progeny are being evaluated to confirm hybrid status and select the best plants for the next generation of crosses. Other steps to be taken in this breeding program are the addition of new *Baptisia* species as parents, use of bridge parents, and selection of the species producing the most fertile crosses for male and female parents.

Introduction

Interspecific and intergeneric crosses are often used to create new hybrids in ornamental plants. As well as combining good ornamental traits, wide crosses are also used to introgress such traits as increased genetic diversity, drought tolerance, or greater ease of propagation into a hybrid. A cross between *Chrysanthemum grandiflorum* (Ramat.) Kitamura var. 'Zhongshanjingui' and *Artemisia vulgaris* L. produced a hybrid that rooted more easily than its chrysanthemum parent (DENG et al., 2012). Interspecific crosses among *Hibiscus paramutabilis* Bailey, *H. sinosyriacus* Bailey, and *H. syriacus* L. were made to incorporate the vigor of the first two species into *H. syriacus* (VAN LAERE et al., 2007). Intergeneric crosses were between a cultivated Brassicaceae (*Erucastrum carminoides* and *Brassica olearaceae* var. alboglabra) to increase the genetic variability of the cultivated (APARAJITA et al., 2009).

The Fabaceae family contains between 670 to 750 genera and 18,000 to 19,000 species (POLHILL et al., 1981). The two genera selected for this project, *Baptisia* and *Thermopsis*, are found mainly in temperate climates such as that of the Southeastern United States. Many species of *Baptisia*, some of them interspecific hybrids, are widely available in nurseries. *Thermopsis* species are not as widely grown as *Baptisia*, especially in the Southeast.

Baptisia and *Thermopsis* belong to the tribe Thermopsidae, which comprises 46 species in six genera (CHEN et al., 1994). Except for *Thermopsis barbata* Benth. ex Royle, which produces reddish-purple flowers, all species in *Thermopsis* have yellow flowers (CHEN et al., 1994; WU and RAVEN, 1994). Flower color in *Baptisia* ranges from white to yellow to blue (LARISEY, 1940). In both *Baptisia* and *Thermopsis*, perfect flowers with superior ovaries are borne on racemes; some *Baptisia* species produce flowers that occur individually in leaf axils

(CHEN et al., 1994; LARISEY, 1940). Seed pods of *Baptisia* are inflated while those of *Thermopsis* are compressed (CHEN et al., 1994).

Seed of species in both genera are hard and require scarification by either acid or mechanical means for best germination (BOYLE and HLADUN, 2005; CENKCI et al., 2007; VOSS et al., 1994). *Baptisia* seeds also contain phenolic compounds in the seed coat that inhibit germination of the seeds themselves as well as seeds of other species (VOSS et al., 1994). A study by Bratcher (BRATCHER et al., 1993) on *Baptisia australis* (L.) R. Br. found that stratification for two to four weeks caused seed to germinate at a lower rate than untreated seed, but stratification for periods longer than four weeks caused a higher rate of germination than that of untreated seed (BRATCHER et al., 1993).

Species in *Thermopsis* and *Baptisia* are all herbaceous; they are the only two genera in Thermopsidae that do not consist of species with woody plants (CHEN et al., 1994). *Thermopsis* contains 23 species and has a wide-spread distribution with species endemic to Asia and much of temperate North America (CHEN et al., 1994). Three *Thermopsis* species, *T. fraxinifolia* Nutt. ex M.A. Curtis, *T. mollis* (Michx.) M.A. Curtis ex A. Gray, and *T. villosa* Fernald & B.G. Schub., are native to the Southeastern United States (CHEN et al., 1994). *Baptisia* contains 15 to 17 species that are endemic to the Southeastern and Midwestern United States (AULT, 2003; CHEN et al., 1994; LARISEY, 1940).

Although polyploidy is wide-spread in the Fabaceae family, no polyploids have been found to exist in *Baptisia*. Only two polyploids have been found in *Thermopsis*, *T. gracillis* Howell and *T. divicarpa* A. Nelson (CHEN et al., 1994). The chromosome numbers of both *Baptisia* and *Thermopsis* are based on x=9, with 2n=18 for all species, except for *T. gracillis* and *T. divicarpa*, which are 2n=36 (CHEN et al., 1994; COOPER, 1936).

Biochemical evidence suggests that *Baptisia* evolved from *Thermopsis* in the Southeastern United States (DEMENT and MABRY, 1975). One phylogeny of Thermopsidae places *T. chinensis* Benth. ex S. Moore and *T. villosa* in the same clade as several species of *Baptisia*, including *B. australis*, based on internal transcribed spacer (ITS) sequences (WANG et al., 2006). Most taxonomists do not recognize interspecific crosses in *Thermopsis*, although Isley postulated that the western *Thermopsis* species had hybridized (DEMENT and MABRY, 1975; ISELY, 1981). However, hybridization readily occurs between species of *Baptisia* (ALSTON and TURNER, 1963; BAETCKE and ALSTON, 1968; DEMENT and MABRY, 1975; LARISEY, 1940; LEEBENS-MACK and MILLIGAN, 1998).

In the genus *Baptisia*, interspecific crosses have been used to create many novel cultivars (AULT, 2003; AVENT, 2002; CULLINA, 2000). *Thermopsis* has not been used to create hybrids to our knowledge and is also not much known or used in the ornamental plant industry. Yet several species of *Thermopsis* have good horticultural qualities. Many species of *Thermopsis*, particularly *T. villosa*, are very tolerant of both drought and heat, making *Thermopsis* a good substitute for lupines in the Southeastern United States (ARMITAGE, 1989). *Baptisia* has proven difficult to propagate vegetatively through cuttings (AULT, 2003; CULLINA, 2000). *Thermopsis* roots more easily than *Baptisia* (HAWKINS et al., 2013) and will bloom two years after germination, whereas *Baptisia* requires three years to bloom from seed (Personal communication, Heather Alley, State Botanical Garden of Georgia, 2013).

The overall goals of this project were to investigate the feasibility of hybridization between *Baptisia* and *Thermopsis* and between species within each genus, create interspecific and intergeneric hybrids, and examine the morphology of the resulting hybrids. To attempt to introgress drought tolerance, earlier flowering, ease of rooting, and ornamental qualities into a

hybrid between *Thermopsis* and *Baptisia*, intergeneric crosses were made between several species of *Baptisia* and *Thermopsis*. Interspecific crosses were also made, since such a cross might produce a hybrid of good ornamental quality, or one that might be used as a bridge parent for further intergeneric crosses.

Materials and Methods

B. australis, T. chinensis, and T. villosa plants were obtained from Northcreek Nurseries, Inc., Landenberg, PA as seed-grown liners. An additional genotype of *T. villosa* was obtained from the State Botanical Garden of Georgia, Athens, GA. *T. lupinoides* (L.) Link plants were vegetatively produced from a stock plant obtained from Plant Delights Nursery, Raleigh, NC. Plants were potted into 7.6 liter trade containers with Fafard 3B Potting Mix (Sun Grow Horticulture, Agawam, MA) consisting of Canadian sphagnum peat (45%), processed pine bark, perlite, and vermiculite, and placed in an enclosed shade house (50% shade) at the University of Georgia (UGA) Horticulture Farm, Watkinsville, GA. All crosses were carried out in the shade house. To increase the diversity of male *Baptisia* parents, pollen was collected from *B. alba* (L.) Vent., *B. bracteata* Muhl. ex Elliott, and *B. lanceolata* (Walt.) Ell. in April 2013 and used to make crosses (Table5.1). The *B. alba* plant from which the pollen was collected was in the Trial Gardens at UGA. The *B. lanceolata* plants were located in Dodge and Laurens County, GA. The *B. bracteata* plant was from Hancock County, GA.

Interspecifc and intergeneric crosses were made from March to May 2013. A total of 2,544 crosses were made; of the crosses 1,550 were interspecific and 994 were intergeneric. Three species of *Thermopsis* were used in the crosses: *T. chinensis*, *T. lupinoides*, and *T. villosa* (Table 5.1). *B. australis* was the only species in its genus to be used as the female parent.

Flowers of the female parents were emasculated immediately before pollination. Pollen was collected from the male parent onto a small paintbrush and used to pollinate the female parent. Reciprocal crosses were made where possible. Since not all species had blooming periods that overlapped or that overlapped for only a brief time, this could not always be accomplished. Seed pods were collected once ripe from May to August 2013. Seeds were extracted from the pods and counted. Seeds were scarified in 0.1M sulfuric acid for 20 minutes, rinsed, and soaked for several hours in plain water to imbibe before being sown in a potting media containing bark, peat moss, and perlite. Germination data was taken at four weeks after sowing. A maximum of 25 progeny of each interspecific and intergeneric cross was potted up into 2.8 liter trade containers with a pine bark based media containing added micronutrients and moved into an enclosed shade house at the UGA Horticulture Farm. In October 2013, the progeny were moved to the field at the UGA Horticulture Farm for overwintering and further evaluation. The seed obtained from the crosses between the genotype of *T. villosa* obtained from the State Botanical Garden and *B. australis* was sown and germinated later than that of the other crosses. The progeny obtained from those crosses was overwintered in a shade house as it was too young to be overwintered in the field.

Statistical analysis on the fertility of the crosses, number of seed per cross, and germination rate was performed using SAS 9.3 (PROC GLM) (SAS Institute, Cary, NC). Percentages were arcsine transformed before analysis to normalize data, and retransformed for reporting. Differences were considered to be significant at the level of $P = 0.05$.

Results

Interspecific crosses had a higher percentage of fertile crosses than intergeneric crosses ($p = 0.005$) (Table 5.2). Number of seed per fertile cross was also higher in interspecific crosses (p

= 0.008) (Table 5.2). However, the germination rate was not different between interspecific and intergeneric crosses (p = 0.238) (Table 5.2).

No differences in fertility were found among female parents used in interspecific crosses in the percent of fertile crosses (p = 0.760) or in germination rate (p = 0.182) (Table 5.3). The number of seeds per fertile cross was different (p = 0.019), with crosses having *Baptisia* as the female parent producing much fewer seed on average than any of the crosses having *Thermopsis* as the female parent (Table 5.3). Intergeneric crosses were also compared to determine whether female parents differed in fertility. Average number of seeds per fertile cross was not different among female parents (p = 0.084) (Table 5.4). Neither germination rate (p = 0.155) nor the percent of fertile crosses (p = 0.231) were different (Table 5.4).

Male parents used in interspecific crosses were compared to determine which had the highest percentage of cross fertility, germination rate, and seed per cross. We found no difference among male parents for percentage of fertile crosses (p = 0.370) or for germination rate (p = 0.087) (Table 5.5). However, we found a difference in the average number of seeds per cross (p = 0.008) (Table 5.5). In intergeneric crosses, the male parent made no difference in percentage of fertile crosses (p = 0.661), number of seeds per fertile cross (p = 0.573), or germination rate (p = 0.656) (Table 5.6).

Although seedlings were still quite juvenile before winter dormancy, preliminary visual examination revealed that several had morphological traits, such as leaflet size (Figure 51.a) and stipule shape (Figures 5.1b and 5.1e), that were intermediate to either parent. Seedlings began to break winter dormancy in March 2014. As of 31 March 2014, 27 of the seedlings had bloomed.

The *T. villosa* x *B. australis* cross and its reciprocal cross produced the most intergeneric seedlings, followed by the *T. villosa* x *B. alba* and *T. chinensis* x *B. alba* crosses. The *T.*

chinensis x *T. lupinoides* cross and its reciprocal produced the most interspecific seedlings. Seedlings of the *T. lupinoides* x *T. chinensis* accounted for 13 of the 27 seedlings that bloomed in March 2014. Examples of seedlings are presented in Figure 5.1. Pictures of some parent species are presented in Figure 5.2 for comparison. Seedlings of interspecific *Baptisia* crosses were slowest to emerge in the spring and were noticeably smaller than seedlings with parentage of any *Thermopsis* species. Detailed data and results for crosses are presented in Table 5.7.

Discussion

Pre- or post-zygotic barriers between parents in interspecific and intergeneric crosses will often preclude fertilization of the ovule after pollination, resulting in low seed set. Since percentage of fertile crosses and number of seed per fertile cross was higher in interspecific crosses than in intergeneric crosses, barriers to fertilization seem to be much lower in the interspecific crosses. Though such a result is typical in many species, it is not always the case. Intergeneric crosses in brooms (genera *Genista* and *Cystisus*, family *Fabaceae*) showed greater fertility than interspecific crosses (BELLENOT-KAPUSTA et al., 2006).

The number of seeds obtained per cross when *B. australis* was used as a female parent was less than when *Thermopsis* was used (Tables 5.3 and 5.4). Lower seed set is possibly due to factors other than ovule number, as B. *australis* is reported to have numerous seeds per pod, as is *Thermopsis villosa* (CRONQUIST, 1980). Counts of 30 seeds per pod have been reported for *B. australis* (EVANS et al., 1989). Although ovule number may vary among genotypes of *B. australis*, a count of ovules in the pods of plants located at the State Botanical Garden of Georgia revealed 12 to 16 ovules per pod (Personal observation). Southeastern *Thermopsis*, such as *T. villosa*, have been reported to have 12-16 ovules and Asian *Thermopsis*, such as *T. chinesis* and *T. lupinoides*, have been reported to contain 10-14 ovules (CHEN et al., 1994). Use of additional

Baptisia species and additional genotypes of *B. australis* as female parents could help determine if the lower seed set is due to the genus, species, or genotype of the *Baptisia* parent.

Since the germination rates for interspecific and intergeneric crosses were not different, mature seeds from intergeneric crosses have a good chance of producing a viable intergeneric hybrid. Several steps should be taken to translate these results into a viable breeding program.

Reciprocal crosses should be evaluated for cross fertility, seed set, and germination rate to determine the best cross. Seed set per flower was found to be higher in crosses of *Baptisia leucophaea* Nutt. and *B. sphaerocarpa* Nutt. when *B. sphaerocarpa* was used as the female parent (LEEBENS-MACK and MILLIGAN, 1998). Higher seed set could be due to greater prezygotic barriers in one female parent (LEEBENS-MACK and MILLIGAN, 1998). In this study, the *T. chinensis* x *T. lupinoides* cross had a lower percentage of fertile crosses compared to the reciprocal cross (21.1% vs. 44.0%), but a higher number of seeds per cross (12.1 vs. 8.4) and a higher germination rate (89.4% vs. 81.1%) (Table 5.7).

Since putative interspecific *Thermopsis* hybrids were obtained, use of these hybrids as bridge parents should be investigated. The blooming period for *T. lupinoides* and *T. chinensis* did not overlap substantially with the blooming period for *B. australis*, while that of *T. villosa* did. A hybrid between *T. villosa* and either *T. lupinoides* or *T. chinensis* might have an intermediate blooming period, making it a more suitable parent in an intergeneric cross with *B. australis* or with other *Baptisia* species.

Future steps include progeny evaluation and production of an F_2 generation. Seedling plants were still too juvenile before they became dormant to thoroughly evaluate for hybrid status.Morphological traits such as leaflet size and shape, leaf pubescence, and stipule size and shape will be compared between the putative hybrids and the parent plants. Progeny must also be

evaluated for possession of desirable traits, such as a compact growth habit and ability to propagate vegetatively. Once the hybrids bloom, the best of them should be crossed to create the next generation of hybrids. Other *Baptisia* species should be added to the breeding program to increase genetic diversity. *B. alba* would be a good addition, since using the species as a male parent resulted in intergeneric crosses with *T. villosa* and *T. chinensis*. Additional genotypes of *B. australis* could be added to increase the genetic diversity and possibly increase seed set when the species is used as the female parent. Pollen of early-blooming *Thermopsis* species should be stored for use in crosses with *Baptisia*. Much work remains to be done to create a desirable hybrid between *Baptisia* and *Thermopsis*. Initial results indicate that creating such a hybrid is feasible.

Literature Cited

Alston, R.E. and Turner, B.L., 1963. Natural Hybridization Among Four Species of *Baptisia* (Leguminosae). *Am J Bot* 50:159-173.

Aparajita, M., Babeeta, C., Nidhi, V., and Shivanna, K.R., 2009. Broadening the genetic base of crop brassicas by production of new intergeneric hybrid. *Czech Journal of Genetics and Plant Breeding* 45:117-122.

Armitage, A.M., 1989. *Herbaceous perennial plants : a treatise on their identification, culture, and garden attributes / Allan M. Armitage*. Athens, Ga. : Varsity Press, 1989.

Ault, J., 2003. Breeding and development of new ornamental plants from North American native taxa. *Acta Horticulturae*:37-42.

Avent, T., 2002. Revenge of the 'Redneck Lupines'. *Horticulture* 99:70.

Baetcke, K.P. and Alston, R.E., 1968. The Composition of a Hybridizing Population of *Baptisia sphaerocarpa* and *Baptisia leucophaea*. *Evolution* 22:157-165.

Bellenot-Kapusta, V., Pesteil, C., and Cadic, A., 2006. Diversity study and breeding of brooms (tribe of Genisteae). *Acta Horticulturae*:29-36.

Boyle, T.H. and Hladun, K., 2005. Influence of seed size, testa color, scarification method, and immersion in cool or hot water on germination of *Baptisia australis* (L.) R. Br. seeds. *HortScience : a publication of the American Society for Horticultural Science* 40:1846-1849.

Bratcher, C.B., Dole, J.M., and Cole, J.C., 1993. Stratification improves seed germination of five native wildflower species. *HortScience* 28:899-901.

Cenkci, S., Kargioglu, M., Dayan, S., and Konuk, M., 2007. Endangered status and propagation of an endemic plant species, *Thermopsis turcica* (Fabaceae). *Asian Journal of Plant Sciences* 6:288-293.

Chen, C.J., Mendenhall, M.G., and Turner, B.L., 1994. Taxonomy of *Thermopsis* (Fabaceae) in North America. *Ann Mo Bot Gard* 81:714-742.

Cooper, D.C., 1936. Chromosome numbers in the Leguminosae. *Am J Bot* 23:231-233.

Cronquist, A., 1980. *Vascular flora of the southeastern United States*. Chapel Hill : University of North Carolina Press, c1980-.

Cullina, W., 2000. A Guide to Growing and Propagating Native Flowers of North America. 1st ed.:322.

Dement, W.A. and Mabry, T.J., 1975. Biological implications of flavonoid chemistry in *Baptisia* and *Thermopsis*. *Biochemical Systematics and Ecology* 3:91-94.

Deng, Y.M., Chen, S.M., Chang, Q.S., Wang, H.B., and Chen, F.D., 2012. The chrysanthemum x *Artemisia vulgaris* intergeneric hybrid has better rooting ability and higher resistance to alternaria leaf spot than its chrysanthemum parent. *Scientia Horticulturae* 134:185-190.

Evans, E.W., Smith, C.C., and Gendron, R.P., 1989. Timing of Reproduction in a Prairie Legume: Seasonal Impacts of Insects Consuming Flowers and Seeds. *Oecologia*:220.

Hawkins, S.M., Chappell, M., and Martin Jr, M.T., 2013. Defining a Protocol for Vegetative Propagation of *Baptisia*, *Eupatorium* and *Thermopsis*. *Journal of Environmental Horticulture* 31:162-168.

Isely, D., 1981. Leguminosae of the United States. III. Subfamily Papilionoideae: tribes Sophoreae, Podalyrieae, and Loteae, p. 181.

Jayavalli, R., Balamohan, T.N., Manivannan, N., and Govindaraj, M., 2011. Breaking the intergeneric hybridization barrier in *Carica papaya* and *Vasconcellea cauliflora*. *Scientia Horticulturae* 130:787-794.

Kashihara, Y., Hirano, T., Murata, N., Shinoda, K., Araki, H., and Hoshino, Y., 2010. Evaluation of pre-fertilization barriers by observation of pollen tube growth and attempts for overcoming post-fertilization barriers in intergeneric hybridization between *Alstroemeria* and *Bomarea* by ovule culture. *Acta Horticulturae*:159-164.

Larisey, M.M., 1940. A Monograph of the Genus *Baptisia*. *Ann Mo Bot Gard* 27:119-244.

Leebens-Mack, J. and Milligan, B.G., 1998. Pollination Biology in Hybridizing *Baptisia* (Fabaceae) Populations. *Am J Bot* 85:500-507.

Polhill, R.M., Raven, P.H., and Stirton, C.H., 1981. Evolution and systematics of the Leguminosae, p. 1-26, Advances in legume systematics. Part 1 [Polhill, R.M.; Raven, P.H. (Editors)]. Royal Botanic Gardens., Kew, Richmond; UK.

Tsai, C.C., Chiang, Y.C., Huang, S.C., Liu, W.L., and Chou, C.H., 2009. Intergeneric hybridization, embryo rescue and molecular detection for intergeneric hybrids between *Ascocenda* and *Phalaenopsis*. *Acta Horticulturae*:413-416.

Van Laere, K., Huylenbroeck, J.M., and Van Bockstaele, E., 2007. Interspecific hybridisation between *Hibiscus syriacus*, *Hibiscus sinosyriacus* and *Hibiscus paramutabilis*. *Euphytica* 155:271-283.

Voss, K., Harnischfeger, G., Lieberei, R., and Mevenkamp, G., 1994. Seed germination behaviour of *Baptisia tinctoria* (L.) R. Br. *Angewandte Botanik* 68:53-59.

Wang, H.C., Yang, J.B., Compton, J.A., and Sun, H., 2006. A phylogeny of Thermopsideae (Leguminosae: Papilionoideae) inferred from nuclear ribosomal internal transcribed spacer (ITS) sequences. *Bot J Linn Soc* 151:365-373.

Wu, Z. and Raven, P.H., 1994. *Flora of China / Wu Zheng-yi and Peter H. Raven, co-chairs of the editorial committee*. Beijing : Science Press ; St. Louis : Missouri Botanical Garden, c1994-.

Tables and Figures

Table 5.1. Species used as parents in Fabaceae breeding project.

Female Parents	**Male Parents**
B. australis	*B. alba*
T. chinensis	*B. australis*
T. lupinoides	*B. bracteata*
T. villosa	*B. lanceolata*
	T. chinensis
	T. lupinoides
	T. villosa

Table 5.2. Fertility of interspecific and intergeneric crosses in Fabaceae breeding project.

Type of Cross	% Crosses with Seed	% Seeds Germinating	No. Seed per Cross	Seedlings Obtained
Interspecific	28.4a	73.7	8.4a	3,633
Intergeneric	10.0b	52.5	2.7b	150
Significance	**	NS	**	

* - significant at P <= 0.05. ** - significant at P <= 0.01, NS – not significant

Table 5.3. Fertility of female parents used in interspecific crosses in Fabaceae breeding project.

Female Parent	**% Fertile Crosses**	**Germination Rate (%)**	**No. Seed per Cross**
B. australis	30.2	46.3	3.0b
T. chinensis	20.2	78.2	11.0a
T. lupinoides	28.9	79.3	9.5a
T. villosa	40.0	93.5	11.5a
Significance	NS	NS	*

* - significant at P <= 0.05. ** - significant at P <= 0.01, NS – not significant.

Table 5.4. Fertility of female parents used in intergeneric crosses in Fabaceae breeding project.

Female Parent	**% Fertile Crosses**	**Germination Rate (%)**	**No. Seed per Cross**
B. australis	6.0	49.2	0.5
T. chinensis	12.0	86.4	7.3
T. lupinoides	0.0	0.0	0.0
T. villosa	18.9	82.3	4.5
T. villosa (BG)	9.0	40.0	2.2
Significance	NS	NS	NS

. * - significant at P <= 0.05. ** - significant at P <= 0.01, NS – not significant.

Table 5.5. Fertility of male parents used in interspecific crosses in Fabaceae breeding project.

Male Parent	% Fertile Crosses	Germination Rate (%)	No. Seed per Cross
B. bracteata	20.0	33.3	3.0c
B. lanceolata	40.0	58.3	3.0c
T. chinensis	44.0	81.1	8.4b
T. lupinoides	30.7	91.5	11.8a
T. villosa	16.2	70.9	10.2ab
Significance	NS	NS	**

* - significant at P <= 0.05. ** - significant at P <= 0.01, NS – not significant.

Table 5.6. Fertility of male parents used in intergeneric crosses in Fabaceae breeding project.

Male Parent	% Fertile Crosses	Germination Rate (%)	No. Seed per Cross
B. alba	13.0	65.1	4.6
B. australis	6.3	30.9	0.7
B. lanceolata	20.0	76.5	5.7
T. chinensis	0.0	0.0	0.0
T. villosa	12.1	85.7	1.0
Significance	NS	NS	NS

* - significant at P <= 0.05. ** - significant at P <= 0.01, NS – not significant.

Table 5.7. Detailed data for Fabaceae crosses.

Female Parent	Male Parent	No. Crosses	No. Crosses with Seed	Fertile Crosses (%)	No. Seed Obtained	No. Seed Germinated	Germination Rate (%)	Seedlings Kept
B. australis	*B. bracteata*	5	1	20.0	3	1	33.3	1
B. australis	*B. lanceolata*	10	4	40.0	12	7	58.3	4
B. australis	*T. chinensis*	5	0	0.0	0	0	0.0	0
B. australis	*T. villosa*	340	41	12.1	42	36	85.7	25
T. chinensis	*B. alba*	25	3	12.0	22	19	86.4	19
T. chinensis	*T. lupinoides*	635	134	21.1	1,617	1,445	89.4	25
T. chinensis	*T. villosa*	130	25	19.2	248	158	63.7	25
T. lupinoides	*B. alba*	25	0	0.0	0	0	0.0	0
T. lupinoides	*B. australis*	10	0	0.0	0	0	0.0	0
T. lupinoides	*T. chinensis*	600	264	44.0	2,228	1,808	81.1	25
T. lupinoides	*T. villosa*	160	21	13.1	221	171	77.4	25
T. villosa	*B. alba*	15	4	26.7	26	23	88.5	23
T. villosa	*B. australis*	459	46	10.0	63	51	81.0	25
T. villosa	*B. lanceolata*	15	3	20.0	17	13	76.5	5
T. villosa	*T. lupinoides*	10	4	40.0	46	43	93.5	25
T. villosa (BG)	*B. australis*	100	9	9.0	20	8	40.0	8

5.1a. *T. chinensis* x *T. villosa* seedling.

5.1b.*T. villosa* x *B. alba* seedling.

5.1c. *T. chinensis* x *B. alba* seedling.

5.1d. *T. chinensis* x *T. lupinoides* seedling.

5.1e. *T. villosa* x *B. australis* seedling

5.1f. *T. villosa* (BG) x *B. australis* seedling

Figure 5.1. Seedlings of interspecific and intergeneric crosses between *Baptisia* and *Thermopsis*.

Figure 5.2a. *B. australis.*

Figure 5.2b. *T. villosa.*

Figure 5.2c. *T. lupinoides.*

Figure 5.2d. *B. lanceolata.*

Figure 5.2e. *B. bracteata*.

Figure 5.2f. *B. bracteata* flowers.

Figure 5.2g. *T. chinensis.*

Figure 5.2. *Baptisia* and *Thermopsis* species used as parents, for comparison with hybrid seedlings.

CHAPTER 6

Conclusions and Next Steps

Conventional breeding of the Melastomaceae species used in this study is not feasible due to the poor seed set from crosses and extremely poor germination of seed. Induction of polyploidy was successful and should be repeated with more plants to obtain more tetraploid specimens of *Dissotis rotundifolia*. Use of paclobutrazol was successful in controlling the growth of *D. rotundifolia* and *Tibouchina fothergillae* x *pilosa* as a short-term solution for maintaining plants in the nursery or preparing them for shipping. Mutation breeding using EMS should also be explored. Any cultivars obtained through mutation breeding should be evaluated for invasive potential before release.

The Fabaceae project is very promising and should be continued to at least an F_2 generation. More species of *Baptisia* should be added as parents. Pollen should be collected and stored from early-flowering species. The interspecific hybrids should be used as bridge parents. A rooting study of the *Baptisia-Thermopsis* putative hybrids should be done to determine if they root more quickly and successfully than the *Baptisia* parent and if they emerge after overwintering.

Literature Cited

Abdullah, T., Malek, A.A., and Ahmad, S.H., 1998. Chemical manipulation of growth and flowering in potted Melastoma decemfidum and Tibouchina semidecandra. *Acta Horticulturae*:297-301.

Abel, S. and Becker, H.C., 2007. The effect of autopolyploidy on biomass production in homozygous lines of *Brassica rapa* and *Brassica oleracea*. *Plant Breeding* 126:642-643.

Al-Khassawneh, N.M., Karam, N.S., and Shibli, R.A., 2006. Growth and flowering of black iris (Iris nigricans Dinsm.) following treatment with plant growth regulators. *Scientia Horticulturae* 107:187-193.

Almasi, K.N., 2000. A non-native perennial invades a native forest. *Biological Invasions* 2:219-230.

Almeda, F., 1997. Chromosome numbers and their evolutionary significance in some neotropical and paleotropical Melastomataceae. *BioLlania*:167-190.

Almeda, F. and Chuang, T.I., 1992. Chromosome-Numbers and Their Systematic Significance in Some Mexican Melastomataceae. *Syst Bot* 17:583-593.

Alston, R.E. and Turner, B.L., 1963. Natural Hybridization Among Four Species of *Baptisia* (Leguminosae). *Am J Bot* 50:159-173.

Aparajita, M., Babeeta, C., Nidhi, V., and Shivanna, K.R., 2009. Broadening the genetic base of crop brassicas by production of new intergeneric hybrid. *Czech Journal of Genetics and Plant Breeding* 45:117-122.

Armitage, A.M., 1989. *Herbaceous perennial plants : a treatise on their identification, culture, and garden attributes / Allan M. Armitage*. Athens, Ga. : Varsity Press, 1989.

Atanassova, B., Filipova, I., and Alexieva, V., 2004. Effect of plant growth regulators Alar, MEIA and paclobutrazol on the phenophase of flowering and some ornamental parameters of mini-carnation (D. caryophyllus f. spray Hort.). *Bulgarian Journal of Agricultural Science* 10:305-309.

Auger, D.L., Coe, E.H., Jr., Birchler, J.A., Kato, A., Gray, A.D., and Ream, T.S., 2005. Nonadditive gene expression in diploid and triploid hybrids of maize. *Genetics* 169:389-397.

Ault, J., 2003. Breeding and development of new ornamental plants from North American native taxa. *Acta Horticulturae*:37-42.

Avent, T., 2002. Revenge of the 'Redneck Lupines'. *Horticulture* 99:70.

Baetcke, K.P. and Alston, R.E., 1968. The Composition of a Hybridizing Population of *Baptisia sphaerocarpa* and *Baptisia leucophaea*. *Evolution* 22:157-165.

Bellenot-Kapusta, V., Pesteil, C., and Cadic, A., 2006. Diversity study and breeding of brooms (tribe of Genisteae). *Acta Horticulturae*:29-36.

Bir, S.S., Cheema, P., and Sidhu, M.K., 1988. SOCGI plant chromosome number reports-VII, p. 219-228.

Bo, J., Zonggen, S., Jinbo, S., Da, Y., Xianyong, S., and Hongfei, L., 2009. Germination and growth of sponge gourd (Luffa cylindrica) pollen tubes and FTIR analysis of the pollen tube wall. *Scientia Horticulturae* 122:638-644.

Boyle, T.H. and Hladun, K., 2005. Influence of seed size, testa color, scarification method, and immersion in cool or hot water on germination of *Baptisia australis* (L.) R. Br. seeds. *HortScience : a publication of the American Society for Horticultural Science* 40:1846-1849.

Bratcher, C.B., Dole, J.M., and Cole, J.C., 1993. Stratification improves seed germination of five native wildflower species. *HortScience* 28:899-901.

Campos, C.C.F., Duarte, J.F., Tristão, R.A., and Borém, D.M.d.C., FLORAL BIOLOGY AND BREEDING MECHANISMS OF TIBOUCHINA HETEROMALLA COGN. IN ROCKY OUTCROPS IN THE SOUTH OF MINAS GERAIS.

Cenkci, S., Kargioglu, M., Dayan, S., and Konuk, M., 2007. Endangered status and propagation of an endemic plant species, Thermopsis turcica (Fabaceae). *Asian Journal of Plant Sciences* 6:288-293.

Chen, C.J., Mendenhall, M.G., and Turner, B.L., 1994. Taxonomy of *Thermopsis* (Fabaceae) in North America. *Ann Mo Bot Gard* 81:714-742.

Christiaens, A., Van Labeke, M.C., Pauwels, E., Gobin, B., De Keyser, E., and De Riek, J., 2012. Flowering Quality of Azalea (Rhododendron simsii.) Following Treatments with Plant Growth Regulators. *ACTA HORTICULTURAE* 1:219-226.

Clausing, G. and Renner, S.S., 2001. Molecular Phylogenetics of Melastomataceae and Memecylaceae: Implications for Character Evolution. *Am J Bot*:486.

Contreras, R.N., Ruter, J.M., and Hanna, W.W., 2009. An oryzalin-induced autoallooctoploid of Hibiscus acetosella 'Panama Red'. *Journal of the American Society for Horticultural Science* 134:553-559.

Cooper, D.C., 1936. Chromosome numbers in the Leguminosae. *Am J Bot* 23:231-233.

Criley, R.A., 1976. Growth retardants for control of ground covers, p. 8-8, Departmental Paper, Hawaii Agricultural Experiment Station.

Cronquist, A., 1980. *Vascular flora of the southeastern United States*. Chapel Hill : University of North Carolina Press, c1980-.

Cullina, W., 2000. A Guide to Growing and Propagating Native Flowers of North America. 1st ed.:322.

Custers, J.B.M., Eikelboom, W., Bergervoet, J.H.W., and Eijk, J.P.v., 1995. Embryo-rescue in the genus *Tulipa* L.; successful direct transfer of *T. kaufmanniana* Regel germplasm into *T. gesneriana* L. *Euphytica* 82:253-261.

Dement, W.A. and Mabry, T.J., 1975. Biological implications of flavonoid chemistry in *Baptisia* and *Thermopsis*. *Biochemical Systematics and Ecology* 3:91-94.

Deng, Y., Chen, S., Chen, F., Cheng, X., and Zhang, F., 2011. The embryo rescue derived intergeneric hybrid between chrysanthemum and Ajania przewalskii shows enhanced cold tolerance. *Plant Cell Reports* 30:2177-2186.

Deng, Y., Chen, S., Lu, A., Chen, F., Tang, F., Guan, Z., and Teng, N., 2010. Production and characterisation of the intergeneric hybrids between Dendranthema morifolium and Artemisia vulgaris exhibiting enhanced resistance to chrysanthemum aphid (Macrosiphoniellasanbourni). *Planta* 231:693-703.

Deng, Y.M., Chen, S.M., Chang, Q.S., Wang, H.B., and Chen, F.D., 2012. The chrysanthemum x Artemisia vulgaris intergeneric hybrid has better rooting ability and higher resistance to alternaria leaf spot than its chrysanthemum parent. *Scientia Horticulturae* 134:185-190.

dos Santos, A.P.M., Fracasso, C.M., Luciene dos Santos, M., Romero, R., Sazima, M., and Oliveira, P.E., 2012. Reproductive biology and species geographical distribution in the Melastomataceae: a survey based on New World taxa. *Annals of Botany* 110:667-679.

Ecker, R.B.A., 1993. Quantitative Genetic Analysis of Growth Rate in Lisianthus. *Plant Breeding* 111:253.

Edeoga, H.O. and Eboka, A.U., 2006. Pollen morphology and systematics in some Nigerian species of Dissotis Benth. (Melastomataceae). *Journal of Economic and Taxonomic Botany* 30:413-419.

Ellison, A.M. and Denslow, J.S., 1993. Seed and seedling ecology of neotropical Melastomataceae. *Ecology* 74:1733.

Evans, E.W., Smith, C.C., and Gendron, R.P., 1989. Timing of Reproduction in a Prairie Legume: Seasonal Impacts of Insects Consuming Flowers and Seeds. *Oecologia*:220.

Francescangeli, N., Marinangeli, P., and Curvetto, N., 2007. Paclobutrazol for height control of two Lilium L.A. hybrids grown in pots, p. 425-430, Spanish Journal of Agricultural Research. Spanish National Institute for Agricultural and Food Research and Technology (INIA), Madrid; Spain.

Goldenberg, R. and Shepherd, G.J., 1998. Studies on the reproductive biology of Melastomataceae in "cerrado" vegetation. *Plant Systematics and Evolution* 211:13-29.

Han, T.H., Jacobsen, E., Jeu, M.J.d., and Eck, H.J.v., 2002. Mapping of quantitative trait loci involved in ornamental traits in *Alstroemeria*. *HortScience : a publication of the American Society for Horticultural Science* 37:585-592.

Hanson, L., McMahon, K.A., Johnson, M.A.T., and Bennett, M.D., 2001. Regular Article: First Nuclear DNA C-values for 25 Angiosperm Families. *Annals of Botany* 87:251-258.

Haque, S., Farooqi, A.H.A., Gupta, M.M., Sangwan, R.S., and Khan, A., 2007. Effect of ethrel, chlormequat chloride and paclobutrazol on growth and pyrethrins accumulation in Chrysanthemum cinerariaefolium Vis. *Plant Growth Regulation* 51:263-269.

Harten, A.M.v., 1998. *Mutation breeding : theory and practical applications / A.M. van Harten*. Cambridge, U.K. : New York : Cambridge University Press, 1998.

Hawkins, S.M., Chappell, M., and Martin Jr, M.T., 2013. Defining a Protocol for Vegetative Propagation of *Baptisia, Eupatorium* and *Thermopsis*. *Journal of Environmental Horticulture* 31:162-168.

He, P., Zhou, J.M., Gill, B.S., and Friebe, B.R., 2003. Allopolyploidy alters gene expression in the highly stable hexaploid wheat. *Plant molecular biology* 52:401-414.

Isely, D., 1981. Leguminosae of the United States. III. Subfamily Papilionoideae: tribes Sophoreae, Podalyrieae, and Loteae, p. 181.

Jayavalli, R., Balamohan, T.N., Manivannan, N., and Govindaraj, M., 2011. Breaking the intergeneric hybridization barrier in Carica papaya and Vasconcellea cauliflora. *Scientia Horticulturae* 130:787-794.

Johansen, I.E., Findsen, S., and Andersen, A.S., 1999. Propagation, growth, retardation, flower induction, and postproduction performance of Tibouchina urvilleana (DC.) Cogn. *Gartenbauwissenschaft* 64:200-205.

Kantartzi, S. and Roupakias, D.G., 2008. Breeding barriers between *Gossypium* spp. and species of the Malvaceae family. *Australian Journal of Botany* 56:241-245.

Karaguzel, O., Baktir, I., Cakmakci, S., and Ortacesme, V., 2004. Growth and flowering responses of Lupinus varius L. to paclobutrazol. *HortScience* 39:1659-1663.

Kashihara, Y., Hirano, T., Murata, N., Shinoda, K., Araki, H., and Hoshino, Y., 2010. Evaluation of pre-fertilization barriers by observation of pollen tube growth and attempts for overcoming post-fertilization barriers in intergeneric hybridization between Alstroemeria and Bomarea by ovule culture. *Acta Horticulturae*:159-164.

Kermani, M.J., Sarasan, V., Roberts, A.V., Yokoya, K., Wentworth, J., and Sieber, V.K., 2003. Oryzalin-induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. *TAG. Theoretical And Applied Genetics. Theoretische Und Angewandte Genetik* 107:1195-1200.

Larisey, M.M., 1940a. A Monograph of the Genus *Baptisia*. *Ann Mo Bot Gard* 27:119-244.

Larisey, M.M., 1940b. A revision of the North American species of the genus *Thermopsis*. *Ann. Mo. Bot. Gdn.* 27:245-258.

Ledesma, N. and Sugiyama, N., 2005. Pollen Quality and Performance in Strawberry Plants Exposed to High-temperature Stress. *Journal of the American Society for Horticultural Science* 130:341-347.

Leebens-Mack, J. and Milligan, B.G., 1998. Pollination Biology in Hybridizing *Baptisia* (Fabaceae) Populations. *Am J Bot* 85:500-507.

Lehrer, J.M., Brand, M.H., and Lubell, J.D., 2008. Induction of tetraploidy in meristematically active seeds of Japanese barberry (Berberis thunbergii var. atropurpurea) through exposure to colchicine and oryzalin. *Scientia Horticulturae* 119:67-71.

Liogier, A.H. and Martorell, L.F., 1982. *Flora of Puerto Rico and adjacent islands : a systematic synopsis / Henri Alain Liogier, Luis F. Martorell*. Río Piedras, P.R. : Editorial de la Universidad de Puerto Rico, 1982. 1a ed.

Luo, Z., Zhang, D., and Renner, S.S., 2008. Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. *Functional Ecology* 22:794-800.

Mann, A., 2009. Efficacy of *Dissotis rotundifolia* on *Trypanosoma brucei brucei* infection in rats. *African journal of biochemistry research* 3:5.

Mendes-Rodrigues, C. and Oliveira, P.E., 2012. Polyembryony in Melastomataceae from Brazilian Cerrado: multiple embryos in a small world. *Plant Biology* 14:845-853.

Michelangeli, F.A., Guimaraes, P.J.F., Penneys, D.S., Almeda, F., and Kriebel, R., 2013. Phylogenetic relationships and distribution of New World Melastomeae (Melastomataceae). *Bot J Linn Soc* 171:38-60.

Mishra, D.K., Mishra, H.R., and Yadava, L.P., 2005. Influence of paclobutrazol on early flowering and aesthetic value of China aster (Callistephus chinensis L. Nees). *Journal of Applied Horticulture (Lucknow)* 7:34-37.

Nazarudin, A., 2012. Plant Growth Retardants Effect on Growth and Flowering of Potted Hibiscus rosa-sinensis L. *J. Trop. Plant Physiol.* 4:12.

Odimegwu, D.C., Ibezim, E.C., Esimone, C.O., Nworu, C.S., and Okoye, F.B.C., 2008. Wound healing and antibacterial activities of the extract of *Dissotis theifolia* (Melastomataceae) stem formulated in a simple ointment base. *Journal of Medicinal Plants Research* 2:11-16.

Olga, P., Nuno, N., Manuela, S., Michelle, S.L., Andreas, M., Luca, C., Wanda, V., Craig, S.P., and Susan, R.W., 2004. Chromosomal Locus Rearrangements Are a Rapid Response to Formation of the Allotetraploid Arabidopsis suecica Genome. *Proceedings of the National Academy of Sciences of the United States of America* 101:18240-18245.

Polhill, R.M., Raven, P.H., and Stirton, C.H., 1981. Evolution and systematics of the Leguminosae, p. 1-26, Advances in legume systematics. Part 1 [Polhill, R.M.; Raven, P.H. (Editors)]. Royal Botanic Gardens., Kew, Richmond; UK.

Porembski, S., Szarzynski, J., Mund, J.P., and Barthlott, W., 1996. Biodiversity and vegetation of small-sized inselbergs in a West African rain forest (Taï, Ivory Coast). *Journal of Biogeography* 23:47-55.

Renner, S.S., 1989. A Survey of Reproductive Biology in Neotropical Melastomataceae and Memecylaceae. *Ann Mo Bot Gard* 76:496-518.

Renner, S.S., 1993. Phylogeny and classification of the Melastomataceae and Memecylaceae. *Nordic Journal of Botany* 13:519-540.

Renner, S.S., Clausing, G., and Meyer, K., 2001. Historical Biogeography of Melastomataceae: The Roles of Tertiary Migration and Long-Distance Dispersal. *Am J Bot*:1290.

Renner, S.S. and Meyer, K., 2001. Melastomeae come full circle: biogeographic reconstruction and molecular clock dating. *Evolution* 55:1315-1324.

Roberts, C.M. and Eaton, G.W., 1988. Response of Tibouchina to chlormequat, paclobutrazol, and fertilizer. *HortScience* 23:1082-1082.

Roberts, C.M., Eaton, G.W., and Seywerd, F.M., 1990. Production of Fuchsia and Tibouchina standards using paclobutrazol or chlormequat. *HortScience* 25:1242-1243.

Ruter, J.M., 1992. Growth and flowering response of butterfly-bush to paclobutrazol formulation and rate of application. *HortScience* 27:929-929.

Sakhanokho, H.F., Islam-Faridi, N., Kelley, R.Y., and Rajasekaran, K., 2009. Induced Polyploidy in Diploid Ornamental Ginger (Hedychium muluense R. M. Smith) Using Colchicine and Oryzalin. *HortScience : a publication of the American Society for Horticultural Science* 44:1809-1814.

Sarkar, D., Tiwari, J.K., Sushruti, S., Poonam, Sanjeev, S., Gopal, J., Singh, B.P., Luthra, S.K., Pandey, S.K., and Pattanayak, D., 2011. Production and characterization of somatic hybrids between Solanum tuberosum L. and S. pinnatisectum Dun. *Plant Cell, Tissue and Organ Culture* 107:427-440.

Sears, E.R., 1976. GENETIC CONTROL OF CHROMOSOME PAIRING IN WHEAT. *Annual Review of Genetics* 10:31-51.

Simão, E. and Takaki, M., 2008. Effect of light and temperature on seed germination in *Tibouchina mutabilis* (Vell.) Cogn. (Melastomataceae). *Biota Neotropica* 8:63-68.

Solt, M.L. and Wurdack, J.J., 1980. Chromosome numbers in the Melastomataceae. *Phytologia* 47:199-220.

Soltis, P.S. and Soltis, D.E., 2000. The role of genetic and genomic attributes in the success of polyploids. In: Ayala, F. J., Fitch, W. M., and Clegg, M. T. (eds.), Variation and evolution in plants and microorganisms: toward a new synthesis 50 years after Stebbins. Proceedings of a colloquium, Irvine, California, USA, 27-29 January, 2000. National Academy Press, Washington; USA.

Srivastava, R. and Srivastava, G.K., 2002. Autopolyploids of Helianthus annuus L. var. morden. *CYTOLOGIA* 67:213-220.

Stadler, L.J., 1929. Chromosome number and the mutation rate in avena and triticum. *Proceedings of the National Academy of Sciences of the United States of America* 15:876-881.

Syiem, D. and Khup, P.Z., 2006. Study of the traditionally used medicinal plant *Osbeckia chinensis* for hypoglycemic and anti-hyperglycemic effects in mice. *Pharmaceutical Biology* 44:613-618.

Tsai, C.C., Chiang, Y.C., Huang, S.C., Liu, W.L., and Chou, C.H., 2009. Intergeneric hybridization, embryo rescue and molecular detection for intergeneric hybrids between Ascocenda and Phalaenopsis. *Acta Horticulturae*:413-416.

Van Laere, K., Huylenbroeck, J.M., and Van Bockstaele, E., 2007. Interspecific hybridisation between Hibiscus syriacus, Hibiscus sinosyriacus and Hibiscus paramutabilis. *Euphytica* 155:271-283.

Voss, K., Harnischfeger, G., Lieberei, R., and Mevenkamp, G., 1994. Seed germination behaviour of *Baptisia tinctoria* (L.) R. Br. *Angewandte Botanik* 68:53-59.

Wadl, P.A., Saxton, A.M., Wang, X.W., Pantalone, V.R., Rinehart, T.A., and Trigiano, R.N., 2011. Quantitative trait loci associated with red foliage in *Cornus florida* L. *Molecular Breeding* 27:409-416.

Wang, H.C., Yang, J.B., Compton, J.A., and Sun, H., 2006. A phylogeny of Thermopsideae (Leguminosae: Papilionoideae) inferred from nuclear ribosomal internal transcribed spacer (ITS) sequences. *Bot J Linn Soc* 151:365-373.

Watson, G.W., 1996. Tree root system enhancement with paclobutrazol. *Journal of Arboriculture* 22:211-217.

Wiens, D., Calvin, C., Wilson, C., Davern, C., Frank, D., and Seavey, S., 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* 71:501.

Wu, Z. and Raven, P.H., 1994. *Flora of China / Wu Zheng-yi and Peter H. Raven, co-chairs of the editorial committee*. Beijing : Science Press ; St. Louis : Missouri Botanical Garden, c1994-.