SLOANE MICHELE SCHEIBER
Characterization of Abelia Taxa for Interspecific Hybridization
(Under the direction of CAROL D. ROBACKER)

Stigmatic receptivity, intra- and interspecific crossability, seed germination, ovule culture techniques, inheritance of foliage variegation, and cold hardiness were evaluated to characterize *Abelia* taxa for interspecific hybridization. *Abelia chinensis* and *A.* ×*grandiflora* 'Francis Mason' were evaluated for stigmatic receptivity. Receptivity of both taxa was highest on the day of anthesis, but stigmas remained receptive throughout the pollination period. Fourteen *Abelia* taxa were assessed for intra- and interspecific crossability. Interspecific hybrids varied in percent seed set dependent on the parentage. Low seed set among intraspecific crosses may be attributed to the presence of a self-incompatibility system. Although seed was derived from intra- and interspecific crosses, germination rates were low and pericarp removal, gibberellic acid treatments, and stratification were tested as a means of increasing germination percentages and rates of *A.* ×*grandiflora*. Stratified seeds with attached pericarps had the highest germination percentage, 63%; though not significantly different than the other treatments. Despite more uniform germination, pericarp removal, GA_3 immersion, and stratification are of no practical benefit due to either reduced germination percentages or the time necessary for stratification. Seeds from interspecific hybridization between 'Francis Mason' and *A. schumannii* failed to germinate prompting development of an ovule culture technique. Ovules were cultured 5 weeks after pollination on Woody Plant Medium containing no growth regulator resulted in the highest ovule recovery rates, 85%, and seedling survival rates, 65%. Segregation ratios from reciprocal crosses between *A. chinensis*, a green-leafed species, and 'Francis Mason', a yellow cultivar of *A.* ×*grandiflora*, and backcross progeny could not be conclusively fit to a model. The best fit was obtained using a 2-

gene model of duplicate recessive epistasis with yellow dominant to green foliage. Twelve taxa were evaluated using laboratory procedures to determine maximum stem and leaf cold hardiness and to evaluate timing of acclimation and deacclimation. ‘Edward Goucher’ and ‘Confetti’ had the least hardy stems and leaves, respectively. ‘John Creech’ ranked in the hardiest group of taxa for both stems and leaves on the majority of test dates and would make a logical choice for incorporation into a breeding program.

INDEX WORDS: Cold hardiness, Foliage variegation, Stigma receptivity, Intra- and interspecific crossability, Ovule culture, Seed germination

CHARACTERIZATION OF ABELIA TAXA FOR INTERSPECIFIC HYBRIDIZATION

by

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I thought I would stop for a short time
And say my thanks
With a little rhyme.

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I can always rely on you
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To the potting up plants
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To all my many friends, I say thank-you
Time came and went
But all along the way
You provided so much encouragement.

As I've ventured through these many years
I've discovered pastures greener than fresh laid sod
All of which were made possible
Thanks be to God.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Compact, pest resistant, flowering evergreen shrubs are in great demand in the nursery and landscape industries. *Abelia* ×*grandiflora* (André) Rehd. meets these criteria with its long flowering period, from May to frost, and glossy semi-evergreen foliage (evergreen in mild climates). However, while it has an abundance of pinkish-white flowers, they are small and only mildly fragrant, and the plants are not hardy below -20°C or zone 6 (Bean, 1970; Krussmann, 1985a; Dirr, 1998a). The nursery industry has stated that *Abelia* R. Br. taxa are important economically, and new selections with increased cold hardiness, richer pink-rose flower colors, unique foliage colors, and compact habits are desired (Dirr, 1998b).

Breeding and selection work in the genus *Abelia* is very limited. *Abelia* ×*grandiflora* is only one of two hybrids ever recorded in the genus *Abelia*. It is derived from a cross between *A. chinensis* R.Br. and *A. uniflora* R.Br. and was first described in 1886 from a plant raised from seed in the Rovelli nurseries, Pallanza, on Lake Maggiore (Bean, 1970). All of the cultivars of *A.* ×*grandiflora* originated as sports. Matsubura (1984) used gamma radiation to induce mutations in *A.* ×*grandiflora* and obtained a dwarf type with variegated leaves, but no cultivar was released. *Abelia* 'Edward Goucher' is a hybrid of *A.* ×*grandiflora* and *A. schumannii* (Graebn.) Rehd., and it was introduced in 1911 by Edward Goucher, USDA, Glenn Dale Plant Introduction Station (Krussmann, 1985a). *Abelia* 'John Creech' is listed as a sister seedling of *A.* 'Edward Goucher' but current thinking places it with *A.* ×*grandiflora* (Dirr, 1997).

The genus *Abelia* contains approximately 30 species, but *A.* ×*grandiflora*, its cultivars, and *A.* 'Edward Goucher' are the primary taxa grown. *Abelia chinensis*, a plant less commonly seen in cultivation, is a fragrant, perpetually-flowering shrub, but it is deciduous with an upright spreading open habit. Furthermore, *A. chinensis* is hardy only to zone 7, being killed back to the ground in cold winters farther north. *Abelia floribunda* Decne., Mexican Abelia, is a wild evergreen species with a scandent shrub-like growth habit that has attractive, large, red flowers, but flowers only on old wood and

lacks cold hardiness (hardy only to zone 8) (Bean, 1970; Krussmann, 1985a; Dirr, 1998a). Interspecific hybridization among these and other species, including *A. uniflora, A. biflora* Turcz., *A. zanderi* (Graebn.) Rehd. and *A. schumannii* offer the potential for new cultivars.

The genus *Abelia* is a member of the family Caprifoliaceae Juss. and its species range in distribution from eastern and central Asia, to Taiwan, the Himalayas, and Mexico (Krussmann, 1985a; Villarreal, 1997). The type specimen, *A. chinensis*, was discovered by Dr. Clarke Abel (for whom the genus was named) in China in 1817. In addition to *Abelia*, the family contains several genera that are noted for their ornamental value including *Viburnum* L. Arrowwood, *Lonicera* L. Honeysuckle, *Sambucus* L. Elderberry, and *Weigela* Thunb.(Bailey, 1976; Sax and Kribs, 1930).

Reproductive Biology and Cytology in *Abelia*

The genus *Abelia* is characterized by small to medium sized shrubs that possess deciduous, semi-evergreen, or evergreen foliage with an opposite to whorled phyllotaxy (Krussmann, 1985a; Everett, 1980). The leaves have entire or serrate margins and short petioles. The floral structure is composed of a tubular to bell-shaped corolla with 2-5 sepals that persist after the corolla has abscised. The corolla colors range from white to pink to purplish-red, and the flowers occur in 1-8 flowered cymes on lateral branches or terminal panicles (Everett, 1980; Griffiths, 1992). The ovary is inferior and 3-celled with only one fertile cell (Rehder, 1937; Hara, 1983). Four stamens, didynamous, are attached to the base of the corolla, and the fruit is a one-seeded leathery achene (Rehder, 1937; Krussmann, 1985a; Hara, 1983).

Cytological studies in *Abelia* are limited and have focused primarily on chromosome counts. The majority of the cytological work in Caprifoliaceae has focused on three genera: *Lonicera, Sambucus,* and *Viburnum.* Sax and Kribs conducted the first extensive chromosome counts in Caprifoliacae in the 1920's, and the study included *A.*

engleriana (Graebn.)Rehd. and *A. schumannii*. During chromosome counts of species in seven of the 18 genera of Caprifoliaceae, Sax and Kribs (1930) found the chromosomes of *Abelia* species to be the smallest among the genera examined. The average size of *Abelia* chromosomes is less than 1 micron.

Chromosome counts have been conducted on seven of the 30 *Abelia* species. All species are tetraploid (2n=4x=36) or (2n=4x=32) with the exception of *A. triflora* R. Br. Both diploid and tetraploid members of the species have been identified (Medra, 1976; Bedi et al, 1982). The species and their chromosome numbers are listed in Table A.1 of the Appendix. Base numbers of *Abelia* are x = 8 and 9, and the primary base number in Caprifoliaceae is x = 9 based on the prevalence of species with n=9 or its multiple (Sax & Kribs, 1930; Rudenburg & Green, 1966; Hounsell, 1968; Bedi et al., 1982). Sax and Kribs (1930) suggested that the loss of two chromosome pairs was responsible for the base number, x=8, observed in *A. engleriana* and *A. schumannii*. However, no explanation as to the cause of the chromosome loss was offered. The incidence of polyploidy in Caprifoliaceae is 26.5%.

Cytological investigations of seven of the 18 genera in Caprifoliaceae indicated that the family has trinucleate pollen. Self-incompatibility has been reported for species of *Lonicera, Sambucus*, and *Viburnum* (Brewbaker, 1957; Brewbaker, 1967; Sedgley, 1994). Bar-Shalom and Mattsson (1977) germinated pollen of *A.* ×*grandiflora* and *A. schumannii* in vitro, indicating short-term pollen viability. However, neither self-incompatibility, pollen cytology, or long-term reproductive viability has been investigated in *Abelia*.

Numerous pollinations must be performed to obtain the large quantities of seeds needed to conduct a breeding program in *Abelia* because the fruit is a one-seeded achene. To maximize pollination success, knowledge of the receptivity of the stigmatic surface is vital for determining the effective pollination period. Effective pollination period as defined by Williams (1970) is the longevity of the ovule minus the time necessary for

pollen tube growth. Stigmatic receptivity can vary widely among taxa. Among woody taxa, Gonzalez et al. (1995) report that stigmatic receptivity is the main factor limiting the effective pollination period in Kiwifruit [*Actinidia deliciosa* (Chev.) Liang and Ferguson]. Stigmatic receptivity averaged 84% during the first 4-days postanthesis, but rapidly declined to 0% by 7-days postanthesis. Tangmicharoen and Owens (1997) report that pistils of Teak (*Tectona grandis* L.f.), a weakly protrandrous species, are receptive only between the hours of 1100-1300 postanthesis. Optimum receptivity of *Vitis coignetiae* Pulliat. was shown to occur 2 days after flowering opening following hand-pollinations performed 0, 2, 4, and 6-days postanthesis (Kimura et al., 1998). Similar results are observed among herbaceous taxa. The stigmas of *Chamelaucium uncinatum* Schauer, a protrandrous species, increase in receptivity as the time from anthesis increases. Stigmas are non-receptive at 1-day postanthesis, receptivity ranges from 10-50% at 3-days postanthesis, and increases to 100% at 10-days postanthesis (O'Brien, 1996). *Cucurbita pepo* L. stigmas are receptive from 1 day prior to anthesis until 2-days postanthesis (Nepo and Pacini, 1993). Shafer et al. (2000) examined stigmatic receptivity in 447 Buffelgrass [*Pennisetum ciliare* (L.) Link.] accessions. Results indicate that accessions of buffelgrass, a protogynous species, vary for the protogynous interval but that stigmas remain receptive throughout the 1,2, or 3 day intervals. Variability in stigmatic receptivity was sited among ten cultivars of *Dianthus plumarius* L.. with very low receptivity occurring 1-day postanthesis and optimum receptivity resulting 4 to 6-days postanthesis depending on the cultivar (Hewage et al., 1999).

Interspecific Compatibility and Embryo / Ovule Culture

Natural species hybrids are known to occur in the *Sambucus, Viburnum, Symphoricarpus* Duh., *Diervilla* Mill., *Weigela* Thunb., and *Lonicera* (Sax and Kribs, 1930; Krussmann, 1985b). Natural hybrids are uncommon in *Lonicera,* but hybridization occurs readily in cultivation and fertile progeny often result. Section *Lonicera,* Subgenus

Caprifolium L. Subsection *Cypheolae* (Raf.) Rehd, and Subgenus *Caprifolium* Subsection *Eucaprifolium* (Spach) Rehd. are noted for prevalent hybridization (Rudenberg and Green, 1966). Gunatilleke and Gunatilleke (1984) reported fruit set ranging from 25 to 91% from interspecific crosses between *L. gracilipes* Miq. × *L. deflexicalyx* Batalin. and *L. tatarica* L. × *L. deflexicalyx*, respectively. In cultivation, *Weigela* and *Viburnum* are also frequently hybridized (Krussmann, 1985b). Benetka (2000) produced fertile hybrids from a cross between *W. florida* (Bunge) A. DC. × *W.* 'Eva Rathke' [*W. coraeensis* Thunb. × *W. floribunda* (Siebold & Zucc.) C. Koch.].

Interspecific hybridization can be difficult or impossible due to a number of factors such as embryo abortion, failure of endosperm development, or failure of viable seeds to germinate (George, 1993; Sharma et al, 1996), and embryo rescue and/or ovule culture have been used to overcome post-zygotic incompatibility. In Caprifoliaceae, embryo rescue has been used to a limited extent in the genera *Sambucus* and *Viburnum*. Koncalova et al. (1983) produced hybrid plants from crosses between *S. nigra* L. × *S. ebulus* L. and *S. nigra* × *S. racemosa* A.Gray., but reciprocal crosses of *S. nigra* × *S. ebulus* failed to produce plants. Therefore, embryos of *S. ebulus*, *S. nigra,* and *S. ebulus* × *S. nigra* were cultured on White's modified medium, but only *S. ebulus* and *S. nigra* embyros retained viability. Zilis and Meyer (1976) successfully cultured embryos of *Viburnum lentago* L., *V. lantana* L., and *V.* × *burkwoodii* Hort. Burkw. & Skipw. on a modified Linsmaier and Skoog medium to overcome seed dormancy and reduced germination time to 45 days compared to 1 to 2 years under natural conditions. Hybrid plants from *V. lantana* 'Mohican' × *V. carlesii* Hemsl. 'Aurora' and *V. lantana* 'Mohican' × *V.* × *juddii* Redh. were obtained by culturing embryos on Woody Plant Medium, however, only 5% of the embryos had normal development (Hoch et al., 1995). George (1993) states that low success rates and hybrid sterility are not uncommon in embryo rescue, but neither factor is relevant if the new hybrid can be asexually propagated.

Success or failure of embryo or ovule culture is contingent upon several factors including development stage of the embryo, media composition, and genotype (George, 1993; Lu & Bridgen, 1996). Timing is particularly critical for the success of embryo or ovule culture (Collins & Grosser, 1984; Hu & Wang, 1986; Honda & Tsutsui, 1997). Dissection of very small embryos is difficult and often results in physical damage to the embryo that prohibits *in vitro* growth. Complex nutrient requirements also increase the difficulty of culturing immature embryos, but embryo viability decreases as age increases due to induction of incompatibility mechanisms. Ovule culture often serves as alternative to embryo rescue because ovules are larger and easier to dissect without physical damage and media requirements are less complex (George, 1993).

Sukno et al. (1999) report few mature plants were derived from interspecific crosses between *Helianthus* L. species when culturing globular or early heart stage embryos, but plant survival significantly increased at later developmental stages (i.e. heart and torpedo). The difficulty of dissecting embryos during the very early developmental stages without inducing damage was noted. Species differences were observed for the efficiency of embryo rescue (number of plantlets obtained / number of cultured embryos), ranging from 3.8 to 34.9%. Yao et al. (1995) cultured embryos derived from self-pollinations and interspecific crosses of Calla Lily (*Zantedeschia* K. Spreng.) 4 to 10 weeks after pollination. Embryos from self-pollinations were fully developed at 10 weeks after pollination and the endosperm was solid and fully mature. Among interspecific hybrids, embryo development was restricted to the globular stage and endosperm degeneration began 5 to 6 weeks after pollination. Based on the results, embryos from additional interspecific crosses were cultured 4 to 7 weeks after pollination and the production of hybrids ranged from 2.5% to 98.2% depending on the parentage. Harvesting embryos 8 weeks after pollination, Yao and Cohen (1996) produced triploid hybrids of Calla Lily, but only 1% of the dissected seeds resulted in mature plants.

Cytological examination of 28, 35, and 42-day old ovules from self-pollinated *Cyclamen persicum* Mill. and *C. persicum* × *C. purpurascens* Mill. showed proembryo development by day 28 but almost complete degeneration by day 42 resulting from failure of endosperm development in the hybrid ovules. Self-pollinated ovules showed normal embryo and endosperm development. Culturing 28-day old ovules of *C. persicum* × *C. purpurascens* resulted in the production of hybrid plants (Ishizaka & Uematsu, 1995). Failure of endosperm development has been observed between interspecific crosses in the genera *Alstroemeria* L. and *Delphinium* L.. Ovules of *Delphinium grandiflorum* L. × *D. nudicaule* Torr. & A.Gray collected 20 to 25 days after pollination resulted in a seedling survival rate of 7.5%, but no seedlings were obtained when ovules were cultured 30 days after pollination (Honda & Tsutsui, 1997). Less than 5% of ovules resulted in mature plants from crosses between *Alstroemeria pelegrina* L. var. *rosea* × *A. magenta* Bayer when 21-day or older ovules were cultured in comparison to survival rates of nearly 20% for 7 and 14 day old ovules (Ishikawa et al., 2001). Endosperm failure is often reported as a post-zygotic barrier to interspecific hybridization (Collins & Grosser, 1984; Hu & Wang, 1986). Regardless if embryo rescue or ovule culture is performed, a compromise must be reached between embryo development and viability (Collins & Grosser, 1984; Hu & Wang, 1986; George, 1993).

Propagation - Asexual and Sexual

Abelia taxa are reproduced commercially by vegetative propagation. Both semi-hardwood and hardwood stem cuttings are easily rooted. Little information is available regarding seed germination requirements, and seed production is considered undesirable because *Abelia* fruits mature over a long time period (Dirr and Heuser, 1987). Everett (1980) noted that nonhybrids germinate within a few weeks when sown on a sand-peat medium in the spring. Observations of untreated *A. chinensis* open-pollinated seeds indicated that germination was slow and non-uniform. Seeds sown in December 1997 did

not begin to germinate until February 1998, with seedlings sporadically emerging over several weeks. Similar germination patterns were observed of seed derived from interspecific crosses and germination percentages were approximately 50%. Although sexual reproduction is undesirable from a commercial standpoint, seed germination is essential for breeding and selection programs.

Abelia fruit is a one-seeded, leathery achene. The dry, indehiscent pericarp of the achene can mimic the function of a seedcoat to delay, reduce, or suppress germination by supplying the embryo with inhibitory compounds, preventing inhibitory compounds from leaving the embryo, mechanically interfering with radicle protrusion, or inhibition of water imbibition or gas exchange (Boesewinkel and Bouman, 1995). Removal or weakening of the pericarp has been shown to increase germination percentages and rates. Yambe and Takeno (1992) treated achenes of *Rosa multiflora* Thunb. with macerating enzymes. Nearly 80% germination was achieved when achenes were treated with 1% Driselase for 36h compared to less than 10% for untreated achenes. Gealy et al. (1985) tested the effect of pericarp removal on the germination of Mayweed (*Anthemis cotula* L.) under a series of constant temperatures. Maximum germination of 87% and 48% for both seeds and achenes, respectively, occurred at 20C. Furthermore, seed germination was earlier and more uniform than achene germination. Acid scarification for 15 min. with 27 N H_2SO_4 or germination in 14 mM GA_3 solutions increased germination percentages to 72% and 75%, respectively. However, germination percentages did reach the level achieved by untreated seeds under optimum temperature conditions indicating that the pericarp acts as a mechanical restraint to germination.

Inhibition of seed germination can result from embryo dormancy or a combination of embryo dormancy and hard seedcoats termed double dormancy. Although environmental conditions are optimum for germination, internal signals prevent germination. The embryo must undergo an after-ripening period to induce the biochemical processes necessary for germination (Dirr & Heuser, 1987). Stratification

and/or gibberellic acid treatments have proven useful for overcoming dormancy requirements. Incorporating 1 mM GA_3 into the germination medium of *Helianthus annuus* L. and *H. petiolaris* Nutt. overcame achenes dormancy. Germination percentages of *H. annuus* and *H. petiolaris* increased by 43 and 51%, respectively, relative to water controls (Seiler, 1998). Upfold and Van Staden (1990) report that achenes of *Tithonia rotundifolia* Mill. must undergo a 12-week after-ripening treatment for maximum germination to occur and that treatment with GA_3 stimulates germination. However, pericarp removal by mechanical or acid scarification did not enhance imbibition.

Inheritance of Foliage Variegation

The presence of distinct markings of different colors on a organ or organism is termed variegation (Marcotrigiano, 1997). Approximately 15 cultivars of *A. ×grandiflora* are commercially available and among its cultivars are several variegated selections: 'Confetti', 'Francis Mason', 'Golden Glow', 'Goldsport', 'Goldspot', 'Goldstrike', and 'Sunrise'. Variegated plants are intriguing in the landscape because of the presence of colorful streaks, spots, or sectors on their foliage and/or flowers. Variegated foliage contributes aesthetic value to the landscape throughout the year by providing highlights and color variations among evergreen foliage as opposed to the brief effectiveness of flowering (Bennett, 1984; Hudak, 1985; Elbert & Elbert, 1987; MacKenzie, 1989; Sheldon, 1991; L1oyd, 1994).

Variegation can be classified in two categories: non-cell lineage and cell lineage types, and is caused by differential gene expression, leaf blisters, viruses, or genetic mosaicism. Differential gene expression is classified as a non-cell lineage type and is the most common and most misunderstood cause of variegation. When genes responsible for the synthesis or destruction of pigments are expressed only in specific sectors or cell layers of an multicellular genetically homogenous organism variegation occurs, and patterns are determined by positional signals regulating gene expression (Marcotrigiano,

1997). Cells of different genotypes coexisting in the same organism are term genetic mosaics and are classified as cell lineage type variegation with a few exceptions. Plant chimeras as defined by Marcotrigiano (1997) are "a specific type of genetic mosaic in which the genetically dissimilar cells are present in the shoot apical meristem, where they continue to give rise to the cells that form the body of the plant. The arrangement of genetically dissimilar cells in the shoot apex is crucial to the stability of a chimeral state and will dictate the plant's phenotype." Chimeras are categorized as sectorial, mericlinal, or periclinal depending upon the arrangement of the genetically different cells within the shoot apical meristem (Marcotrigiano, 1997; Marcotrigiano & Gradziel, 1997).

The terms variegation and chimera are often used interchangeably in the literature, particularly in popular literature. Since all variegated plants are not chimeras, determining the mechanism controlling plant variegation is beneficial for reproduction of the phenomenon in future generations. Chimeral variegation patterns are not transmitted sexually and must be propagated asexually to reproduce the phenotype in future generations. Several factors provide evidence of nonchimeral variegation and include 1) maintenance of variegation patterns from plants derived from adventitious shoots 2) variegation patterns that do not appear to follow cell lineage patterns, however, exceptions do occur and 3) uncultivated species exhibiting the same pattern. However, the strongest evidence is provided by sexual transmission of the variegated phenotype to future generations (Marcotrigiano, 1997).

Observations of an open-pollinated *A. chinensis* population showed that eight of the 200 seedlings possessed yellow, variegated foliage suggesting nonchimeral, nuclear inheritance of the trait. The mode of inheritance for foliage variegation is not universal. Studies conducted in *Aglaonema* Schott. found foliar variegation to be controlled by a single locus, multiallelic system in which variegation is dominant to nonvariegation (Henny, 1983; Henny, 1986b; Henny, 1992). A single dominant nuclear gene controls foliar variegation in *Dieffenbachia* Schott. (Henny, 1982; Henny, 1986a). Lawrence

(1974) found that inheritance of variegated foliage patterns in Russian Wild Ryegrass (*Elymus junceus* Fisch.) was governed by dominant epistasis. A single homozygous recessive gene controls the inheritance of pale-green foliage and pinkish-white variegation in *Phaseolus vulgaris* L. and *Brassica campestris* L., respectively (Wyatt, 1981; Orakwue & Crowder, 1983). Reisch and Watson (1984) proposed a similar hypothesis for *Vitis* L. species to explain the occurrence of green and white variegation patterns in F_1 and S_1 seedling populations. Yellow striping in both purple and green foliage varieties of Pearl Millet [*Pennisetum typhoides* (Burm) S. & H.] was determined to be recessive to the normal non-striped foliage and under the control of three complementary recessive genes (Gill et al., 1969). Variegated foliage is a horticulturally desirable trait and determination of the mode of inheritance is beneficial for breeding programs and sexual propagation (Jaynes, 1974; Henny, 1986; Henny, 1992).

Adaption to Environmental Stresses

To improve adaptability to environmental stresses, techniques to evaluate plants for stress tolerance must be incorporated into the breeding program. A limited number of studies have been conducted within the genus *Abelia* and the majority of the work has centered upon *A.* ×*grandiflora*. Francois and Clark (1978) examined salt tolerance among 10 ornamental shrubs and found that *A.* ×*grandiflora* was very poorly adapted to salinity, being severely damaged or killed at EC_e's of 4 mmho/cm. Investigations of boron tolerance among 25 ornamental shrub species indicated *A.* ×*grandiflora* is semi-tolerant to boron at concentrations of 2.5 ppm and 7.5 ppm (Francois and Clark, 1979). *Abelia* ×*grandiflora* was found to be relatively tolerant to ozone at 2.5× ambient levels in studies conducted by Findley et al. (1997).

Breeders are particularly concerned with the adaption of woody plants to freezing stress because cold, more than any other environmental factor, limits the northern distribution range (Dirr et al., 1993). The universal problem of freeze damage is of major

economic importance even in subtropical regions (Weiser, 1970). Freezing stress occurs when temperatures are lowered to below 0°C and ice forms either inside (intracellular) or outside (extracellular) cell walls within plant tissues (Burke et al., 1976). Most plants have the ability to develop tolerance to extracellular freezing in response to exposure to fall/winter conditions. During extracellular freezing ice crystals form first in vessel elements of the xylem and then spread rapidly through the vessels to all other parts of the plant. The plasma membrane prevents the growth of ice crystals into the cytoplasm, thereby preventing direct injury since there is no contact between the ice and the protoplasm. However, a greater negative osmotic potential is generated by the extracellular ice causing diffusion of liquid water out of the protoplasm, resulting in dehydration. Cell injury is dependent upon the degree of freeze-induced dehydration. Fortunately, if injury is not too severe the cells are able to repair themselves (Chen, 1994). Numerous hardy woody species are able to survive extracellular freezing even to the temperature of liquid nitrogen (196°C) when fully acclimated (Burke et al., 1976).

Intracellular freezing will occur if temperatures fall below a critical point and ice crystals form inside the cytoplasm. Ice formation within cells causes disruption of the plasma membrane. Upon thawing, cytoplasmic components and cell turgor are lost, causing cell death (Chen, 1994; Burke et al., 1976). Such freezing occurs in tender plants that lack the ability to acclimate and in hardy plants before acclimation. It can also occur in plants that have the ability to supercool. Supercooling is a freezing avoidance mechanism involving the reduction or elimination of ice nucleation centers in cells and/or development of effective barriers to nucleation by ice in or around adjacent cells. However, should the homogenous nucleation temperature be reached or external ice nucleators initiate nucleation, death will result due to intracellular freezing. Another freezing avoidance mechanism, freezing point depression, results when water is withdrawn from a cell or other compartment. Consequently, the solute concentration

increases and the freezing point decreases by a few degrees. Few species have freezing point depressions of more than 4°C (Burke et al., 1976).

Cold acclimation is the transition from a tender to hardy status and involves a number of morphological, biochemical, and biophysical changes that are not fully understood. The potential for cold acclimation varies among species and different levels of cold hardiness have been exhibited among plant species and even cultivars within a species. Even within a single plant, individual tissues display differential hardiness (Chen, 1994). Alexander and Havis (1980) examined branch, lower stem and root parts from a deciduous and an evergreen azalea cultivar following acclimation to determine which organ was the most susceptible to cold injury. Results indicated that branches > lower stems > roots in levels of cold hardiness.

Cold hardiness is influenced by a number of factors that include provenance and plant environmental conditions (Lindstrom & Dirr, 1994). Cultural practices such as pruning dates, water regimes, and transplant dates can also influence cold hardiness (Davies et al., 1978; Marini, 1986; Haynes et al., 1991; Harber et al., 1992; Lindstrom, 1992; Anisko & Lindstrom, 1995). Woody plant species having wide geographic ranges have wide differences in cold hardiness. Plants from warm provenances are less cold hardy at specific times in early winter because they acclimate more slowly than accessions from colder origins (Flint, 1972). Furthermore, hardiness rankings vary due to timing and rates of acclimation and deacclimation in response to environmental conditions.

Temperature is a major factor controlling a plant's ability to acclimate, deacclimate, and ultimately develop maximum cold hardiness potential (Levitt, 1980; Chen, 1994). Flint (1972) found considerable variation in twig hardiness among 38 ecotypes of *Quercus rubra* L. Estimated extreme minimum temperatures ranged from -23C for accessions collected in Union County, Georgia to -46C for accessions from Cass County, Minnesota. Similar variations have been observed in *Cornus stolonifera* Michx.

(Smithberg & Weiser, 1968). Several authors have reported that variations in cold hardiness throughout the fall, winter, and early spring months are correlated to temperature fluctuations. Correlations between increases and decreases in flower bud hardiness and fluctuations in ambient temperatures prior to sampling dates have been reported in *Rhododendron* L. and *Vitis* (Pellett et al., 1991; Hubackova, 1996). To explain the effect of temperature on cold hardiness, Anisko et al. (1994) developed an empirical cold hardiness model based on the lowest survival temperatures of *Acer rubrum* L., *Betula nigra* L., *Fraxinus pennsylvanica* Marsh., *Liquidambar styraciflua* L., *Prunus serotina* J.F. Ehrh., and *Quercus alba* L. collected at weekly intervals during the winters of three consecutive years. The model demonstrates that a plants ability to harden and deharden in response to fluctuating temperatures is modulated by the accumulation of heat and chill hours.

Cold hardiness ratings are available for several taxa of *Abelia*, but the ratings are based on field observations in one or a few locations. Therefore, ratings for a given taxon often differ in the literature and are listed in Table A.2 of the Appendix. Little or no information is provided regarding the cultural practices, environmental conditions, or provenances of the taxa observed. Numerous laboratory procedures such as differential thermal analysis (DTA), electrical conductivity, and artificial freezing via freezing chambers have been adopted to examine the cold hardiness potentials of various plant taxa. Differential thermal analysis involves the attachment of thermocouplers to specific tissue (e.g. flower buds) to record the temperature of plant samples during freezing in order to deduce specific points of water crystallization, thereby identifying exotherms created by latent heat of fusion (Weiser, 1970; Burke et al., 1976). The electrical conductivity method measures the conductivity of electrolytes that have permeated from within in the cell following freezing damage. The greater the damage to the cell induced by freezing the greater the leakage of electrolytes and consequently the higher the

conductivity. Both differential thermal analysis and electrical conductivity provide objective measures of freeze damage.

Freezing chambers provide a subjective measure of freezing injury. The method involves subjection of sample representatives of a given taxa to gradually lower temperatures to determine lowest survival temperatures (Levitt, 1980; Lindstrom & Dirr, 1989). Levitt (1980) describes basic requirements for the method: 1) inoculation of plants to ensure freezing 2) a standard rate of cooling 3) a single freeze for a standard length of time 4) a standard rate of warming and 5) standardized postthawing conditions. Furthermore, hardiness estimations must be made throughout the year to provide a comprehensive profile of freezing. Included in the profile are 1) timing and rates of fall acclimation 2) mid-winter hardiness potential 3) stability of hardiness during fluctuating temperatures and 4) timing and rates of deacclimation. Strong correlations have been found between cold hardiness observed in the field and artificial freezing via freezing chambers for accessions evaluated at the same location on multiple test dates (Levitt, 1980; Dirr & Lindstrom, 1990).

Conclusion

Since breeding and selection work has been limited in *Abelia*, beneficial information regarding compatibility and reproductive biology has either been lost or is simply unavailable. Furthermore, knowledge of production procedures and environmental adaptability is restricted to observational data or trial and error practices without the benefit of scientifically derived evidence. Investigations of stress physiology, reproductive biology, propagation procedures and modes of inheritance are needed to optimize development of new interspecific hybrids in the genus *Abelia.*

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CHAPTER 2

STEM AND LEAF COLD HARDINESS OF 12 *ABELIA* TAXA[1]

[1]Scheiber, S.M., Carol D. Robacker, and Orville M. Lindstrom. Submitted to the Journal of Environmental Horticulture, 11/13/01.

STEM AND LEAF COLD HARDINESS OF 12 *ABELIA* TAXA

Keywords: cold tolerance, acclimation, deacclimation, *Abelia*

Abstract

Twelve taxa of *Abelia* were evaluated using laboratory procedures to determine maximum stem and leaf hardiness and to evaluate timing of acclimation and deacclimation over a two-year period. Among the 12 *Abelia* taxa evaluated, 'John Creech' ranked in the hardiest group of taxa for both stems and leaves on the majority of test dates. Stems and leaves of 'John Creech' survived to at least -25C(-13F) and -21C (-6F), respectively, in January 2001. 'Edward Goucher' and Confetti™ had the least hardy stems and leaves, respectively. Stems of 'Edward Goucher' survived to at least -16C(3F) in January 2000, and Confetti™ leaves survived to only -14(7F) in December 2000. *Abelia* ×*grandiflora* consistently ranked among the first to attain cold hardiness in the fall and among the last to lose cold hardiness in the spring in both test seasons. Stems were equal in hardiness or hardier than leaves on the majority of test dates in both test seasons. Laboratory results often differed from published hardiness ratings. Differences in lowest survival temperatures and attainment and retention of cold hardiness closely followed temperature fluctuations just prior to sampling dates. Timing of acclimation and deacclimation are generally not reflected in hardiness ratings, but in regions commonly affected by rapidly fluctuating spring temperatures, the capacity of a plant to retain hardiness is essential for survival and landscape performance.

Taxa used in this study: *A. chinensis* R. Br., *A.* 'Edward Goucher', *A.* ×*grandiflora* (André) Rehd., *A.* ×*grandiflora* 'Compacta', *A.* ×*grandiflora* 'Confetti', *A.* ×*grandiflora* 'Francis Mason', *A.* ×*grandiflora* 'Golden Glow', *A.* ×*grandiflora* 'John Creech', *A.* ×*grandiflora* 'Little Richard', *A.* ×*grandiflora* 'Prostrata', *A.* ×*grandiflora* 'Sherwoodii', *A. zanderi* (Graebn.) Rehd.

Significance to the Industry

Abelia are limited in their range of adaptability due more to cold than any other environmental factor. The northern landscape distribution of *Abelia* is limited by both stem and leaf hardiness. *Abelia* ×*grandiflora* is appealing due to its lustrous, dark, evergreen foliage. However, it becomes semi-evergreen in more northern climates and is not hardy below -20C(-4F). Evaluations of 12 *Abelia* taxa for stem and leaf hardiness in the southeastern United States revealed that 'John Creech' ranked in the hardiest group of taxa for both stems and leaves on the majority of test dates. In addition, 'John Creech' retained cold hardiness later in the spring than did the other taxa. 'Edward Goucher' and Confetti™ had the least hardy stems and leaves, respectively. Midwinter hardiness and timing of acclimation and deacclimation are important criteria for the selection of plant materials for landscapes and parental germplasm for cultivar development. Cold hardiness data indicate 'John Creech' would make a logical choice for incorporation into a breeding program or landscape.

Introduction

The genus *Abelia* contains 30 species that vary in many traits including cold hardiness, flower color, and growth habit (3,9,11). *Abelia* ×*grandiflora* (André) Rehd. is widely used in the landscape because of its prolific floral displays of pinkish-white flowers and glossy semi-evergreen to evergreen foliage (3,6,11). However, it becomes semi-evergreen in more northern climates, and the plants are not reported to be hardy below -20C(-4F) (2,4,6,8,11,18). The universal problem of freeze damage is of major economic importance even in subtropical regions (5,19). Breeders are concerned with the cold acclimation of woody plants because cold, more than any other environmental factor, limits the northern distribution range (7). Interspecific hybridization among various taxa of *Abelia* R. Br. offers the potential for new cultivars with improved cold hardiness.

Hardiness ratings are available for a number of species, but these ratings are based on field observations in a few locations and may not be applicable to different geographic regions (2,4,6,8,11,16,18). Studies conducted by Lindstrom and Dirr (13) have indicated a strong correlation between cold hardiness observed in the field and laboratory tests when plants were evaluated on multiple dates. Cold hardiness evaluations are needed for selection of superior parental germplasm and assurance of improved hardiness among progeny. The objective of this study was to evaluate 12 taxa of *Abelia* for stem and leaf cold hardiness.

Materials and Methods

Twelve taxa of *Abelia* (Tables 2.1-2.4) were evaluated for stem and leaf cold hardiness. Two deciduous species, *A. chinensis* R. Br. and *A. zanderi* (Graebn.) Rehd., and the evergreen species, *A.* ×*grandiflora* (André) Rehd., were evaluated. *Abelia* 'Edward Goucher', a cross between *A.* ×*grandiflora* and *A. schumannii*, and eight *A.* ×*grandiflora* cultivars, 'Compacta', Confetti™, 'Francis Mason', 'Golden Glow', 'John Creech', 'Little Richard', 'Prostrata', and 'Sherwoodii' were also evaluated. All taxa were obtained from commercial sources and public arboreta. A specimen of *A.* ×*grandiflora*, unknown origin, was collected from the University of Georgia campus, Athens, GA and included in the study.

Each representative of the 12 taxa was clonally propagated and grown in a #1 (3.8 l) container. Eight plants of each taxon, except Confetti™ and *A. zanderi*, were planted into a field in Griffin, GA, in a randomized complete block design in mid-September, 1998. Confetti™ and *A. zanderi* were added to the plot in mid-October 1999. The research plot was located under a canopy of Pecan trees [*Carya illinoinensis* (Wangenh.) C. Koch], drip irrigated as needed, and fertilized twice per year.

As described by Lindstrom and Dirr (13), 36 uniform stem tips, each approximately 10 cm (4 in) in length, were collected from each taxon on 16 October, 13

November, and 11 December 1999; 8 January, 12 February, 11 March, 17 October, 14 November, and 13 December 2000; and 16 January, 13 February, 13 March, and 17 April 2001 and prepared for testing within two hours of collecting. To prepare the stems and leaves for freezing, the terminal 7 cm (2.8 in) were removed, and leaves, if present, were removed from the stems. Four stems and leaves of each taxon were wrapped in cheesecloth and placed in a 25 × 200 mm test tube (1 × 8 in). A total of 9 test tubes per taxon were prepared. Tubes were then submerged in an ethylene glycol-water solution (1:1) in a Forma Scientific Model 2425 temperature bath (Forma Scientific, Marietta, OH) precooled to -2C±0.5(28F±1).

Stem and leaf temperatures were measured by thermocouples placed next to the samples and recorded by a Campbell Scientific datalogger (Model CR7-X, Campbell Scientific, Inc., Logan, UT). Crushed ice crystals were applied to the wet cheesecloth of stem and leaf samples to insure that the samples did not undercool. Temperature of the samples was held constant at -2C±0.5(28F±1) for approximately 14 hrs. Samples were then cooled at a rate of not greater than 4C(7F) per hour. Four stems and leaves of each taxon were removed from the bath at progressively lower 3C(5F) temperature intervals. Controls were prepared and kept at 4C (39F) for the duration of the freezing test.

Frozen samples were allowed to thaw overnight at 4C±2(39F±4). Samples were then removed from the tubes and placed in disposable, round, 100 × 15 mm (3.9 × 0.6 in) petri dishes containing filter paper saturated with distilled water to maintain 100 percent relative humidity. The petri dishes were placed on their sides in the dark at 22C±2(72F±4) for 7-10 days when samples were visually evaluated for injury. Stems and leaves showing brown discoloration and breakdown of cells in the cambium and phloem were rated as dead. Browning was observed with the naked eye and with the aid of a stereomicroscope when needed. Controls and samples not injured in the freezing tests were identified by green coloration or no discoloration or no breakdown of cells in the cambium and phloem. The number of stems and leaves killed at each temperature was

recorded and from these data the lowest survival temperatures (LSTs) were determined for each taxon. The LST is the lowest test temperature at which survival was observed (15). In many cases, no variability was observed among replicates when determining the LST. Where variability was present, the standard error was calculated. The sensitivity of the laboratory evaluation detected only cold hardiness differences greater than 3C(5F). The lower limit of the freeze bath was -27C(-17F) on all test dates.

Monthly LSTs were compared within and between test seasons among both species and cultivars to assess timing of acclimation and deacclimation and to determine maximum stem and leaf hardiness. Among species, the timing of acclimation and deacclimation of stems only were evaluated due to the deciduous nature of *A. chinensis* and *A. zanderi*. Timing of acclimation and deacclimation for both stems and leaves were assessed for cultivars.

Results and Discussion

Species Comparisons

Stem hardiness. *Abelia chinensis, A. ×grandiflora,* and *A. zanderi* survived to at least -24C(-11F) (Table 2.1). The mean lowest survival temperatures for each species were -25.5C±0.87(-13.9F±1.45) for *A. chinensis* in December 1999, -25.5C±0.87 (-13.9F±1.45) for *A. zanderi* and -24.75C±0.75(-12.55F±1.25) for *A. ×grandiflora* in February 2000. Published reports of hardiness ratings for *A. chinensis, A. ×grandiflora,* and *A. zanderi* range from zone 6 to 8, zone 5 to 7, and zone 5 to 6, respectively, depending on the authority (2,4,6,8,11,18). Dirr (6) reported that *A. chinensis* will survive to zone 5 as a herbaceous perennial.

Significant variation in the timing of acclimation and deacclimation occurred. *Abelia ×grandiflora* consistently ranked among the first to attain cold hardiness in the fall and among the last to lose cold hardiness in the spring in both test seasons. During the first test season, *A. ×grandiflora* was significantly hardier than *A. chinensis* in October

and significantly hardier than both species in March. In the second test season, *A. zanderi* was significantly less hardy in October than *A. chinensis* and *A. ×grandiflora,* but significantly more hardy than *A. chinensis* in March and April. No differences in LSTs were found between *A. ×grandiflora* and *A. zanderi* in March or April. As of the April 2001 test date, the deacclimation process of stems of *A. ×grandiflora* and *A. zanderi* was not completed. Between the first and second test season, *Abelia chinensis* and *A. ×grandiflora* were more cold hardy in October but less cold hardy in February. Stems of *A. zanderi* remained unchanged in hardiness or were less cold hardy on all test dates except in March 2001. In March 2000, only the controls survived, but, stems survived to approximately -10C(14F) in 2001. No differences were found for any other test dates between seasons one and two.

Leaf hardiness. Leaf hardiness of *A. ×grandiflora* was equal to or significantly greater than both *A. chinensis* and *A. zanderi* on the majority of test dates in both test seasons (Table 2.2). The mean lowest survival temperature recorded for *A. ×grandiflora* was -23.25C±0.75(-9.85F±1.25) in January 2001. During the first test season, leaves of *A. chinensis* and *A. zanderi* abscised following the January test date. Mean low temperatures were lower in October of the second test season resulting in the abscission of *A. chinensis* leaves after the November test date. Despite colder temperatures in year two, *A. zanderi* did not defoliate, and only the controls survived the March and April sampling dates.

Cultivar Comparisons

Stem hardiness. 'John Creech' and 'Edward Goucher' were ranked in the hardiest and least hardy groups of cultivars, respectively, on 10 out of 13 test dates (Table 2.3). Stems of 'John Creech' survived to approximately -23C(-9F) in February 2000 during the first test season and approximately -26C(-15F) in January 2001 of the second test season. The mean lowest survival temperatures recorded for 'Edward Goucher' in season one and two

were -16.5C±0.87(2.3F±1.45) in January 2000 and -15.75C±0.75(3.65F±1.25) in December 2000, respectively. Dependent on the source, 'Edward Goucher' has been reported hardy to zone 5 or 6 (6,8,11). It was expected that 'Edward Goucher' would be among the least hardy taxa based upon its parentage. It is an interspecific hybrid between *A.* ×*grandiflora* and *A. schumannii*. Published hardiness rankings, based on field observations, vary for both *A.* ×*grandiflora* and *A. schumannii*. *A.* ×*grandiflora* is reported hardy from zone 5 to zone 7, and hardiness rankings for *A. schumannii* range from zone 6 to zone 8 (2,4,6,8,11,18).

Plants attained cold hardiness later and lost cold hardiness earlier in the first test season compared to the second test season; however, no clear trends in ranking of the cultivars were identified. In October 1999, only 'Francis Mason' and 'Golden Glow' survived the freezing test. All cultivars survived to at least -3C(26F) in October 2000, and 'Compacta', 'Little Richard', 'Prostrata', and 'Sherwoodii' survived to approximately -7C(19F). During the first test season, it was November before cultivars achieved LSTs equivalent to those reached by October 2000. Stems remained unchanged in hardiness or were more cold hardy on all sampling dates during the second test season relative to the corresponding test date in the first test season, with the exception of the January and February sampling dates. 'Edward Goucher' was less cold hardy in January 2001 than in January 2000. In February 2001, 6 of the 10 cultivars were less cold hardy than on the corresponding test date in season one.

Leaf Hardiness. Confetti™ was ranked among the least hardy group of cultivars on 9 out of 12 test dates. Despite its ranking among the least hardy cultivars, a mean lowest survival temperature of -14.25C±0.75(-6.35F±1.25) was recorded in December 2000 (Table 2.4). On 12 out of 13 test dates, 'John Creech' was ranked in the hardiest group of cultivars. The LST recorded for 'John Creech' was -21C(-6F) in January 2001. Among the remaining cultivars, mean LSTs ranged between -10.5C±0.87(13.1F±1.45) and -18.75C±0.75(-1.75F±1.25), and all were recorded in January 2001.

Leaves vs. stems

Stems of all evergreen taxa were equal in hardiness or significantly hardier than leaves on 9 out of 13 test dates (Tables 2.1-2.4). The majority of occasions when leaves were more hardy than stems occurred as plants were acquiring cold hardiness in October 1999 and as plants began to lose hardiness in March 2000 and April 2001. During the midwinter test dates of December through February, only two instances occurred where leaves were significantly hardier than stems: *A.* ×*grandiflora* and 'Edward Goucher' in January 2001. Field observations revealed that evergreen taxa often appeared semi-evergreen during the midwinter months, but plant survival was not affected by leaf hardiness. 'John Creech' ranked as the hardiest cultivar for both stems and leaves on the majority of test dates. The least hardy cultivars for stems and leaves were 'Edward Goucher' and Confetti™, respectively.

Temperature Effects

Temperature is a major factor controlling a plant's ability to acclimate, deacclimate, and ultimately develop maximum cold hardiness (5,12). Several authors have reported that variations in cold hardiness throughout the fall, winter, and early spring months are correlated to temperature fluctuations. An empirical cold hardiness model developed by Anisko et al. (1) to explain the effect of temperature on cold hardiness demonstrates that a plant's ability to harden and deharden in response to fluctuating temperatures is modulated by the accumulation of heat and chill hours. Pellett et al. (17) reported rapid dehardening of flower buds of Flame Azalea [*R. calendulaceum* (Michx.) Torr.], Roseshell Azalea [*R. prinophyllum* (Small) Millias] and Swamp Azalea [*R. viscosum* (L.) Torr.] in response to increases in air temperature just prior to the testing dates. Significant correlations were reported between the LSTs of grapevine buds and the mean maximum and average temperatures that the grapevines were exposed to prior to sampling throughout the winter season (10). However, significant correlations

between mean minimum temperature and cold hardiness were only found during midwinter.

In the present study, differences in monthly LSTs, as well as timing of acclimation and deacclimation both among and within taxa can be attributed to differences in high and/or low temperatures 7 to 10 days prior to the test dates (Fig. 2.1-2.3). The mean high and low temperatures were lower during the second test season for all sampling periods except February. Average low temperatures were nearly 20C(33F) lower one week prior to the test date in October 2000 than October 1999 (Fig. 2.1). The average low temperatures were 11.4C(20.5F) lower in the 10 days preceding the October 2000 sampling date than in October 1999 (Fig. 2.1), but average high temperatures were only 0.8C(1.4F) lower. Stems of all taxa were more hardy in October 2000 than October 1999 and decreases in LSTs of 3 to 10C (5 to 17F) were recorded. In February 2000, increases in stem LSTs ranging from 3 to 11C (5 to 18F) were observed. Mean high and low temperatures in February 1999 were 2.2C(3.9F) and 2.4C(4.3F) lower, respectively, during the 10 days preceding the sampling date than in February 2000 (Fig. 2.2). Similar temperature and LST trends were observed during the March sampling dates. In March 2000, the mean high and low temperatures 10 days prior to the sampling date were 6.0C(10.8F) and 4.8C(8.6F) higher, respectively, than in March 2001 (Fig. 2.3). Stems of all taxa, except *A. chinensis*, were at least 2C(3F) more hardy in March 2001. Only the controls of *A. chinensis* survived during either March sampling date. The temperature data suggest that low temperature is more critical for acclimation of *Abelia* taxa than high temperature; however, no trend could be identified for deacclimation.

Temperature fluctuations just prior to sampling dates significantly affect lowest survival temperatures and timing of acclimation and deacclimation among *Abelia* taxa. Timing of acclimation and deacclimation can be more critical in southern landscapes than actual midwinter hardiness because of unexpected cold spells in the early fall and late spring (5,14,15,16). Although midwinter hardiness varied little among all taxa, ‘John

Creech' had the greatest stem and leaf hardiness on the majority of test dates. In addition, 'John Creech' retained cold hardiness later in the spring than did the other taxa. Midwinter hardiness and timing of acclimation and deacclimation are important criteria for the selection of superior parental germplasm to assure improved hardiness among resulting cultivars. Based on the data, 'John Creech' would make a logical choice for incorporation into a breeding program.

Table 2. 1. Mean lowest survival temperatures (LST °C ± SE) for stems of 3 *Abelia* species from October 1999 to April 2001.

	Date													
	Oct. 1999	Oct. 2000	Nov. 1999	Nov. 2000	Dec. 1999	Dec. 2000	Jan. 2000	Jan. 2001	Feb. 2000	Feb. 2001	March 2000	March 2001	April[z] 2000	April 2001
Deciduous Taxa														
A. chinensis	C[y]	-9.75[†]	-12.75[†]	-9.75[†]	-25.5[††]	-23.25[†]	-23.25[†]	-21.75[†]	-24	-15.75[†]	C[y]	C[y]	—	C[y]
A. zanderi[x]	—	-3.75[†]	-15.75[†]	-9.75[†]	-24.75[†]	-20.25[†]	-20.25[†]	-23.25[†]	-25.5[††]	-15.75[†]	C[y]	-9.75[†]	—	-1.5[††]
Evergreen Taxa														
A. × *grandiflora*	-1.5**	-6.75[†]	-9.75[†]	-13.5[††]	-22.5[††]	-21.75[†]	-18.75[†]	-19.5[††]	-24.75[†]	-15.75[†]	-3.75[†]	-6.75[†]	—	-3

[z]Test not conducted in April of the first test season.
[y]Only control (C) survived.
[x]Added to the study after the first test date, 10-16-99.
[†]Standard error of 0.75.
[††]Standard error of 0.87.

Table 2.2. Mean lowest survival temperatures (LST °C ± SE) for leaves of 3 *Abelia* species from October 1999 to April 2001.

	Date													
	Oct. 1999	Oct. 2000	Nov. 1999	Nov. 2000	Dec. 1999	Dec. 2000	Jan. 2000	Jan. 2001	Feb. 2000	Feb. 2001	March 2000	March 2001	April[z] 2000	April 2001
Deciduous Taxa														
A. chinensis	C[y]	-1.5[††]	-4.5[††]	-2.25[†]	-7.5[††]	—[x]	-13.5[††]	—[x]	—[x]	—[x]	—[x]	—[x]	—	—[x]
A. zanderi[w]	—	C[y]	C[y]	C[y]	-6.75[†]	-15.75[†]	-12.75[†]	-12.75[†]	—[x]	-4.5[††]	—[x]	C[y]	—	C[y]
Evergreen Taxa														
A. × *grandiflora*	C[y]	-0.75[†]	-8.25[†]	-4.5[††]	-12.75[†]	-6.75[†]	-9.75[†]	-23.25[†]	-9.75[†]	-15.75[†]	-3	-7.5[††]	—	-4.5[††]

[z]Test not conducted in April of the first test season.
[y]Only control (C) survived.
[x]The species is deciduous and the leaves had abscised by the given test date.
[w]Added to the study after the first test date, 10-16-99.
[†]Standard error of 0.75.
[††]Standard error of 0.87.

Table 2.3. Mean lowest survival temperatures (LST °C ± SE) for stems of 9 *Abelia* cultivars from October 1999 to April 2001.

Taxa	Date													
	Oct. 1999	Oct. 2000	Nov. 1999	Nov. 2000	Dec. 1999	Dec. 2000	Jan. 2000	Jan. 2001	Feb. 2000	Feb. 2001	March 2000	March 2001	April[z] 2000	April 2001
'Compacta'	C[y]	-6.75[†]	-11.25[†]	-9.75[†]	-19.5[††]	-21.75[†]	-18	-23.25[†]	-22.5[††]	-19.5[††]	-3	-9	—	-3.75[†]
'Confetti'[x]	—	-4.5[††]	-3.75[†]	-9	-19.5[††]	-22.5[††]	-21	-22.5[††]	-19.5[††]	-14.25[†]	-4.5[††]	-8.25[†]	—	C[y]
'Edward Goucher'	C[y]	-3.75[†]	-2.25[†]	-6	-8.25[†]	-15.75[†]	-16.5[††]	-12.75[†]	-15.75[†]	-12.75[†]	-3.75[†]	-6.75[†]	—	-1.5[†]
'Francis Mason'	-1.5[††]	-4.5[††]	-3.75[†]	-8.25[†]	-15	-21.75[†]	-12.75[†]	-21.75[†]	-22.5[††]	-12.75[†]	-2.25[†]	-6	—	C[y]
'Golden Glow'	-0.75[†]	-5.25[†]	-4.5[††]	-9.75[†]	-13.5[††]	-18.75[†]	-17.25[†]	-22.5[††]	-15.75[†]	-4.5[††]	-2.25[†]	-7.5[††]	—	-0.75[†]
'John Creech'	C[y]	-4.5[††]	-4.5[††]	-13.5[††]	-19.5[††]	-21.75[†]	-21.75[†]	-25.5[††]	-23.25[†]	-22.5[††]	-6.75[†]	-9	—	C[y]
'Little Richard'	C[y]	-6.75[†]	-9.75[†]	-12.75[†]	-16.5[††]	-21.75[†]	-23.25[†]	-21.75[†]	-22.5[††]	-15.75[†]	-2.25[†]	-6	—	-1.5[††]
'Prostrata'	C[y]	-6.75[†]	-12.75[†]	-10.5[††]	-17.25[†]	-22.5[††]	-17.25[†]	-24.75[†]	-17.25[†]	-11.25[†]	-3	-9	—	C[y]
'Sherwoodii'	C[y]	-6.75[†]	-6.75[†]	-9.75[†]	-15.75[†]	-21.75[†]	-16.5[††]	-24	-19.5[††]	-14.25[†]	-2.25[†]	-7.5[††]	—	C[y]

[z]Test not conducted in April of the first test season.
[y]Only control (C) survived.
[x]Added to the study after the first test date, 10-16-99.
[†]Standard error of 0.75.
[††]Standard error of 0.87.

Table 2.4. Mean lowest survival temperatures (LST °C ± SE) for leaves of 9 *Abelia* cultivars from October 1999 to April 2001.

Taxa	Date													
	Oct. 1999	Oct. 2000	Nov. 1999	Nov. 2000	Dec. 1999	Dec. 2000	Jan. 2000	Jan. 2001	Feb. 2000	Feb. 2001	March 2000	March 2001	April[z] 2000	April 2001
'Compacta'	-0.75[†]	C[y]	C[y]	-4.5[††]	-10.5[††]	-15.75[†]	-11.25[†]	-15.75[†]	-15.75[†]	-14.25[†]	-0.75[†]	-4.5[††]	—	-1.5[††]
'Confetti'[x]	—	C[y]	-2.25[†]	C[y]	-3.75[†]	-14.25[†]	-5.25[†]	-10.5[††]	C[y]	-6.75[†]	C[y]	C[y]	—	C[y]
'Edward Goucher'	C[y]	-0.75[†]	C[y]	-3.75[†]	-3.75[†]	-15.75[†]	-9.75[†]	-18	-13.5[††]	-2.25[†]	-3.75[†]	C[y]	—	C[y]
'Francis Mason'	C[y]	-1.5[††]	C[y]	-3.75[†]	-12.75[†]	-9.75[†]	-9.75[†]	-18.75[†]	-14.25[†]	-13.5[††]	-1.5[††]	-4.5[††]	—	-3.75[†]
'Golden Glow'	-0.75[†]	-1.5[††]	C[y]	-5.25[†]	-9.75[†]	C[y]	-13.5[††]	-16.5[††]	-12.75[†]	-5.25[†]	-2.25[†]	-0.75[†]	—	-0.75[†]
'John Creech'	-0.75[†]	-3.75[†]	-4.5[††]	-7.5[††]	-9.75[†]	-15.75[†]	-13.5[††]	-21	-13.5[††]	-14.25[†]	-11.25[†]	C[y]	—	-0.75[†]
'Little Richard'	C[y]	C[y]	-0.75[†]	-0.75[†]	-0.75[†]	-7.5[††]	-11.25[†]	-18.75[†]	-15.75[†]	-10.5[††]	-2.25[†]	-7.5[†]	—	-3.75[†]
'Prostrata'	-0.75[†]	C[y]	C[y]	-6.75[†]	-9	-11.25[†]	-9.75[†]	-15.75[†]	-12	-12	-2.25[†]	-6	—	-3
'Sherwoodii'	C[y]	-1.5[††]	-2.25[†]	-4.5[††]	-7.5[††]	-13.5[††]	-12.75[†]	-16.5[††]	-10.5[††]	-0.75[†]	C[y]	-5.25[†]	—	C[y]

[z]Test not conducted in April of the first test season.
[y]Only control (C) survived.
[x]Added to the study after the first test date, 10-16-99.
[†]Standard error of 0.75.
[††]Standard error of 0.87.

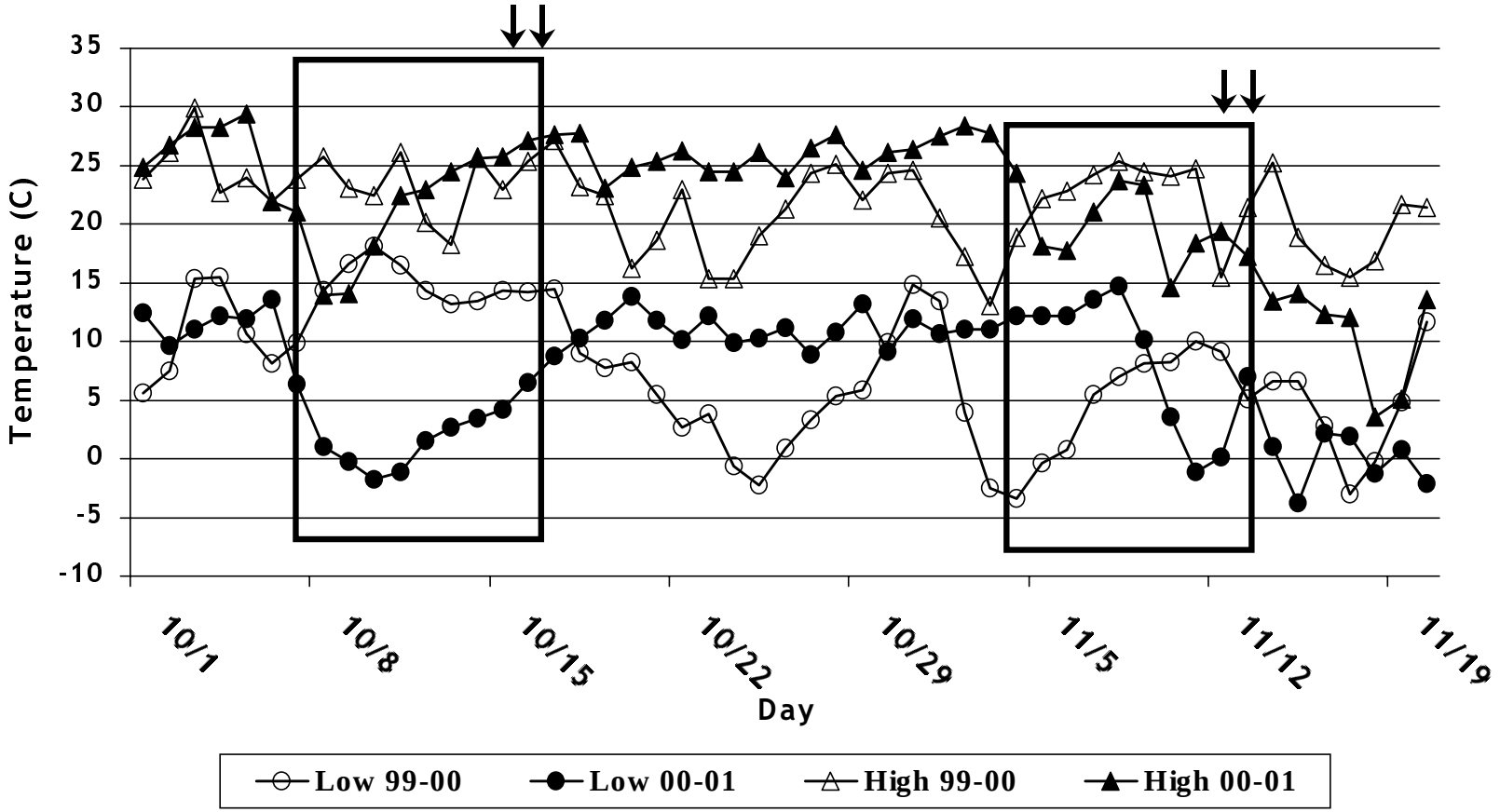

Fig. 2.1. Low and high temperatures for the period of October 1, 1999 through November 20, 1999 and October 1, 2000 through November 20, 2000. Arrows indicate the sampling dates. Boxes indicate the 10-day period prior to the sampling date.

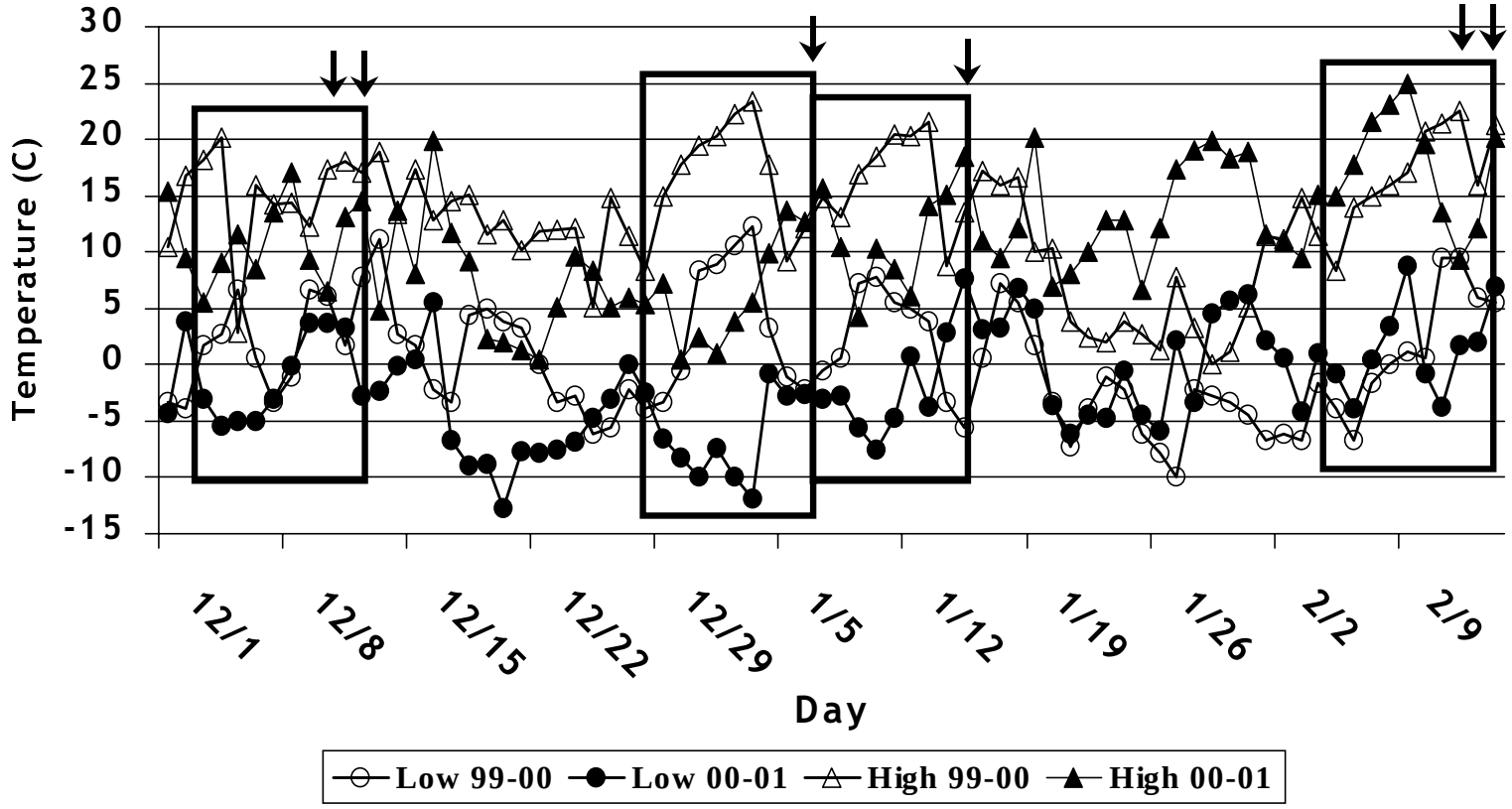

Fig. 2.2. Low and high temperatures for the period of December 1, 1999 through February 12, 2000 and December 1, 2000 through February 12, 2001. Arrows indicate the sampling dates. Boxes indicate the 10-day period prior to the sampling date.

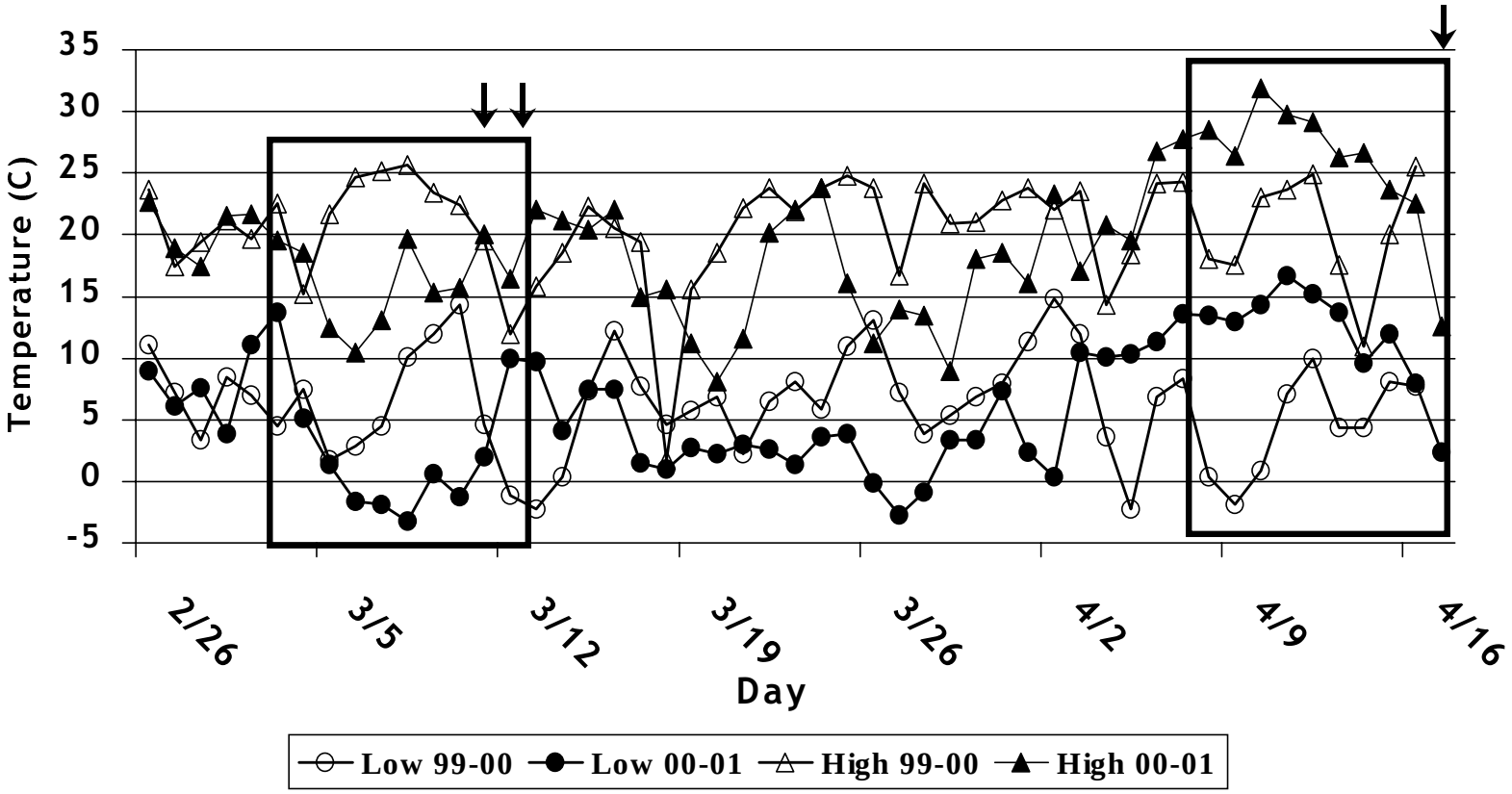

Fig. 2.3. Low and high temperatures for the period of February 26, 2000 through April 17, 2000 and February 26, 2001 through April 17, 2001. Arrows indicate the sampling dates. Boxes indicate the 10-day period prior to the sampling date.

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CHAPTER 3

STIGMATIC RECEPTIVITY AND INTERSPECIFIC HYBRIDIZATION IN *ABELIA*[2]

[2]Scheiber, S.M. and Carol D. Robacker. To be submitted to HortScience.

STIGMATIC RECEPTIVITY AND INTERSPECIFIC HYBRIDIZATION IN *ABELIA*

Keywords: *Abelia chinensis, Abelia engleriana, Abelia floribunda, Abelia* ×*grandiflora, Abelia serrata, Abelia spathulata***,** ovule culture, embryo rescue, self-incompatibility

Intra- and interspecific crossability was assessed among 12 taxa of *Abelia.* Seed set was generally higher among interspecific hybrids than intraspecific hybrids. Success rate of interspecific crosses varied dependent on the parentage, but previously unrecorded interspecific hybrids were obtained between *A. chinensis* × *A. engleriana, A. chinensis* × *A. spathulata, A. chinensis* × *A. serrata,* and *A. chinensis* × *A. zanderi*. *Abelia chinensis* and *A.* ×*grandiflora* 'Francis Mason' were evaluated for stigmatic receptivity. Flowers were pollinated 1-, 2-, or 3-days prior to anthesis; the day of anthesis; and 1-, 2-, 3-, 4-, or 5-days postanthesis. Receptivity of both taxa was highest on the day of anthesis, but stigmas remained receptive throughout the pollination period.

Introduction

Abelia ×*grandiflora* (André) Rehd. has been a staple in landscapes for nearly a century. It is noted for prolific displays of pinkish-white flowers from May to frost, resistance to environmental stresses, and glossy evergreen foliage (Bean, 1970; Krussmann, 1985; Dirr, 1998a). Derived from a cross between *A. chinensis* R. Br. and *A. uniflora* R. Br., *A.* ×*grandiflora* was first described in 1886 from a plant raised from seed in the Rovelli nurseries, Pallanza, on Lake Maggiore (Bean, 1970). *Abelia* ×*grandiflora* is one of only two interspecific hybrids ever developed and released within the genus. *Abelia* 'Edward Goucher', a hybrid of *A.* ×*grandiflora* and *A. schumannii* (Graebn.) Rehd., was introduced by Edward Goucher, USDA, Glenn Dale Plant Introduction Station (Krussmann, 1985).

The genus *Abelia* R. Br. is a member of the family Caprifoliaceae Juss. and contains approximately 30 species that vary widely in flower color, size, leaf retention, and growth habit. Despite the potential for interspecific hybridization, breeding work in the genus had been limited. Since the introduction of *A.* 'Edward Goucher' in 1911, only sports and seedling selections of *A.* ×*grandiflora* and open-pollinated selections of *A. chinensis* have been introduced into commercial production (Bean, 1970; Dirr, 1994; Dirr, 1998a). Interest has been expressed by nursery owners in the development of new cultivars through interspecific hybridization (Dirr, 1998b).

Cytological and reproductive biology studies in *Abelia* are limited and have focused primarily on chromosome counts. Bar-Shalom and Mattsson (1977) reported in vitro germination of *A.* ×*grandiflora* and *A. schumannii* pollen, indicating pollen viability, but no information was presented concerning viability of the female reproductive system. Knowledge of the receptivity of the stigmatic surface is vital for determining the effective pollination period and optimizing returns on pollination efforts. Gonzalez et al. (1995) reports that stigmatic receptivity is the main factor limiting the effective pollination period in Kiwifruit [*Actinidia deliciosa* (Chev.) Liang and

Ferguson]. Stigmatic receptivity can vary widely among woody taxa, ranging from a few hours in teak (*Tectona grandis* L.f.) to several days in almond [*Prunus dulcis* (Mill.) D.A. Webb] (Sharma et al., 1990; Tangmicharoen and Owens, 1997).

Numerous pollinations must be performed to obtain the large quantities of seeds needed to conduct a breeding program in *Abelia* because the fruit is a one-seeded achene. Since breeding work has been limited in *Abelia*, information regarding interspecific crossability and reproductive biology is unavailable. The purpose of this study is to evaluate stigmatic receptivity and assess interspecific crossability to maximize pollination success and ultimately develop new interspecific hybrids.

Materials and Methods

Stigma Receptivity

Plant material and pollinations. *Abelia* ×*grandiflora* 'Francis Mason' and *A. chinensis* were used in the study. Reciprocal crosses between *A. chinensis* and 'Francis Mason' were performed in mid-August 1999 in a greenhouse. The flowers were pollinated at the following intervals: 1-, 2-, or 3-days prior to anthesis; the day of anthesis; and 1-, 2-, 3-, 4-, or 5-days postanthesis. Pollen was collected from freshly dehiscing anthers and applied directly to the stigma. Pollinations were bagged with glassine envelopes (BioQuip Products, Gardena, CA).

In vivo germination tests. Styles were removed from the pistil 48h after pollination and placed in a fixative of seven parts 95% ethanol and three parts glacial acetic acid. Prior to staining, the stylar tissue was softened by soaking the styles in petri plates containing 1N NaOH for 1h. After softening, the styles were placed on a glass slide, stained with aniline blue (1.0 g of aniline blue, 63.9 g K_3PO_4 in 1L H_2O, Martin, 1958), and squashed with a cover slip. The styles were incubated in 150 × 15 mm petri dishes at 100% RH at 5°C for 24h before examination with fluorescence microscopy. The callose lining and the

callose plugs in the pollen tubes fluoresce when stained with aniline blue, allowing differentiation of the pollen tubes from the non-fluorescing stylar tissue.

Data collection and analysis. Receptivity was determined by *in vivo* germination tests in which the presence or absence of pollen tube growth within the style was evaluated. Percentage of styles with pollen tube growth was calculated for each replicate as the number of styles with pollen tubes present/total number of styles examined. Each replicate consisted of five pollinations performed on the same day. Treatments were replicated six times, over six days. In addition, seed set data were collected and used as a measure of female fertility or post-fertilization barriers. Again, five pollinations of each type were performed per day for six days. Percentage of crosses setting seed was calculated. The experiment was conducted as a split plot design. Data were analyzed by analysis of variance.

Intra- and Interspecific Crosses

Plant material and pollinations. Fourteen taxa of *Abelia* were evaluated for intraspecific and/or interspecific crossability. The species used were *A. chinensis, A. engleriana* (Graebn.) Rehd., *A. floribunda* Decne., *A. mosanensis* Chung ex Nakai, *A. schumannii, A. serrata* Sieb. & Zucc., *A. spathulata* Sieb. & Zucc., and *A. zanderi* (Graebn.) Rehd. The *A. ×grandiflora* cultivars used were ‘Compacta’, ‘John Creech’, ‘Francis Mason’, ‘Golden Glow’, and ‘Prostrata’. All taxa were obtained from commercial sources and public arboreta. A specimen of *A. ×grandiflora*, unknown origin, was collected from the University of Georgia campus, Athens, GA and included in the study. All taxa were clonally propagated. Plant materials were maintained and pollinations were performed in a greenhouse. Flowers were emasculated 1-day preanthesis, pollinated on the day of anthesis, and bagged with glassine envelopes. Bags were removed two days after pollination. Pollen was collected from freshly dehiscing anthers and applied directly to the stigma.

Experiment 1 - Self pollinations of *A. chinensis, A.* 'Edward Goucher', and *A.* ×*grandiflora* 'John Creech', 'Francis Mason' and 'Golden Glow' were performed by bagging two inflorescences per taxon prior to anthesis and shaking the bags to disseminate pollen. Pollinations were performed in June 1998 in a greenhouse. Inflorescences remained bagged until fruit ripening and seed set was assessed.

Experiment 2 - Self-seed set was assessed among 4 *A.* × *grandiflora* genotypes by examining pollen tube growth in the style with fluorescence microscopy as previously described. Self pollinations of the species *A.* ×*grandiflora,* and three of its cultivars: 'Compacta', 'Francis Mason', and 'Prostrata' were performed by both bagging inflorescences and hand pollinations. Bagged inflorescences were pollinated by shaking the bags to disseminate pollen. Hand-pollinated flowers were emasculated and pollinated 1-day preanthesis. Pollen was collected from freshly dehiscing anthers and applied directly to the stigma. All pollinations were bagged with glassine envelopes. Styles were harvested 48h after pollination.

Experiment 3 - *Abelia chinensis, A.* 'Edward Goucher', and *A.* ×*grandiflora* 'John Creech', 'Francis Mason' and 'Golden Glow' were crossed in a diallel mating design excluding self pollinations in June-August 1998. Intra- and interspecific crosses were assessed by seed set and seedlings obtained. Percentage of seed set per cross combination was calculated as total number of seeds set/total number of pollinations. Percentage of seedlings obtained was calculated as the number of seedlings that germinated/total number of pollinations.

Experiment 4 - Pollinations among *A. chinensis, A. engleriana, A. floribunda, A. schumannii, A. serrata,* and *A. zanderi* were performed in June-October 2000. Crossability was assessed by seed set and seedlings obtained. Percentage of seed set and percentage of seedlings obtained was calculated as in Experiment 3.

Experiment 5 - Pollinations among *A.* 'Edward Goucher', three accessions of *A. chinensis, A. engleriana, A. floribunda, A.* ×*grandiflora* 'Francis Mason', *A. mosanensis,*

A. schumannii, A. serrata, A. spathulata, and *A. zanderi* were performed in June 2001. Embryo rescue through ovule culture was performed in an attempt to bypass post-zygotic barriers to hybridization. Ovules were collected 5 weeks after pollination, and cultured on Woody Plant Medium (WPM) (Lloyd & McCown, 1981) containing MS vitamins, 3% sucrose, and solidified with 0.8% agar (Scheiber, 2001). Percent ovules recovered was calculated as total number of ovules cultured/total number of pollinations performed. Plant survival was calculated as the number of plants surviving the hardening-off process/total number of pollinations performed.

Complete diallels could not be performed in the studies to assess interspecific crossability in Experiments 4 and 5 due to incongruent flowering cycles and the inability to successfully store pollen.

Results and Discussion

Stigma Receptivity

Stigmatic receptivity of *A. chinensis* and 'Francis Mason' was highest on the day of anthesis and gradually declined as days from anthesis increased (Tables 3.1 and 3.2). However, no significant differences in receptivity of 'Francis Mason' were observed from 1-day preanthesis through 4-days postanthesis. *Abelia chinensis* stigmas were 100% receptive on the day of anthesis and 1-day postanthesis, though receptivity was not significantly different from 3-days preanthesis through 3-days postanthesis. For both species, stigmas remained receptive throughout the pollination period with receptivity exceeding 50% from 3-days preanthesis through 5-days postanthesis. Similar reports in decline of stigmatic receptivity as time increased from anthesis have been reported among numerous taxa including: *Actinidia deliciosa,* cultivars of *Prunus dulcis*, and *Cucurbita pepo* L. (Sharma et al., 1990; Nepi & Pacini, 1993; Gonzalaz et al., 1995). Within Caprifoliaceae, retention of stigmatic receptivity throughout the flowering cycle was

reported for *Lonicera deflexicalyx* Batalin., *L. gracilipes* Miq., and *L. tatarica* L. (Gunatilleke & Gunatilleke, 1984).

Seed set generally declined relative to increasing days from anthesis for 'Francis Mason'. Significant declines in percent seed set were observed for postanthesis pollinations but not for preanthesis pollinations. The reduction in seed set was less apparent for *A. chinensis* with a significant decline in percent seed set recorded only at 5-days preanthesis. Regardless of days from anthesis, seed set never equaled stigma receptivity for either species.

Intra- and Interspecific Crossability

Experiment 1. Self pollinations of *A. chinensis, A.* 'Edward Goucher', and *A.* ×*grandiflora* 'John Creech', 'Francis Mason' and 'Golden Glow produced no seed. These plants have successfully been used as maternal and paternal parents in interspecific crosses performed in the same environment (Table 3.3). Failure of self pollinations suggests that self-incompatibility is occurring within some taxa of *Abelia*.

Experiment 2. Observations of styles with fluorescence microscopy following self-pollinations (bagged and hand pollinated) revealed failure of pollen germination and/or abortion of pollen tube growth on the stigma or in the upper style in 'Compacta' and 'Francis Mason' (data not shown) and provide further evidence for the presence of a self-incompatibility system in *Abelia*. Pollen tube growth was observed in 26% and 14% of the styles from bagged and hand pollinated flowers, respectively, of *A.* ×*grandiflora.* Bagged and hand pollinated flowers of 'Prostrata' revealed pollen tube growth in only 8% of the styles examined. No information is available regarding self-incompatibility in other species in the genus. Self-incompatibility has been reported in Caprifoliaceae, in the genera *Lonicera* L., *Sambucus* L., and *Viburnum* L. (Brewbaker, 1957; Brewbaker, 1967; Sedgley, 1994). Conformation of incompatibility is needed in *Abelia* taxa to eliminate the need for the time consuming activity of emasculation.

Experiment 3. When *A. chinensis* was used as the maternal parent in crosses to *A.* ×*grandiflora* cultivars, on average 59% of the crosses produced seed, and 38% yielded seedlings (Table 3.3). Crosses between *A. chinensis* and *A.* 'Edward Goucher' yielded a seed set of 33%, and 10% of the crosses produced plants. Dependent on the maternal parent, seed set ranged between 12-60% if *A. chinensis* was the paternal parent. Seed set did not exceed 25% if *A. chinensis* was not included in the parentage. *Abelia chinensis* is the maternal parent of *A.* ×*grandiflora* and is in the parentage of *A.* 'Edward Goucher' (*A.* ×*grandiflora* × *A. schumannii*).

Among *A.* ×*grandiflora* cultivars, the highest seed set recorded was 16% from the cross between 'John Creech' and 'Francis Mason'. Seedling survival from the cross was less than 10%. No seed was set between reciprocal crosses of 'Francis Mason' and 'Golden Glow'. 'Francis Mason' originated as a variegated branch sport of *A.* ×*grandiflora*, and the foliage is yellow to yellow-green with a mottled appearance (Dirr, 1994; Kelly & Hillier, 1995). The origin of 'Golden Glow' is unknown, but morphologically its yellow foliage differs from 'Francis Mason' only by the presence of a distinct yellow margin (Dirr, 1998). Failure of seed set from crosses between the cultivars offers additional support for the presence of a self-incompatibility system in *Abelia*. *Abelia* 'John Creech' was originally listed as a sister seedling of *A.* 'Edward Goucher'; however, morphologically it resembles *A.* ×*grandiflora* and is currently regarded as an *A.* ×*grandiflora* cultivar (Dirr, 1998). Pseudo-self compatibility can account for the low seed set observed when 'John Creech' was included in the parentage. Percentage of seedlings obtained generally declined relative to percentage seed set among all crosses. Failure of seeds to germinate from interspecific hybrids is attributed to post-fertilization barriers.

Experiment 4. The cross of *A. chinensis* to *A.* ×*grandiflora* 'Francis Mason' gave the highest seed set, 72% (Table 3.4). A seedling recovery of 48% was recorded for the cross of *A. chinensis* × *A. zanderi*, the highest of any crossing combination. As maternal

parents, *A. chinensis* , *A. engleriana*, and 'Francis Mason' had the highest seed set and seedling recovery percentages. Depending on the paternal parent, seed set ranged from 2-100%, and seedlings obtained ranged from 0-48%. The highest percentages were recorded for *A. zanderi*. *Abelia serrata* appeared to function well as a paternal parent with seed set ranging between 21 and 60%, but only seeds generated from *A. engleriana* × *A. serrata* germinated. Due to the fragility of the flowers, *A. serrata* was not used as maternal parent. All crosses of *A. chinensis* × *A. schumannii* and *A.* ×*grandiflora* × *A. schumannii* yielded seed, but none of the seeds germinated.

The low number of seedlings obtained in these crosses can be attributed to post-zygotic barriers. Such barriers include: 1) disruption of cell division and differentiation due to undesirable genetic interactions between species; 2) unfavorable interactions between nuclear and cytoplasmic genes in zygotic cells; 3) failure of endosperm development; and 4) embryo-endosperm incompatibility (Emsweller & Uhring, 1962; Fehr, 1991; George, 1993). Reciprocal crosses, parental selection, and embryo/ovule culture are techniques to circumvent post-zygotic barriers. In Caprifoliaceae, embryo rescue has been used to a limited extent in the genera *Sambucus* and *Viburnum*. Koncalova et al. (1983) produced hybrid plants from crosses between *S. nigra* L. × *S. edulus* L. and *S. nigra* × *S. racemosa* A.Gray, but reciprocal crosses of *S. nigra* × *S. edulus* failed to produce plants. Embryos of *S. edulus* × *S. nigra* were cultured, but failed to germinate. Zilis and Meyer (1976) successfully cultured embryos of *V. lentago* L., *V. lantana* L., and *V.* × *burkwookii* Hort. Burkw. & Skipw. to overcome seed dormancy and reduced germination. Hybrid plants from *V. lantana* 'Mohican' × *V. carlesii* Hemsl. 'Aurora' and *V. lantana* 'Mohican' × *V.* × *juddii* Rehd. were obtained by culturing embryos. However, only 5% of the embryos had normal development (Hoch et al., 1995).

Experiment 5. Due to the low success rates, selected crosses were repeated, and embryos were rescued via ovule culture in an attempt to increase progeny numbers or

recover previously unattainable hybrids. The highest ovule recovery and plant survival percentages were obtained from the cross 'Francis Mason' × *A. schumannii* (Table 3.5). An ovule recovery percentage of 69% was recorded, and plant survival exceeded 59%. In Experiment 4, all crosses between 'Francis Mason' and *A. schumannii* set seed, but no seedlings germinated (Table 3.4). Crosses between *A. chinensis* and *A. serrata* also resulted in 69% ovule recovery. Results were similar to seed set percentages in the previous study. While only one plant was produced from *A. chinensis* × *A. serrata*, no plants were produced via conventional seed propagation. George (1993) states that low success rates and hybrid sterility are not uncommon in embryo rescue, but neither factor is relevant for commercial production if the new hybrid can be asexually propagated. *Abelia* taxa are reproduced commercially by vegetative propagation (Dirr & Heuser, 1987).

Among the remaining crossing combinations, high ovule recovery and plant survival percentages were recorded for *A. chinensis* × *A. spathulata* and *A. chinensis* × *A. engleriana*. Ovule recovery and seedling survival were 40% and 28%, respectively, for *A. chinensis* × *A. spathulata* (Table 3.5). Without ovule culture, no plants were produced from the cross of *A. chinensis* × *A. engleriana* (Table 3.4). Using ovule culture, ovule recovery exceeded 41% and plant survival ranged from 5-21%. The reciprocal cross produced no plants via ovule culture, but plants were produced from seed (Table 3.4). Success or failure of embryo or ovule culture is contingent upon several factors including development stage of the embryo, media composition, and genotype (George, 1993; Lu & Bridgen, 1996). Timing is particularly critical for the success of embryo and/or ovule culture (Collins & Grosser, 1984; Hu & Wang, 1986; Honda & Tsutsui, 1997). Embryo maturity may have been a factor in the failure of *A. engleriana* ovules.

Abelia floribunda had the lowest ovule recovery and plant survival rates when used as either the maternal or paternal species among all the taxa assessed. A total of 754

crosses involving *A. floribunda* were performed, but only 6 ovules were recovered, and no plants were produced.

Interspecific hybridization among genetically diverse species is often difficult or impossible, and may require specialized techniques (Fehr, 1991). Genetic diversity has not been defined in *Abelia* and systematic studies are limited. Species estimates range in number from fewer than 30 to greater than 60 (Rehder, 1937; Bean, 1970; Bailey, 1976; Krussmann, 1985; Griffiths, 1992; The International, 2001). Rehder subdivided the genus into two sections, Euabelia Rehd. and Zabelia Rehd., based on morphological characteristics (Krussmann, 1985a). Crossability within section Euabelia, between subsections Uniflorae Rehd. and Rupestrus Zab. has been previously reported in the genus [i.e. *A.* ×*grandiflora* (*A. chinensis* × *A. uniflora*) and *A.* 'Edward Goucher' (*A.* ×*grandiflora* × *A. schumannii*)] (Bean, 1970; Krussmann, 1985). *Abelia chinensis* belongs to section Rupestrus, and *A. uniflora* belongs to section Euabelia subsection Uniflorae Rehd., the subsection that also contains *A. engleriana* and *A. schumannii* (Krussmann, 1985).

Interspecific hybridization between species originating from the same geographic location was generally greater than compatibility between species that have been classified by morphological characteristics. All the species evaluated are native to Asia with the exception of *A. floribunda*, a native of Mexico. Morphologically, *A. floribunda* is categorized in the same section, Euabelia, as all the other species examined except *A. zanderi*, yet no interspecific crosses were produced. *Abelia zanderi*, a member of section Zabelia, was successfully crossed to *A. chinensis* and *A. engleriana*. Furthermore, 21% and 27%, respectively, of the crosses of *A. chinensis* × *A. engleriana* and the reciprocal resulted in seedlings. Geographically *A. chinensis, A. engleriana,* and *A. zanderi* originated in China (Bean, 1970; Krussman, 1985).

Intra-specific hybridization was limited by the apparent presence of a self-incompatibility system. Self-incompatibility is advantageous for breeding interspecific

hybrids by eliminating the need for emasculation. Success rates of interspecific crosses varied dependent on the parentage. However, previously unrecorded interspecific hybrids were obtained between *A. chinensis* × *A. engleriana, A. chinensis* × *A. spathulata, A. chinensis* × *A. serrata,* and *A. chinensis* × *A. zanderi*. These hybrids may have potential in the development of new cultivars.

Table 3.1. Effect of days from anthesis on the stigma receptivity of *Abelia* ×*grandiflora* 'Francis Mason' determined by pollen tube growth in the style and seed set.

Days From Anthesis	Mean Pollen Tube Growth[z]	Mean Seed Set[y]
3-days preanthesis	66.67c[x]	30.56abc
2-day preanthesis	69.17c	43.89abc
1-day preanthesis	96.67ab	61.11a
Anthesis	97.62a	60.42a
1-day postanthesis	91.11abc	48.89ab
2-days postanthesis	95.83ab	19.44bc
3-days postanthesis	88.89abc	24.44bc
4-days postanthesis	73.33abc	31.67abc
5-days postanthesis	71.67bc	15.56c

[z]Calculated as number of styles with pollen tubes/number of styles examined by fluorescence microscopy.
[y]Calculated as the number of seed collected/number of crosses performed.
[x]Mean separation within columns by Fisher's LSD test, $P = 0.05$.

Table 3.2. Effect of days from anthesis on the stigma receptivity of *Abelia chinensis* determined by pollen tube growth in the style and seed set.

Days From Anthesis	Mean Pollen Tube Growth[z]	Mean Seed Set[y]
3-days preanthesis	76.67abc[x]	16.67b
2-day preanthesis	80.00abc	36.11ab
1-day preanthesis	96.67ab	52.78ab
Anthesis	100.00a	58.28a
1-day postanthesis	100.00a	61.67a
2-days postanthesis	87.14ab	62.45a
3-days postanthesis	84.17ab	70.00a
4-days postanthesis	68.33bc	57.22a
5-days postanthesis	51.67c	47.22ab

[z]Calculated as number of styles with pollen tubes/number of styles examined by fluorescence microscopy.
[y]Calculated as the number of seed collected/number of crosses performed.
[x]Mean separation within columns by Fisher's LSD test, $P = 0.05$.

Table 3.3. Experiment 3: Crossing combinations, number of crosses per combination, number of seeds set per crossing combination, and number of seedlings obtained per crossing combination from intra- and interspecific crosses among *A.* ×*grandiflora* cultivars, *A.* 'Edward Goucher' and *A. chinensis*.

♀ \ ♂	*chinensis*			'Edward Goucher'			'Francis Mason'			'Golden Glow'			'John Creech'		
	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings
chinensis	X	X	X	135	44	13	177	127	68	71	42	32	81	64	50
'Edward Goucher'	117	37	30	X	X	X	78	5	5	56	9	7	119	28	20
'Francis Mason'	231	139	83	65	13	7	X	X	X	53	0	0	91	3	3
'Golden Glow'	161	20	14	56	2	0	74	0	0	X	X	X	87	1	0
'John Creech'	118	28	15	56	8	2	95	15	9	52	2	2	X	X	X

[z]X denotes that the cross was not performed.

Table 3.4. Experiment 4: Crossing combinations, number of crosses per combination, number of seeds set per crossing combination, and number of seedlings obtained per crossing combination from interspecific crosses among 7 *Abelia* species.

♀ \ ♂	*chinensis*			*engleriana*			*floribunda*			*grandiflora* 'Francis Mason'			*schumannii*			*serrata*			*zanderi*		
	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings	Crosses	Seeds	Seedlings
chinensis	X[z]	X	X	64	17	0	266	6	0	177	127	68	11	11	0	148	40	0	104	58	50
engleriana	22	12	6	X	X	X	X	X	X	X	X	X	X	X	X	35	21	3	31	12	3
floribunda	86	6	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
grandiflora 'Francis Mason'	231	139	83	X	X	X	X	X	X	X	X	X	7	7	0	X	X	X	X	X	X
serrata	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	52	1	0
zanderi	4	0	0	48	5	0	X	X	X	X	X	X	X	X	X	42	9	0	X	X	X

[z]X denotes that the cross was not performed.

Table 3.5. Experiment 5: Crossing combinations, number of crosses per combination, number of ovules recovered per crossing combination, and number of seedlings obtained per crossing combination from interspecific crosses among 9 *Abelia* species and 'Edward Goucher'.

♂ / ♀	*chinensis*[z]			*chinensis* - H[y]			*chinensis* - K[x]			*engleriana*			*floribunda*			*grandiflora* 'Francis Mason'			*mosanensis*			*schumannii*			*serrata*			*spathulata*			*zanderi*			'Edward Goucher'		
	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings	Crosses	Ovules	Seedlings
chinensis	X[w]	X	X	X	X	X	X	X	X	29	12	6	188	3	0	X	X	X	92	0	0	X	X	X	26	18	0	25	10	7	X	X	X	X	X	X
chinensis-H	X	X	X	X	X	X	X	X	X	66	30	3	X	X	X	X	X	X	X	X	X	X	X	X	74	31	1	X	X	X	X	X	X	X	X	X
chinensis-K	X	X	X	X	X	X	X	X	X	66	20	6	X	X	X	X	X	X	X	X	X	X	X	X	74	28	0	X	X	X	X	X	X	X	X	X
engleriana	29	0	0	32	0	0	25	0	0	X	X	X	81	1	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
floribunda	73	0	0	X	X	X	X	X	X	30	0	0	X	X	X	21	0	0	18	0	0	X	X	X	39	0	0	12	0	0	44	0	0	24	0	0
grandiflora 'Francis Mason'	X	X	X	X	X	X	X	X	X	X	X	X	60	0	0	X	X	X	X	X	X	29	20	17	X	X	X	X	X	X	X	X	X	X	X	X
zanderi	X	X	X	X	X	X	X	X	X	X	X	X	84	2	0	X	X	X	19	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
'Edward Goucher'	X	X	X	X	X	X	X	X	X	X	X	X	78	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[z]*A. chinensis* obtained from Nurseries Caroliniana.
[y]*A. chinensis* - H obtained from Sir Harold Hillier Gardens and Arboretum.
[x]*A. chinensis* - K obtained from The Royal Botanical Garden - Kew.
[w]X denotes that the cross was not performed.

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CHAPTER 4

THE EFFECT OF PERICARP REMOVAL, GIBBERELLIC ACID, AND STRATIFICATION ON SEED GERMINATION OF *ABELIA ×GRANDIFLORA* (ANDRE) REHD.[3]

[3]Scheiber, S.M. and Carol D. Robacker. Submitted to HortScience, 11/13/01.

THE EFFECT OF PERICARP REMOVAL, GIBBERELLIC ACID, AND STRATIFICATION ON SEED GERMINATION OF *ABELIA ×GRANDIFLORA* (ANDRE) REHD.

Keywords: achene, GA_3, *Abelia*

Seed germination within *Abelia* has been described as slow and inconsistent. Experiments were conducted to test procedures to increase germination percentages and rates. The effect of pericarp removal was examined in combination with stratification for 60 days at 4C, immersion in 100 mg/l gibberellic acid for 24h, and no treatment. Treatments were replicated five times with 15 seeds per replication. Seeds were sown on sphagnum peat, and grown under mist in the greenhouse. Weekly germination counts were recorded for eight weeks. Seeds with attached pericarps germinated at a significantly higher percentage than those without pericarps. Stratified seeds with attached pericarps had the highest germination percentage, 63%; though not significantly different than the other treatments. Germination was significantly more uniform for stratified seeds with pericarps and GA_3 immersed seeds without pericarps relative to their controls. Despite more uniform germination, pericarp removal, GA_3 immersion, and stratification are of no practical benefit due to either reduced germination percentages or the time necessary for stratification.

Introduction

Abelia ×*grandiflora* (André) Rehd. is an important shrub in the nursery and landscape industries. It flowers from May to frost, has an abundance of pinkish-white flowers, and glossy semi-evergreen foliage (evergreen in mild climates) (Bean, 1970; Krussmann, 1985; Dirr, 1998a). However, the flowers are small and only mildly fragrant, and the plants are not hardy below -20°C or zone 6 (Bean, 1970; Krussmann, 1985; Dirr, 1998a). Nursery owners and gardeners are interested in new *Abelia* cultivars with increased cold hardiness, richer pink-rose flower colors, unique foliage colors, and compact habits (Dirr, 1998b).

Abelia taxa are reproduced commercially by vegetative propagation of semi-hardwood and hardwood stem cuttings. Little information is available regarding seed germination requirements, and seed production is considered undesirable because *Abelia* fruits mature over a long time period, and the seedlings would be highly variable (Dirr and Heuser, 1987). Everett (1980) noted that non-hybrids germinate within a few weeks when sown on a sand-peat medium in the spring. Observations of untreated *A. chinensis* open-pollinated seeds indicated that germination was slow and non-uniform. Seeds sown in December 1997 did not begin to germinate until February 1998, with seedlings sporadically emerging over several weeks. Similar germination patterns were observed of seed derived from interspecific crosses and germination percentages were approximately 50%. Although sexual reproduction is undesirable from a commercial standpoint, seed germination is essential for breeding and selection programs.

Inhibition of seed germination can result from hardened seedcoats, embryo dormancy, or a combination of both factors termed double dormancy (Dirr & Heuser, 1987; Boesewinkel & Bouman, 1995). *Abelia* fruit is a one-seeded, leathery achene. The dry, indehiscent pericarp of the achene can mimic the function of a seedcoat and delay, reduce, or suppress germination. The pericarp can supply the embryo with inhibitory compounds, prevent inhibitory compounds from leaving the embryo, mechanically

interfere with radicle protrusion, or inhibit water imbibition or gas exchange (Boesewinkel & Bouman, 1995). Removal or weakening of the pericarp has been shown to increase germination percentages and rates in *Rosa multiflora* Thunb. and *Anthemis cotula* L. (Yambe & Takeno, 1992; Gealy et al., 1985). In seeds with embryo dormancy, internal signals prevent germination even when environmental conditions are optimum for germination. The embryo must undergo an after-ripening period to induce the biochemical processes necessary for germination (Dirr & Heuser, 1987; Salisbury & Ross, 1992; Boesewinkel & Bouman, 1995). Stratification and/or gibberellic acid treatments have proven useful for overcoming dormancy requirements. Incorporating 1 mM GA_3 into the germination medium of *Helianthus annuus* L. and *H. petiolaris* Nutt. overcame achene dormancy (Seiler, 1998). Upfold and Van Staden (1990) report that achenes of *Tithonia rotundifolia* Mill. must undergo a 12-week after-ripening treatment for maximum germination to occur and that treatment with GA_3 stimulates germination. Stratification promoted germination of dormant achenes of *Polygonom convolvulus*, *Solidago* species, and *Bidens laevis* (Metzger, 1992; Leck et al., 1994; Walck et al., 1997). In the present study, pericarp removal, gibberellic acid treatments, and stratification were tested as a means of increasing germination percentages and rates of seedling germination of *A.* ×*grandiflora*.

Materials and Methods

Seeds of self-pollinated *A.* ×*grandiflora* were collected from a stock plant maintained under greenhouse conditions at the Georgia Station, Griffin, GA in December 1998. Two treatments, pericarp removal and seed preconditioning, were examined to determine their effects on germination percentages and rates of seedling germination. Prior to treatment, seeds were stored in paper envelopes at room temperature. To test the effect of pericarp removal, one half of the seeds were dissected from their pericarps with the aid of a stereomicroscope and are referred to as seeds without pericarps. Pericarps

were removed from seeds immediately prior to any subsequent preconditioning treatment. The remaining seeds with intact pericarps are referred to as seeds with pericarps. Both seeds with pericarps and seeds without pericarps received a cold, moist stratification pretreatment, gibberellic acid (GA_3) pretreatment, or no pretreatment. In the stratification pretreatment, the seeds were mixed with moist, milled sphagnum peat, sealed in polyethylene bags, and placed in the dark for 60 days at 4C. For GA_3 preconditioning, seeds with and without pericarps were immersed in 100 mg/l GA_3 at room temperature for 24h and were immediately sown following treatment. All seeds were sown in February 1999. The experiment was conducted as a 2 × 3 factorial of pericarp removal and seed preconditioning (total treatments = 6) with 5 replicates (15 seeds per rep) per treatment. Seeds were sown on milled sphagnum peat, and grown under mist (10 sec every 32 min) with bottom heat (22C) in the greenhouse. Weekly germination counts were recorded for eight weeks. Seeds were considered germinated when the hypocotyl had emerged.

Statistical data for pericarp removal and seed preconditioning were initially analyzed by ANOVA procedures. Based on ANOVA results, data were sorted by presence or absence of the pericarp and preconditioning treatment and were modeled and analyzed using the following logistic growth function (SAS Institute, 1990):

$$Y = \beta_o / \{1 + [(1-p)/p]\, e^{[-\beta_1 (day-\beta_2)]}\}$$

where β_o = asymptote of final germination percentage, β_1 = relative rate of germination, β_2 = time until asymptote of p is reached, and p = point where a predetermined value of maximal germination is obtained (90%). Significant differences between parameters were determined using 95% confidence intervals.

Results and Discussion

Analysis of final germination percentages (8 weeks) by ANOVA indicated no significant interaction between pericarp removal and seed preconditioning (data not

shown), though significant effects due to pericarp removal were found. Seeds with pericarps had a significantly higher germination percentage than seeds without pericarps when treated with GA_3 or stratification. Similar results were observed in *Anemone coronaria* L. Partial removal of the pericarp promoted germination, but complete removal retarded germination.

Analysis of seeds with pericarps by non-linear regression revealed that stratification resulted in the highest germination percentage, 62.5%; though not significantly different than the other treatments (Table 4.1). Relative rate of germination was similar for all treatments. However, significant differences were found among stratification, GA_3, and no pretreatment for time until 90% of the maximum germination (Table 4.1, Fig. 4.1). Stratification significantly decreased the time until 90% maximum germination was reached. Between days 7 and 14, germination increased by 47% (75% of the final germination percentage, 62.5%). By day 14, stratified seeds reached 90% of maximal germination or 51% germination compared to 1% and 0% germination for seeds receiving GA_3 or no pretreatment, respectively. For seeds receiving GA_3 or no pretreatment, 90% of maximal germination was not achieved until after day 35. Walck et al. (1997) reported a similar decrease in time until the onset of germination following stratification of *S. altissima* L., *S. nemoralis* Ait., and *S. shortii* Torr. & Gray achenes (seeds with pericarps) for 12 weeks. Despite the significant effect of stratification, the 3 week reduction in time until 90% maximal germination does not compensate for the 60 days required for the stratification process.

Final germination percentages were significantly higher for seeds without pericarps receiving no pretreatment than for stratified or GA_3-treated seeds (Table 4.1, Fig. 4.2). Relative rate of germination and time until 90% of maximum germination were not significantly different between the control and stratified seeds. No differences were observed between GA_3 treatment and stratification for any parameters. Germination was significantly more rapid and more uniform for GA_3-treated seeds than untreated

seeds. Maximum germination of GA_3-treated seeds occurred between day 7 and day 14. Seeds receiving no pretreatment did not achieve 90% of maximum germination until day 28. Similar results were reported for seeds of *Citrumelo* 'Swingle' (*Citrus paradisi* Macfad. × *Poncirus trifoliata* Rafin.) with time until germination reduced following immersion in 50 mg/l or 250 mg/l GA_3. While uniform germination is desirable for maximizing efficiency and minimizing resources in transplanting and crop management, the low germination percentages in comparison to no pretreatment negate its benefits.

Control seeds with pericarps germinated at a higher percent (56%), than those without pericarps (49%), though the difference was not significant (Table 4.1, statistics not shown). Comparisons between seeds receiving GA_3-pretreatment revealed that final germination percentages of seeds with pericarps were significantly higher than seeds without pericarps, 56% and 38%, respectively. It is possible that removal of the pericarp made the unprotected embryo vulnerable to damage from the pretreatments. Pericarps can imitate the function of a seedcoat by providing structural protection from interactions with the environment, pathogens, insects, and chemicals (Boesewinkel & Bouman, 1995). Direct penetration of GA_3 to the unprotected embryo may have reduced the germination percentage due to a concentration effect. Shahi et al. (1991) report that *Cymbopogon martini* Stapf. and *Cenchrus ciliaris* L. treated with 5 mg/l GA germinated at 68% and 98%, respectively, but treatment with 20 mg/l GA reduced germination relative to the control. Citrumelo 'Swingle' seeds immersed in 50 mg/l GA_3 increased germination percentages by 10% relative to the control, but immersion in 250 mg/l GA_3 reduced germination. Similar results were found for stratified seeds. A final germination percentages of 63% was recorded for seeds with pericarps, significantly higher than the final germination percentage recorded for seeds without pericarps, 34%. Following stratification, it was observed that a number of seeds had fungal growth. On stratified seeds with pericarps, this was not observed. Apparently, removal of the pericarp allowed

entry of microorganisms which had ample time to damage the embryo during the stratification process.

Pericarp removal, immersion in gibberellic acid (GA_3) and stratification had no practical benefit on the germination of *Abelia* seed. Since the fruit of *Abelia* is a one-seeded achene, numerous pollinations must be performed to obtain the large quantities of seeds needed to conduct a breeding program. Results indicate that untreated achenes offer optimal efficiency and maximum returns on pollinations.

Table 4.1. Final germination percentages and non-linear regression parameter estimates for emergence of *Abelia ×grandiflora* separated by pericarp treatment following immersion in gibberellic acid, stratification, and no pretreatment.

	β_o[z]	β_1[y]	β_2[x]	r^2
	Seeds with pericarps - all treatments			
Gibberellic Acid (GA_3)	56.24a[w]	0.20a	37.33b	0.82
Stratification	62.53a	0.57a	15.34c	0.78
No Pretreatment	56.09a	0.25a	37.75a	0.82
	Seeds without pericarps- all treatments			
Gibberellic Acid (GA_3)	38.00b	1.83a	15.14b	0.86
Stratification	34.10b	1.61ab	8.25ab	0.31
No Pretreatment	48.94a	0.34b	21.43a	0.81

[z] Final germination percentage.
[y] Relative rate of emergence.
[x] Time (days) until 90% of final emergence is reached.
[w] Parameter separation among preconditioning treatments within pericarp treatments. Parameter separation determined by 95% asymptotic confidence intervals.

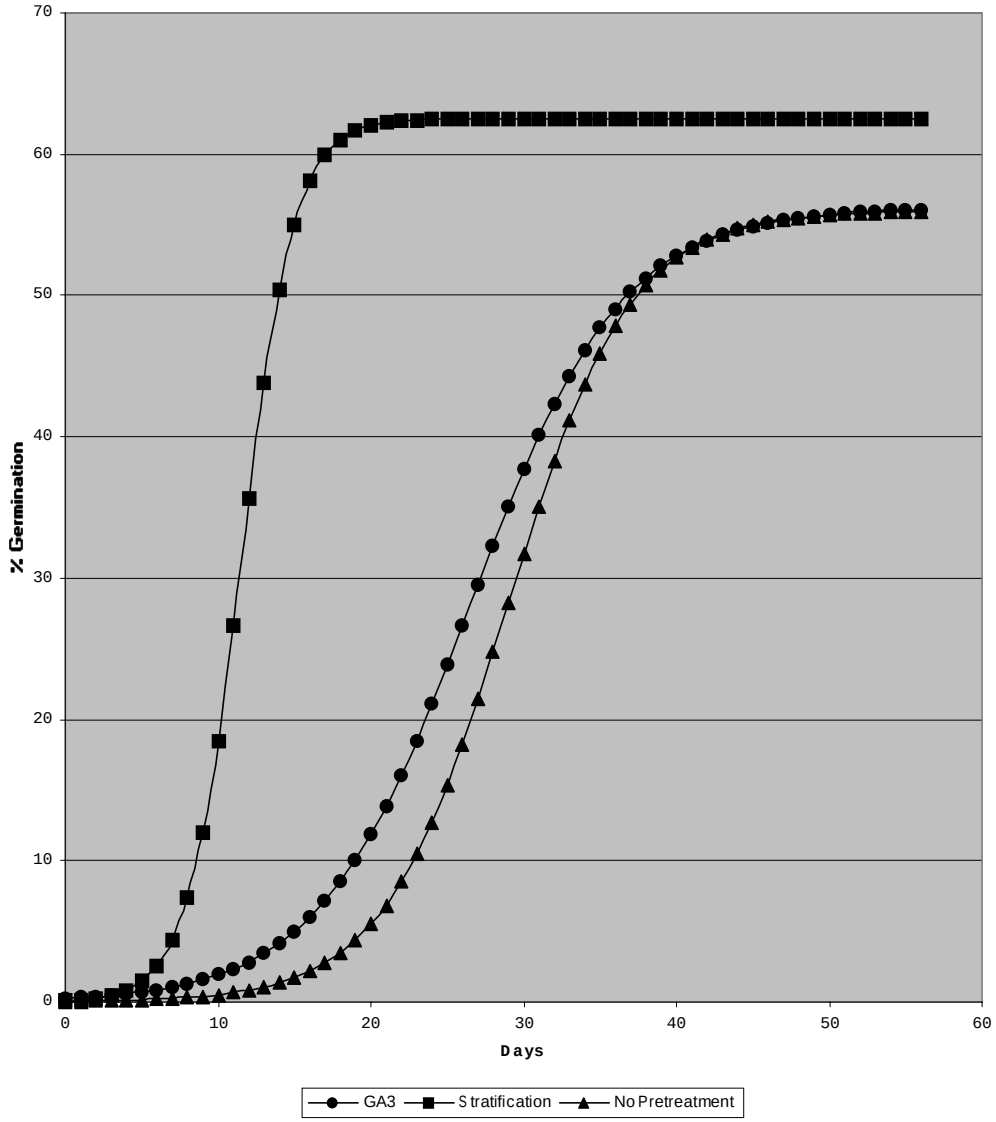

Fig. 4.1. Emergence of *Abelia* ×*grandiflora* seeds with pericarps receiving stratification for 60 days at 4C, immersion in 100 mg/l gibberellic acid (GA_3) for 24h, or no pretreatment. Model parameter estimates are presented in Table 1.

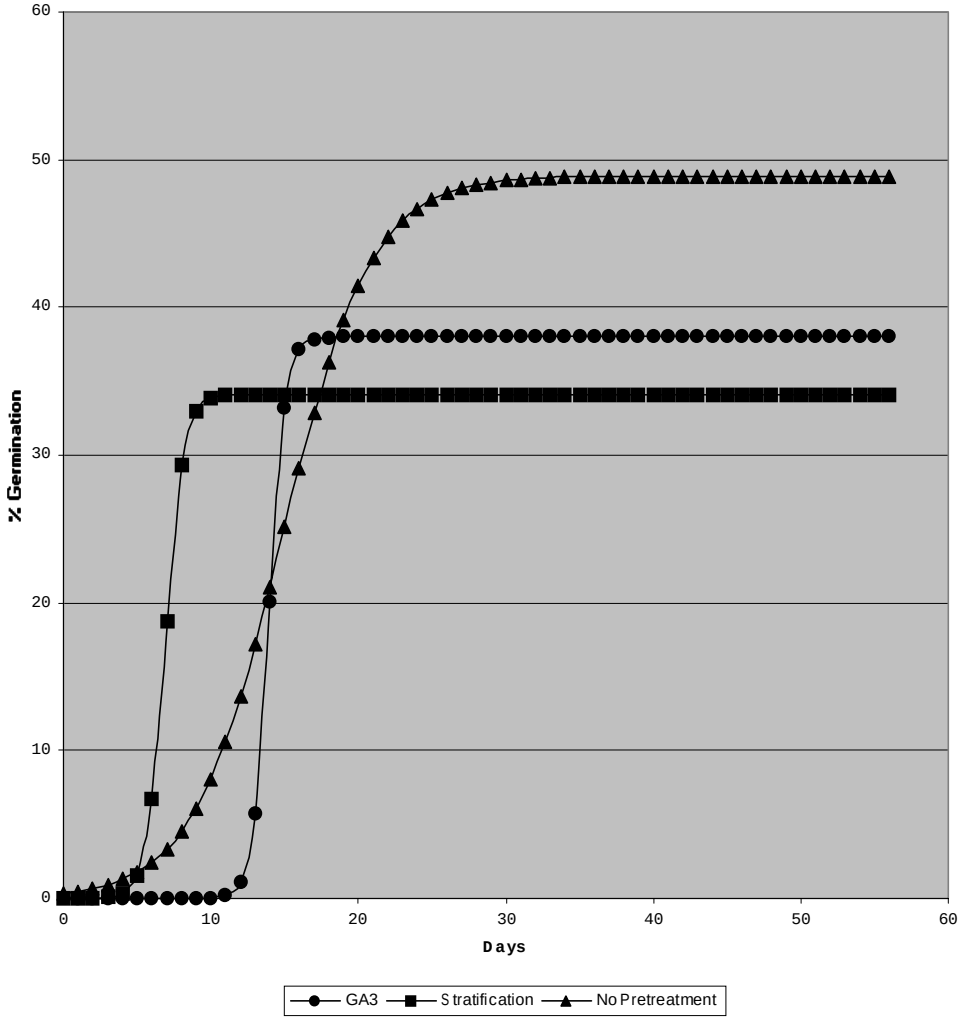

Fig. 4.2. Emergence of *Abelia* ×*grandiflora* seeds without pericarps receiving stratification for 60 days at 4C, immersion in 100 mg/l gibberellic acid (GA_3) for 24h, or no pretreatment. Model parameter estimates are presented in Table 1.

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CHAPTER 5

INTERSPECIFIC HYBRIDIZATION BETWEEN *ABELIA* ×*GRANDIFLORA* 'FRANCIS MASON' AND *A. SCHUMANNII* VIA OVULE CULTURE[4]

[4]Scheiber, S.M. and Carol D. Robacker. To be submitted to Euphytica.

INTERSPECIFIC HYBRIDIZATION BETWEEN *ABELIA ×GRANDIFLORA* ‘FRANCIS MASON’ AND *A. SCHUMANNII* VIA OVULE CULTURE

Keywords: *Abelia ×grandiflora* ‘Francis Mason’, *Abelia schumannii*, embryo rescue

Interspecific hybridization among species of *Abelia* offers the potential for new cultivars. Seeds from interspecific hybridization between *A. ×grandiflora* ‘Francis Mason’ × *A. schumannii* failed to germinate, and ovule culture was employed. Ovules were dissected from the achenes 4, 5, and 6 weeks after pollination and cultured on either Woody Plant Medium (WPM) or Linsmaier and Skoog (LS) Medium containing 0, 9.8, or 39.2 μM 2iP. All media contained coconut water (5% v/v), sucrose (30 g/l), agar (8 g/l), and MS vitamins (1 mg/l). Embryos recovered, spontaneous embryo germination, root formation, and plant survival were recorded. Number of weeks following pollination significantly affected all parameters examined, with ovules harvested 5 weeks after pollination producing the best results. Plant survival was greatest for embryos cultured on Woody Plant Medium with 0 μM 2iP. Eight-five percent of the cultured ovules produced embryos that rooted and 65% survived the hardening off process when ovules were cultured 5 weeks after pollination on WPM containing no growth regulator.

Introduction

Lustrous dark semi-evergreen to evergreen foliage, profuse pinkish-white flowers that open from May to frost, heat and drought tolerance, and pest resistance have made *Abelia* ×*grandiflora* (André) Rehd. a staple in landscapes of the southeastern United States for nearly a century (Bean, 1970; Krussmann, 1985; Dirr, 1998a). A limited number of cultivars of *A.* ×*grandiflora* are commercially available. These vary little in form, foliage, or floral characteristics, with the exception of a few variegated selections. The first variegated cultivar, *A.* ×*grandiflora* 'Francis Mason', was introduced in the 1950s and is characterized by small white flowers, yellow to yellow-green evergreen foliage, and young copper-colored shoots (Dirr, 1994).

The most popular cultivar, *A.* 'Edward Goucher', is an interspecific hybrid derived from a cross between *A.* ×*grandiflora* and *A. schumannii* (Graebn.) Rehd. and released in 1911 (Bean, 1970). 'Edward Goucher' and *A. schumannii* produce large pinkish-purple flowers, graceful arching habit, and dark green foliage. However, 'Edward Goucher' has semi-evergreen foliage and *A. schumannii* is deciduous. Cold hardiness (zone 7 or -18°C) limits the northern distribution of both taxa, but *A.* ×*grandiflora* and its cultivars are hardy to -23°C or zone 6 (Bean, 1970; Krussmann, 1985; Dirr, 1998a; Scheiber, 2001).

'Edward Goucher' and *A.* ×*grandiflora* are important economically to the nursery and landscape industry, but nursery owners and gardeners are interested in new *Abelia* cultivars (Dirr, 1998b). Interspecific hybridization between *A.* ×*grandiflora* 'Francis Mason' and *A. schumannii* offers the potential for new cultivars with unique floral and foliage characteristics and improved cold hardiness. Therefore, we attempted to obtain hybrids of 'Francis Mason' × *A. schumannii*, but the seeds failed to germinate. Embryo abortion, failure of endosperm development, or failure of viable seeds to germinate make interspecific hybridization difficult or impossible (George, 1993; Sharma et al, 1996). Ovule and/or embryo culture have been used to overcome post-zygotic incompatibility in

a number of woody ornamental genera including *Ilex* L., *Hydrangea* L., and *Viburnum* L. (Hu, 1975; Zilis and Meyer, 1976; Hoch et al., 1995; Reed, 2000; Sanberro et al., 2001). In the present study, we conducted experiments to develop an ovule culture technique to obtain interspecific hybrids between *A.* ×*grandiflora* 'Francis Mason' × *A. schumannii*.

Materials and Methods

Plant material. *Abelia* ×*grandiflora* 'Francis Mason' was used as the maternal parent in the study. 'Francis Mason' is characterized by white, slightly fragrant, 2-2.5 cm long flowers subtended by 5 sepals. The evergreen to semi-evergreen leaves are 1-2 cm long, half as wide, lustrous yellow to yellow green with a richer yellow marginal border or mottled. The paternal parent, *Abelia schumannii,* is deciduous with 1-3 cm long leaves that are dark green above, paler beneath, and less lustrous than *A.* ×*grandiflora*. The sepal number is two, and the flowers are 2.5-3 cm in length and pinkish-purple.

Pollinations. Flowers of *A.* ×*grandiflora* 'Francis Mason' were emasculated one day prior to anthesis and fresh pollen was applied directly to the stigma. Pollinations were bagged with 7 × 10.8 cm or 9.2 × 12.7 cm glassine envelopes (BioQuip Products, Gardena, CA). Pollinations were performed in June-July, 2000 under greenhouse conditions.

Ovule culture. Achenes, the one-seeded fruit of *Abelia*, were collected 4, 5, and 6 weeks after pollination. Achenes were dipped for 1 min in 70% ethanol, soaked for 20 min in 20% sodium hypochlorite containing a trace amount of Tween 20, and rinsed three times with sterile distilled water. Following disinfection, ovules were dissected from the achenes with the aid of a stereoscope. Two ovules were placed in each petri plate and each plate was sealed with Tenderskin® Hypoallergenic Paper Tape (The Kendall Company, Mansfield, MA). The petri plates were arranged in a completely randomized design and maintained at 27 to 30C with 16 h of light provided by 110-W wide-spectrum fluorescent bulbs (70 μmol•m^{-2}•s^{-1}).

Culture media consisted of either Woody Plant Medium (WPM) (Lloyd & McCown, 1981) or Linsmaier and Skoog (LS) (Linsmaier & Skoog, 1965) salts containing MS vitamins, 3% sucrose, and solidified with 0.8% agar. Both media were supplemented with 0, 9.8, or 39.2 μM of the growth regulator, 6-(y,y-dimethylallylamino) purine (2iP). Filter-sterilized coconut water (50 ml l^{-1}) was added to the media after autoclaving. The pH was adjusted to 5.5-5.6 with 1 N NaOH. The media were autoclaved for 20 min at 121C prior to dispensing 8-ml aliquots in 60 × 15 mm petri plates under sterile conditions.

Embryos failing to germinate in less than 4 weeks were dissected from the ovule. Following either spontaneous germination or dissection, embryos were transferred to media containing no plant growth regulators and no coconut water. After roots reached a length of approximately 2 cm, embryos were transplanted from in vitro culture to a 1:1 sphagnum peat:perlite mixture. To harden off the embryos, the transplanted seedlings were placed under intermittent mist (4 sec. every 8 min) in a greenhouse. Seedlings were removed from the mist when new shoot growth was observed and transplanted into 8.75 × 8.75 × 10 cm cells containing Metro Mix 300 (The Scotts Company, Marysville, OH).

Data analysis. Data were collected on number of embryos that germinated spontaneously, total number of embryos recovered (germinated and dissected embryos), number of plantlets transplanted from in vitro culture to media, and plant survival following the hardening-off process, and recorded as a percentage of the ovules cultured. The experiment was conducted as a 2 × 3 × 3 factorial of media, plant growth regulator (PGR), and weeks after pollination (total treatments = 18) with 5 replicates (4 ovules per rep) per treatment. Data were analyzed by analysis of variance using a general linear model (SAS Institute, 1990). When treatment effects were significant, treatment means were separated by Fisher's LSD test.

Seed germination and cytological examination. Ripened seeds were collected to examine the status of the embryo in mature seeds. Prior to sowing or cytological examination,

seeds were stored for 3 months in paper envelopes at room temperature. Seeds were sown in milled sphagnum peat, and germinated under mist (10 sec every 32 min) with bottom heat (22C) in the greenhouse. Seeds were considered germinated when the hypocotyl had emerged. Seeds for cytological examination were soaked in water for 24h before embryos were dissected with the aid of a stereomicroscope.

Characterization of hybrids. Morphological examination was used to confirm hybrid identity of F_1 plants. Floral and foliage characteristics of mature greenhouse-grown hybrid and parental plants were evaluated. Floral characteristics included color, size, and sepal number. Foliage was evaluated for variegation and glossiness.

Results and Discussion

Embryo development and hybrid verification. Cultured ovules swelled (Fig. 5.1), and in many cases, embryos germinated (Fig. 5.2) within 4 weeks of culture. Following further development and root formation (Figures 5.3 and 5.4), plantlets were transferred to the greenhouse. A total of 115 hybrid plants were successfully hardened off.

Morphological examination of F_1 plants confirmed their hybrid identity. All plants examined had pinkish-purple flowers with two sepals (Figures 5.5, 5.6, and 5.7); both features are characteristic of the paternal plant, *A. schumannii.* Flower size was intermediate between the two parent species. Foliage variegation segregated in a 1:1 ratio of green to yellow, and all the F_1 plants lacked the glossy appearance of the maternal parent, *A.* ×*grandiflora* ‘Francis Mason’.

Media and growth regulator effects. No significant interactions were found for any parameter examined. The only parameter significantly affected by media and PGR was plant survival (Tables 5.1 and 5.2). Ovules cultured on WPM had a survival rate of 38.9%, significantly greater than the 26.9% when cultured on LS medium (Table 5.1). Survival rates were significantly higher, 44.0%, for ovules initially cultured on media containing 0 μM 2iP than the final survival rates of 28.1% and 25.5% for ovules initially

cultured on media containing 8.9 and 39.2 μM 2iP, respectively (Table 5.2). In addition, embryos on 2iP-containing media exhibited hyperhydricity, unorganized callus growth, and growth inhibition of ovules after 4 weeks in culture. Hyperhydricity and unorganized callus growth were particularly apparent among germinated and ungerminated embryos, respectively, grown on media containing 39.2 μM 2iP. The data suggest that residual 2iP effects may account for differences in plant survival rates between media with and without 2iP.

Abelia is a member of the family Caprifoliaceae Juss., and embryo rescue has been used within the family to a limited extent in the genera *Sambucus* L. and *Viburnum* (Zilis & Meyer, 1976; Koncalova, 1983; Hoch et al, 1995). Zilis and Meyer (1976) successfully cultured embryos of *V. lentago* L., *V. lantana* L., and *V.* × *burkwoodii* Hort. Burkw. & Skipw. on a modified LS medium without plant growth regulators to overcome seed dormancy. Germination time was reduced to 45 days compared to 1 to 2 years under natural conditions. Hybrid plants from *V. lantana* 'Mohican' × *V. carlesii* Hemsl. 'Aurora' and *V. lantana* 'Mohican' × *V.* × *juddii* Rehd. were obtained by culturing embryos on WPM containing 2iP concentrations of 0, 1, 10, or 40 μM. However, after 4 weeks in culture large amounts of callus, senescence, and death were observed, and only 5% of the embryos developed normally (Hoch et al., 1995).

Weeks after pollination effect. Number of weeks after pollination significantly affected all parameters (Table 5.3). No significant interactions were found. Ovules cultured 5 weeks after pollination had the highest percentage of spontaneous embryo germination, embryos recovered, embryos transplanted from in vitro culture to media, and plant survival. Culturing ovules 4 weeks after pollination significantly reduced response percentages for all parameters. Germination was 21% higher for ovules cultured 5 weeks after pollination than for 4-week-old ovules. No significant differences in germination were found between ovules cultured 5 or 6 weeks after pollination. Reed (2000) reported similar germination responses for intraspecific ovules of *Hydrangea macrophylla*

(Thunb.) Ser. Ovules cultured 5 to 6 weeks after pollination germinated at a rate 22% higher than ovules cultured 3 to 4 weeks after pollination.

Significant differences in percent embryo recovery and percent transplanted from in vitro culture were observed among ovules cultured 4, 5, and 6 weeks after pollination. Embryos were recovered from 86% of the ovules cultured 5 weeks after pollination, and 91% of the recovered embryos were transplanted compared to 66% and 76% for 4- and 6-week-old ovules, respectively. Embryo recovery and transplants from in vitro culture were significantly higher for 6-week-old ovules compared to 4-week-old ovules. Plant survival following the hardening-off process was highest for ovules cultured 5 weeks after pollination. Culturing ovules 4 and 6 weeks after pollination resulted in a significant decrease in survival rates. No significant differences were found between ovules cultured 4 and 6 weeks after pollination.

Timing is critical for the success of ovule culture (Collins & Grosser, 1984; Hu & Wang, 1986; Honda & Tsutsui, 1997). Complex nutrient requirements increase the difficulty of culturing immature embryos, but embryo viability decreases as age increases due to induction of incompatibility mechanisms. Therefore, a compromise must be reached between embryo development and viability (Collins & Grosser, 1984; Hu & Wang, 1986; George, 1993).

Examination of 4-week-old ovules with a stereoscope revealed that the ovules were approximately one-half the size of 5- and 6-week-old ovules suggesting that 4-week-old ovules were not sufficiently developed for removal from maternal tissues. Reed (2000) noted the small size and difficulty of removing 3- to 4-week-old ovules from maternal tissues of *Hydrangea* compared to 5- to 6-week-old ovules. No size differences were noted between 5- and 6-week-old ovules of *Abelia*. Similar results were observed with cultured ovules of *Delphinium grandiflorum* L. × *D. nudicaule* Torr & A. Gray. Seedling survival rates of 7.5% were reported for ovules collected 20 to 25 days after pollination, but no seedlings were obtained when ovules were cultured 30 days after

pollination (Honda & Tsutsui, 1997). Ishikawa et al. (2001) cultured ovules from crosses between *Alstroemeria pelegrina* L. var. *rosea* × *A. magenta* Bayer and reported that 19.8% of 7- and 14-day-old ovules produced plantlets compared to 4.9%, 1.6%, and 0.4% for ovules cultured 21, 28, and 35 days after pollination, respectively. The reduction in seedling formation was attributed to failure of endosperm development. Endosperm failure is often reported as a post-zygotic barrier to interspecific hybridization (Collins & Grosser, 1984; Hu & Wang, 1986).

However, in the present study, the endosperm was well developed in both 5-and 6-week-old ovules. During the dissection of ungerminated ovules, several of the 6-week-old ovules had swollen to nearly twice their original size, but when dissected, no embryo was present. Dissection of fully-ripened seeds revealed that approximately one-half of the dissected seeds contained brown, slightly shriveled ovules with intact endosperm but no embryo, and no seedlings were obtained from sown seeds. The reduction in embryo recovery, rooting, and survival between ovules cultured 5 and 6 weeks after pollination suggests that embryos were weakened or aborted due to embryo-endosperm incompatibility. Emsweller and Uhring (1962) reported similar results for ovules derived from crosses between *Lilium speciosum* Thunb. 'Album' and *L. auratum* Lindl. Cultured ovules from the cross contained fully developed endosperms and embryos, but no seedlings were obtained from mature seeds. Dissection of stored seed revealed intact endosperm, but most of the embryos were at various stages of decomposition with the exception of a few normal embryos. Embryo-endosperm incompatibility was determined as the cause of embryo death.

The ovule-culture protocol developed for the cross between *A.* ×*grandiflora* 'Francis Mason' and *A. schumannii* was successful in generating a large number of hybrids. Whether this protocol will be successful in generating interspecific hybrids among *Abelia* species such as *A. chinensis* R.Br., *A. engleriana* (Graebn.) Rehd., *A. floribunda* Decne., *A.* ×*grandiflora, A. serrata* Sieb. & Zucc., and *A. spathulata* Sieb. &

Zucc. needs to be determined. Greenhouse and field observations have shown that the fruits of *Abelia* species develop and ripen at different rates under natural conditions. The optimum number of weeks after pollination for ovule culture may vary depending on the maternal parent, and additional research is needed to determine optimum harvest dates for the various species of Abelia.

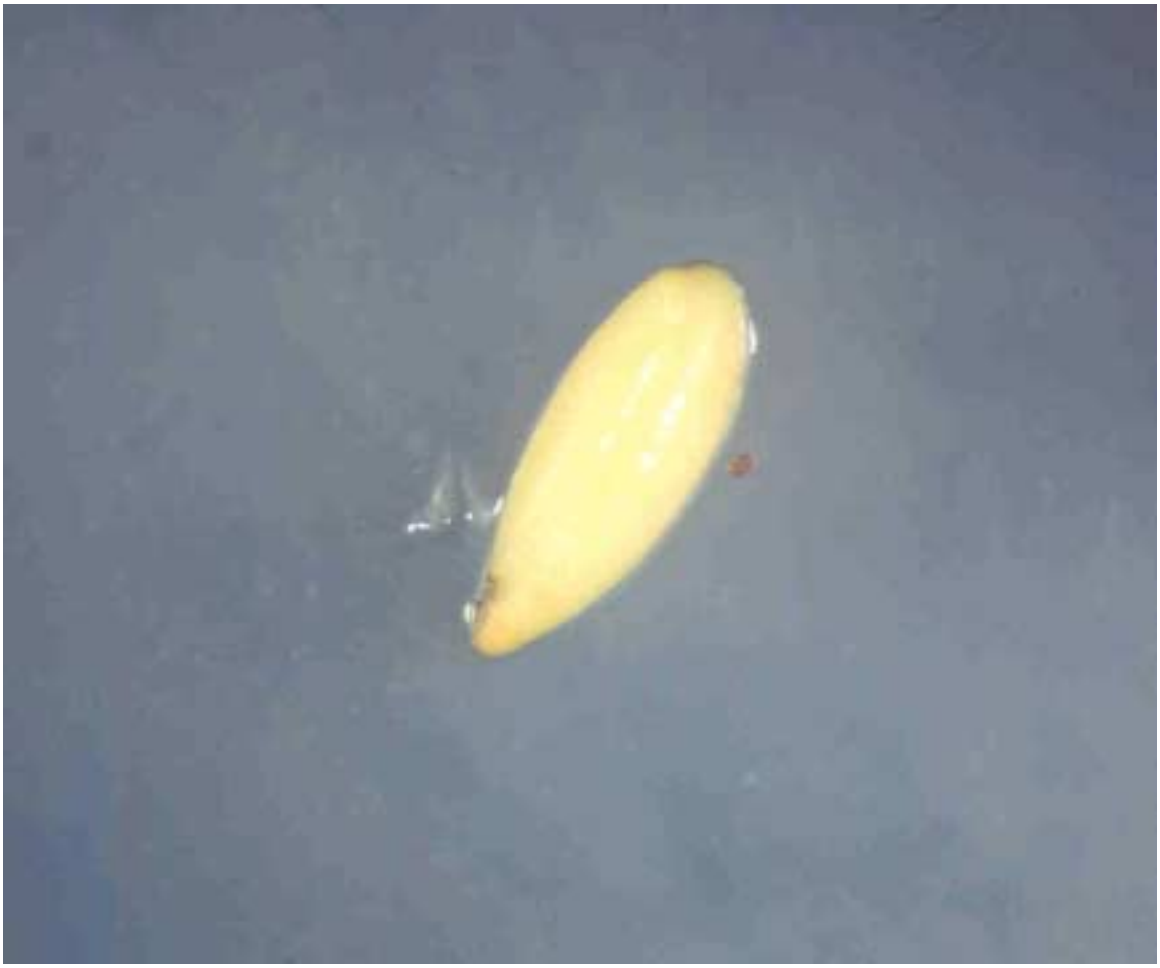

Fig. 5.1. An ungerminated ovule 6 weeks after pollination on Woody Plant Medium containing 8.9 μM 2iP.

Fig. 5.2. Germination of an ovule 6 weeks after pollination on Woody Plant Medium containing 8.9 μM 2iP.

Fig. 5.3. Rooting development of an embryo obtained from an ovule 6 weeks after pollination on Woody Plant Medium containing no growth regulator.

Fig. 5.4. Seedlings just prior to transplanting from in vitro culture to media that were derived from ovules 5 weeks after pollination on Linsmaier and Skoog Medium containing no growth regulator.

Fig. 5.5. *Abelia* ×*grandiflora* ‘Francis Mason’ × *A. schumannii* seedling.

Fig. 5.6. Flower colors of *A.* ×*grandiflora* 'Francis Mason' - maternal parent (A), *A. schumannii* - paternal parent (B), and *A.* ×*grandiflora* 'Francis Mason' × *A. schumannii* - F_1 hybrid (C).

Fig. 5.7. Flower colors and sepal numbers of *A.* ×*grandiflora* 'Francis Mason' - maternal parent (A), *A. schumannii* - paternal parent (B), and *A.* ×*grandiflora* 'Francis Mason' × *A. schumannii* - F_1 hybrid (C).

Table 5.1. Effect of basal salt media on percentage of spontaneously germinating embryos, percentage of total number of embryos recovered, percentage of seedlings transplanted from in vitro culture to soil, and plant survival percentage following the hardening-off process.

	Mean % of ovules cultured[z]			
Media	**Germination**	**Embryos[y]**	**Transplants**	**Plants**
LS	35.52a[x]	59.52a	46.33a	26.9b
WPM	34.82a	65.41a	54.30a	38.9a

[z]Calculated as number of embryos spontaneously germinating, embryos recovered, seedlings transplanted from in vitro culture to soil, and plants surviving the hardening-off process/number of ovules cultured, respectively.
[y]The percentage of embryos recovered including both spontaneously germinated and dissected embryos.
[x]Mean separation within columns by Fisher's LSD test, $P = 0.05$.

Table 5.2. Effect of plant growth regulator, 2iP, on percentage of spontaneously germinating embryos, percentage of total number of embryos recovered, percentage of seedlings transplanted from in vitro culture to soil, and plant survival percentage following the hardening-off process.

	Mean % of ovules cultured[z]			
2iP (μM)	**Germination**	**Embryos[y]**	**Transplants**	**Plants**
0	32.50a[x]	66.33a	58.00a	44.00a
8.9	41.17a	61.22a	49.33a	28.11b
39.2	31.83a	59.83a	43.61a	25.50b

[z]Calculated as number of embryos spontaneously germinating, embryos recovered, seedlings transplanted from in vitro culture to soil, and plants surviving the hardening-off process/number of ovules cultured, respectively.
[y]The percentage of embryos recovered including both spontaneously germinated and dissected embryos.
[x]Mean separation within columns by Fisher's LSD test, $P = 0.05$.

Table 5.3. Effect of number of weeks after pollination on percentage of spontaneously germinating embryos, percentage of total number of embryos recovered, percentage of seedlings transplanted from in vitro culture to media, and plant survival percentage following the hardening-off process.

	Mean % of ovules cultured[z]			
Weeks after pollination	**Germination**	**Embryos[y]**	**Transplants**	**Plants**
4	23.6b[x]	41.4c	27.5c	21.7b
5	44.1a	86.2a	78.1a	53.8a
6	37.8ab	59.8b	45.3b	22.1b

[z]Calculated as number of embryos spontaneously germinating, embryos recovered, seedlings transplanted from in vitro culture to soil, and plants surviving the hardening-off process/number of ovules cultured, respectively.
[y]The percentage of embryos recovered including both spontaneously germinated and dissected embryos.
[x]Mean separation within columns by Fisher's LSD test, $P = 0.05$.

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CHAPTER 6

INHERITANCE OF YELLOW FOLIAGE VARIEGATION IN *ABELIA* ×*GRANDIFLORA* 'FRANCIS MASON'[5]

[5]Scheiber, S.M. and Carol D. Robacker. To be submitted to Euphytica.

INHERITANCE OF YELLOW FOLIAGE VARIEGATION IN *ABELIA* ×*GRANDIFLORA* ‘FRANCIS MASON’

Keywords: *Abelia chinensis, A.* ×*grandiflora* ‘Francis Mason’, duplicate recessive epistasis, carotenoid biosynthesis

Inheritance of yellow variegated foliage was studied in crosses between *A. chinensis*, a green leafed species, and ‘Francis Mason’, a yellow variegated cultivar of *A.* ×*grandiflora*. Segregation ratios from reciprocal crosses between *A. chinensis* and ‘Francis Mason’ and backcross progeny could not be conclusively fit to a model. The best fit was obtained using a 2-gene model of duplicate recessive epistasis with yellow foliage dominant to green foliage. Evidence is presented that a homozygous dominant at the Y_1 loci is lethal. Observational data indicate that penetrance of foliage color is photoregulated. This study has demonstrated that yellow foliage can readily be transferred to new genotypes for use in development of new cultivars.

Introduction

Abelia ×*grandiflora* (André) Rehd. and its cultivars have been widely used in landscapes for nearly a century because of their profuse pinkish-white flower, lustrous dark semi-evergreen to evergreen foliage, and tolerance to heat, drought, and pests (Bean, 1970; Krussmann, 1985; Dirr, 1998). Among its cultivars are several variegated selections: Confetti™, 'Francis Mason', 'Golden Glow', 'Goldsport', 'Goldspot', 'Goldstrike', and 'Sunrise'. Variegated plants add variety in the landscape because of the presence of distinct markings of different colors on their foliage and/or flowers. Variegated foliage contributes aesthetic value to the landscape throughout the year as opposed to the brief effectiveness of flowering (Elbert & Elbert, 1987; MacKenzie, 1989; Sheldon, 1991).

Variegation can occur by differential gene expression, leaf blisters, viruses, or chimeras (Marcotrigiano, 1997). The mode of inheritance for foliage variegation is not universal, and mechanisms can vary from a single recessive gene to dominant epistasis (Lawrence, 1974; Orakwue & Crowder, 1985). 'Francis Mason' originated as a variegated branch sport of *A.* ×*grandiflora* at Mason's Nurseries in New Zealand in the 1950s (Dirr, 1994). The evergreen to semi-evergreen foliage of 'Francis Mason' is yellow to yellow-green with a mottled appearance, and the young shoots are copper colored. *Abelia chinensis* is deciduous with dark green leaves, pale beneath, and less lustrous than *A.* ×*grandiflora*. In the present study, segregation ratios of progeny from reciprocal crosses between *A. chinensis* and *A.* ×*grandiflora* 'Francis Mason' and their backcross progeny were evaluated to determine the mode of inheritance of yellow variegated foliage for use in future breeding endeavors.

Materials and Methods

Reciprocal crosses between *A. chinensis* and 'Francis Mason' were performed in June-July 1998 in a greenhouse. Pollinations were bagged with glassine envelopes

(BioQuip Products, Gardena, CA). The F_1 progeny were backcrossed reciprocally to both *A. chinensis* and 'Francis Mason' in June-July 1999. Seeds were sown on milled sphagnum peat, and grown under mist (10 sec every 32 min) with bottom heat (22C) in the greenhouse. The F_1 progeny and BC_1 progeny were grown in the greenhouse. Backcrosses were repeated in January-February 2001, and ovule culture as described by Scheiber (2001) was used to generate BC_1 progeny. Ovules were collected 5 weeks after pollination, and cultured on Woody Plant Medium (WPM) (Lloyd & McCown, 1981) containing MS vitamins, 3% sucrose, and solidified with 0.8% agar.

The F_1 and BC_1 progeny were visually evaluated for the presence or absence of variegated foliage. Number of progeny from backcrosses were low in 1999 due to low seed germination. Backcrosses were repeated in 2001 and ovule culture was used in an attempt to obtain more progeny. Although ovule culture did increase the number of backcross progeny, numbers were still low and data from 1999 and 2001 were combined. Ratios were calculated for all crosses and the data used to determine the mode of inheritance. Hypotheses were evaluated by the chi-square goodness of fit test for observed segregation ratios.

Results and Discussion

Segregation of F_1 and backcross progeny. Segregation ratios from reciprocal crosses between *A. chinensis* and 'Francis Mason' and backcross progeny suggest that yellow variegated foliage in *Abelia* is controlled by duplicate recessive epistasis with yellow foliage dominant to green foliage. Progeny of *A. chinensis* × 'Francis Mason' and the reciprocal cross segregated in 1:1 ratios of green to yellow seedlings, indicating that one parent was heterozygous for foliage color. Backcrosses of a green F_1 plant from *A. chinensis* × 'Francis Mason' and the reciprocal to *A. chinensis* (BC1-BC4 in Table 6.1) resulted in 133 green seedlings. However, a single yellow seedling was observed among the BC4 progeny. The fruit of *Abelia* is a single-seeded achene and pollinator error may

account for the yellow seedling. Backcross progeny of yellow F_1 plants from 'Francis Mason' × *A. chinensis* and the reciprocal to *A. chinensis* (BC5-BC8 in Table 6.1) segregated in a 1:1 ratio of yellow to green. Initially, a single-dominant gene hypothesis was proposed with yellow foliage dominant to green foliage and 'Francis Mason' heterozygous (Yy) for yellow foliage. Variegation governed by a single gene has been reported in numerous taxa including *Phaseolus vulgaris* L., *Brassica campestris* L., and *Vitis* L. species (Wyatt, 1981; Orakwue & Crowder, 1983; Reisch & Watson, 1984). However, reports of variegation as a dominant trait are unusual. Studies conducted by Henny (1982, 1983, 1986a, 1986b, 1992) indicate that variegation is dominant to non-variegation in *Aglaonema* Schott. and *Dieffenbachia* Schott. Single locus, multiallelic systems control variegation in both genera.

Based on the one-gene hypothesis, yellow progeny of 'Francis Mason' × *A. chinensis* and the reciprocal that were backcrossed to 'Francis Mason' (BC13-BC16 in Table 6.1) were expected to segregate in a 3:1 ratio of yellow to green. Progeny of BC13 and BC14 segregated as expected. However, ratios of 1:2, yellow to green, were observed among BC15 and BC16 progeny, suggesting that foliage color is controlled by more than one gene. Based on the data, the ratios were fitted to a two-gene model of duplicate recessive epistasis. Identical ratios were expected for BC1-BC8 using either a single-dominant gene or duplicate recessive epistasis model. For BC9-BC12, either a 1:1 or 3:5 ratio of yellow to green was expected using a two-gene model, and a 1:1 ratio was expected using a one-gene model. Analysis indicated that all four crossing combinations fit a 1:1 hypothesis, but the 3:5 ratio yielded a better fit for BC9, BC10, and BC12. Segregation ratios of 3:1, yellow to green, for a one-gene model, and 9:7 or 3:1, yellow to green, for a two-gene model were expected for BC13-BC16. Backcross 13 produced 12 yellow and 4 green seedlings, and perfectly fit the 3:1 ratio expected in the one-gene model. However, BC14 was a poor fit, and neither BC15 nor BC16 fit the 3:1 hypothesis. When the 9:7 ratio was analyzed, only BC15 failed to support the hypothesis.

One possible explanation for the poor fit of BC15 and BC16 to a two-gene model of duplicate recessive epistasis is a lethal dosage effect if Y_1 is in the homozygous dominant condition. If Y_1Y_1 is lethal, BC13-BC16 would be the only crosses affected, and the segregation ratios would be altered to either a 1:1 or 2:1, yellow to green (Table 6.2). Analysis indicated that all four combinations fit this model at a significance level of P=0.05. Despite the statistical evidence for the presence of a lethal dosage effect, no unusual observations of seedling death were noted.

Variegation governed by more than one gene is not unusual. Dominant epistasis was reported as the mechanism controlling variegated foliage patterns in Russian Wild Ryegrass (*Elymus junceus* Fisch.) (Lawrence, 1974). Gill et al. (1969) reported that three complimentary recessive genes control yellow striping in both purple and green foliage varieties of Pearl Millet [*Pennisetum typhoides* (Burm) S. & H.]. Kohle (1970) concluded that light green foliage was dominant to dark green foliage in *Vigna* Savi. and governed by two complimentary genes.

Light effects on foliage color. Carotenoids are the pigments responsible for the yellow and orange appearance of foliage, and they contribute to photosynthesis by functioning as light-harvesting pigments and protecting chlorophyll from oxidative destruction during high irradiance (Salisbury & Ross, 1992). Biosynthesis of carotenoids involves a complex pathway under the regulation of numerous nuclear encoded genes (Bartley et al., 1994; von Lintig, 1997; Cunningham & Gantt, 1998; Vishnevetsky et al., 1999). Carotenoid biosynthesis is photoregulated, and transfer from low to high light intensity can significantly increase production (Rau, 1985; Bartley et al., 1994).

Observation of ‘Francis Mason’ grown under a canopy of deciduous trees, in a greenhouse covered by 45% shade cloth, and full sun have shown that when grown in full to partial shade the foliage of ‘Francis Mason’ is solid green. As light intensity increases, the foliage develops a yellow coloration. After long periods of exposure to high light intensities, the leaves can become bleached. Interior leaves that are well-shaded remain

green. Progeny of *A. chinensis* and 'Francis Mason' and backcross progeny revealed similar responses at both the seedling stage grown in a shaded greenhouse and mature field-grown specimens grown in full sun. Similar responses were found by Kunst and Wrischer (1983) in that pigment concentrations in the leaves of *Ligustrum ovalifolium* Hassk. 'Aureum'. varied in response to changing light intensities. As light intensities increased, carotenoid concentrations increased 200-300%, and chlorophyll concentrations declined to 50-70% of the level present in green leaves. If leaves were exposed to lower light intensities, carotenoid concentrations decreased and chlorophyll concentrations increased. In addition, bleaching of foliage was reported in *L. ovalifolium* 'Aureum' and *Acer negundo* L. var. *odessanum* H.Rothe following prolonged exposure to high light conditions (Wrischer et al., 1975; Kunst, 1983). Therefore, in this study, the F_1 and backcross seedlings of *Abelia* were exposed to sun, to encourage production of carotenoids, so that the yellow phenotype was expressed.

Lethal dosage effects, low progeny numbers, or a combination of these factors may account for the skewed ratios observed among progeny of yellow parents. This study has demonstrated, however, that yellow foliage is controlled by major nuclear genes, and can readily be transferred to new genotypes for use in development of new cultivars.

Table 6.1. Combined segregation data from 1999 and 2001 for yellow and green foliage among seedlings from crosses involving *A. chinensis, A.* ×*grandiflora* 'Francis Mason' and backcross progeny.

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
A. chinensis × 'Francis Mason'	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2y_2$		64	34	30	1:1	0.25	0.75-0.50
'Francis Mason' × *A. chinensis*	$Y_1y_1Y_2y_2$	$y_1y_1Y_2Y_2$		73	39	34	1:1	0.34	0.75-0.50
A. chinensis × ['Francis Mason' × *A. chinensis* (green)]	$y_1y_1Y_2Y_2$	$y_1y_1Y_2$–	BC1	71	0	71	0:1	0.00	0.99
['Francis Mason' × *A. chinensis* (green)] × *A. chinensis*	$y_1y_1Y_2$–	$y_1y_1Y_2Y_2$	BC2	31	0	31	0:1	0.00	0.99
A. chinensis × [*A. chinensis* × 'Francis Mason' (green)]	$y_1y_1Y_2Y_2$	$y_1y_1Y_2$–	BC3	12	0	12	0:1	0.00	0.99
[*A. chinensis* ×'Francis Mason' (green)] × *A. chinensis*	$y_1y_1Y_2$–	$y_1y_1Y_2Y_2$	BC4	18	1	17	0:1	0.06	0.90-0.75
A. chinensis × ['Francis Mason' × *A. chinensis* (yellow)]	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2$–	BC5	41	19	22	1:1	0.22	0.75-0.50
['Francis Mason' × *A. chinensis* (yellow)] × *A. chinensis*	$Y_1y_1Y_2$–	$y_1y_1Y_2Y_2$	BC6	21	13	8	1:1	1.19	0.50-0.25
A. chinensis × [*A. chinensis* × 'Francis Mason' (yellow)]	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2$–	BC7	48	25	23	1:1	0.08	0.90-0.75

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
[*A. chinensis* × 'Francis Mason' (yellow)] × *A. chinensis*	$Y_1y_1Y_2-$	$y_1y_1Y_2Y_2$	BC8	26	11	15	1:1	0.62	0.50-0.25
'Francis Mason' × ['Francis Mason' × *A. chinensis* (green)]	$Y_1y_1Y_2y_2$	$y_1y_1Y_2y_2$	BC9	24	9	15	3:5	0.00	0.99
		$y_1y_1Y_2Y_2$					1:1	1.50	0.25-0.10
['Francis Mason' × *A. chinensis* (green)] × 'Francis Mason'	$y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC10	28	10	18	3:5	0.04	0.90-0.75
	$y_1y_1Y_2Y_2$						1:1	2.29	0.25-0.10
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (green)]	$Y_1y_1Y_2y_2$	$y_1y_1Y_2y_2$	BC11	22	12	10	3:5	2.78	0.10-0.05
		$y_1y_1Y_2Y_2$					1:1	0.18	0.75-0.50
[*A. chinensis* × 'Francis Mason' (green)] × 'Francis Mason'	$y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC12	12	4	8	3:5	0.09	0.90-0.75
	$y_1y_1Y_2Y_2$						1:1	1.33	0.25-0.10
'Francis Mason' × ['Francis Mason' × *A. chinensis* (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC13	16	12	4	9:7	2.29	0.25-0.10
		$Y_1y_1Y_2Y_2$					3:1	0.00	0.99
['Francis Mason' × *A. chinensis* (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC14	28	17	11	9:7	0.24	0.75-0.50
	$Y_1y_1Y_2Y_2$						3:1	3.05	0.10-0.05

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC15	30	10	20	9:7	6.41	0.025-0.01
		$Y_1y_1Y_2Y_2$					3:1	27.78	< 0.005
[*A. chinensis* × 'Francis Mason' (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC16	16	6	10	9:7	2.29	0.25-0.10
	$Y_1y_1Y_2Y_2$						3:1	12.00	< 0.005

Table 6.2. Lethal dosage effect of Y_1Y_1 on backcross progeny of *A. chinensis* and *A.* ×*grandiflora* 'Francis Mason'.

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
'Francis Mason' × ['Francis Mason' × *A. chinensis* (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC13	16	12	4	1:1	4.00	0.05-0.025
		$Y_1y_1Y_2Y_2$					2:1	0.50	0.50-0.25
['Francis Mason' × *A. chinensis* (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC14	28	17	11	1:1	1.29	0.50-0.25
	$Y_1y_1Y_2Y_2$						2:1	0.41	0.75-0.50
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC15	30	10	20	1:1	3.33	0.10-0.05
		$Y_1y_1Y_2Y_2$					2:1	15.00	< 0.005
[*A. chinensis* × 'Francis Mason' (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC16	16	6	10	1:1	1.00	0.50-0.25
	$Y_1y_1Y_2Y_2$						2:1	6.23	0.025-0.005

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CHAPTER 7

CONCLUSIONS

Prolific displays of pinkish-white flowers from May to frost, resistance to environmental stresses, and glossy evergreen foliage have made *Abelia* ×*grandiflora* (André) Rehd. a staple in landscapes for nearly a century (Bean, 1970; Krussmann, 1985a; Dirr, 1998a). Derived from a cross between *A. chinensis* R. Br. and *A. uniflora* R. Br., *A.* ×*grandiflora* is one of only two interspecific hybrids ever developed and released within the genus (Bean, 1970). The genus contains approximately 30 species that vary widely a number of traits including flower color, flower size, growth habit, and cold hardiness. Despite the potential, breeding work in the genus had been limited. Interest has been expressed by nursery owners in the development of new cultivars through interspecific hybridization (Dirr, 1998b).

Information regarding compatibility and reproductive biology has either been lost or is simply unavailable. Knowledge of production procedures and environmental adaptability is restricted to observational data or trial and error practices without the benefit of scientifically derived evidence. To optimize development of new interspecific hybrids in the genus *Abelia,* studies were conducted to evaluated stigmatic receptivity, intra- and interspecific crossability, seed germination, ovule culture techniques, inheritance of foliage variegation, and cold hardiness.

Numerous pollinations must be performed to obtain the large quantities of seeds needed to conduct a breeding program in *Abelia* because the fruit is a one-seeded achene. Gonzalez et al. (1995) reports that stigmatic receptivity is the main factor limiting the effective pollination period in Kiwifruit. Receptivity can vary widely among woody taxa, ranging from a few hours to several days (Sharma et al., 1990; Tangmicharoen and Owens, 1997). To optimize returns on pollination efforts, *A. chinensis* and *A.* ×*grandiflora* ‘Francis Mason’ were evaluated for stigmatic receptivity. Flowers were pollinated 1-, 2-, or 3-days prior to anthesis; the day of anthesis; and 1-, 2-, 3-, 4-, or 5-days postanthesis. Receptivity of both taxa was highest on the day of anthesis, but stigmas remained receptive throughout the pollination cycle despite significant declines

in both receptivity and seed set capability. Optimal periods for pollination appear to be species dependent, ranging from 3 days for 'Francis Mason' to 6 days for *A. chinensis*. Given the lengthy receptive period, pre- and postanthesis flowers are advantageous for use in the production of interspecific hybrids.

Interspecific hybridization is possible in *Abelia*. Assessment of 12 *Abelia* taxa for intra- and interspecific compatibility indicated seed set was generally higher among interspecific hybrids than intraspecific hybrids. Interspecific hybrids varied in percent seed set dependent on the parentage. Intraspecific seed set was very low among cultivars of *A.* ×*grandiflora* and did not exceed 16%. Low seed set among intraspecific crosses may be attributed to the presence of a self-incompatibility system. Seed set of interspecific hybrids was highest between species originating in the same geographic region and decreased as geographic distance increased.

Although seed was derived from intra- and interspecific crosses, germination rates were low and studies were conducted on *A.* ×*grandiflora* seeds to determine means to increase germination percentages and rates. Each *Abelia* seed is enclosed in a leathery achene. The dry, indehiscent pericarp of the achene can mimic the function of a seedcoat and delay, reduce, or suppress germination (Boesewinkel & Bouman, 1995). The effect of pericarp removal was examined in combination with stratification for 60 days at 4C, immersion in 100 mg/l gibberellic acid for 24h, and no pretreatment. Seeds with attached pericarps germinated at a significantly higher percentage than those without pericarps. Stratified seeds with attached pericarps had the highest germination percentage, 63%; though not significantly different than the other treatments. Germination was significantly more uniform for stratified seeds with pericarps and GA_3-immersed seeds without pericarps relative to their controls. Despite more uniform germination, pericarp removal, GA_3 immersion, and stratification are of no practical benefit due to either reduced germination percentages or the time necessary for stratification.

Seeds from interspecific hybridization between *A.* ×*grandiflora* 'Francis Mason' and *A. schumannii* failed to germinate, prompting development of an ovule culture technique. Three main factors were evaluated: number of weeks after pollination to harvest the developing ovules, basal salt medium, and concentration of the growth regulator, 2iP. Ovules were dissected from the fruiting structure 4, 5, and 6 weeks after pollination and cultured on either Woody Plant Medium (WPM) (Lloyd & McCown, 1981) or Linsmaier and Skoog (LS) (Linsmaier & Skoog, 1965) containing 0, 9.8, or 39.2 μM 2iP. Data were collected on embryos recovered, spontaneous embryo germination, root formation, and final plant survival. Number of weeks following pollination significantly affected all parameters examined, with the best response occurring on embryos harvested 5 weeks after pollination. Final plant survival was greatest for embryos cultured on Woody Plant Medium and 0 μM 2iP. Eight-five percent of the cultured ovules produced embryos that rooted and 65% survived the hardening off process when ovules were cultured 5 weeks after pollination on WPM containing no growth regulator. The technique was successfully used to obtain previously unrecorded interspecific hybrids between *A. chinensis* × *A. engleriana, A. chinensis* × *A. spathulata, A. chinensis* × *A. serrata,* and *A. chinensis* × *A. zanderi*.

Abelia ×*grandiflora* 'Francis Mason' is a variegated cultivar characterized by yellow to yellow green evergreen foliage with a mottled appearance, and young copper-colored shoots (Dirr, 1994). Variegated plants add variety to the landscape because of the presence of colorful streaks, spots, or sectors on their foliage and/or flowers. The mode of inheritance for foliage variegation is not universal and determination of the controlling mechanism in *Abelia* is beneficial in future breeding endeavors. Segregation ratios from reciprocal crosses between *A. chinensis* and *A.* ×*grandiflora* 'Francis Mason' and backcross progeny indicate that foliage color in *Abelia* is controlled by duplicate recessive epistasis and yellow foliage is dominant to green foliage. Observational data suggests that expression of foliage color is regulated by light intensity.

Evaluation for adaptability to environmental stresses is a vital aspect of cultivar development. Breeders are concerned with the cold acclimation of woody plants because cold, more than any other environmental factor, limits their distribution range (Dirr et al., 1993). The northern distribution of *Abelia* is limited by both stem and leaf hardiness. Twelve taxa of *Abelia* were evaluated using laboratory procedures to determine maximum stem and leaf cold hardiness and to evaluate timing of acclimation and deacclimation over a two year period. 'John Creech' ranked in the hardiest group of taxa for both stems and leaves on the majority tests. In addition, 'John Creech' retained cold hardiness later in the spring than did the other taxa. 'Edward Goucher' and 'Confetti' had the least hardy stems and leaves, respectively. Stems were generally equal in hardiness or hardier than leaves on all test dates in both test seasons. Laboratory results were consistent with field observations, but often differed from published hardiness ratings. Studies conducted by Lindstrom and Dirr (1989) have indicated a strong correlation between cold hardiness observed in the field and laboratory tests when plants were evaluated on multiple dates. Temperature fluctuations just prior to sampling dates appear to significantly affect lowest survival temperatures and timing of acclimation and deacclimation among *Abelia* taxa. Midwinter hardiness and timing of acclimation and deacclimation are important criteria for the selection of superior parental germplasm to assure improved hardiness among resulting cultivars. Based on the data, 'John Creech' would make a logical choice for incorporation into a breeding program.

Advances have been made in the development of new *Abelia* cultivars through interspecific hybridization. Protocols for assessment of cold hardiness have been evaluated and ovule culture techniques were developed. Information regarding stigmatic receptivity and the mode of inheritance of foliage variegation is now available. Finally, several previously unrecorded hybrids were obtained.

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APPENDIX

Table A.1. Chromosome Numbers in *Abelia* R. Br.

Species	Chromosome Number	Source
A. chinensis R. Br.	2n=32	Goldblatt & Johnson, 1996
A. coreana Nakai	2n=36	Goldblatt & Johnson, 1991
A. engleriana (Graebn.)Rehd.	2n=32	Federov, 1974
A. × *grandiflora* (André) Rehd.	2n=32	Goldblatt & Johnson, 1990
A. schumannii (Graebn.)Rehd.	2n=32	Federov, 1974
A. triflora R. Br.	2n=36	Goldblatt, 1981
A. triflora R. Br.	2n=18	Bedi, Bir, & Gill, 1982
A. uniflora R. Br.	2n=36	Federov, 1974

Table A.2. Cold Hardiness Ratings in *Abelia* R. Br.

Taxa	Zone	Source
A. biflora Turcz.	7	Krussmann, 1985a
A. buddleoides W.W. Sm.	8	Griffiths, 1994
A. buddleoides W.W. Sm.	8	Krussmann, 1985a
A. chinensis R. Br.	7	Bailey Hortorium, 1976
A. chinensis R. Br.	7	Beckett & Beckett, 1983
A. chinensis R. Br.	(6)7	Dirr, 1998
A. chinensis R. Br.	7	Griffiths, 1994
A. chinensis R. Br.	8	Krussmann, 1985a
A. chinensis R. Br.	8	Rehder, 1937
A. 'Edward Goucher'	6	Dirr, 1998
A. 'Edward Goucher'	5	Griffiths, 1994
A. 'Edward Goucher'	5	Krussmann, 1985a
A. engleriana (Graebn.) Rehd.	6	Bailey Hortorium, 1976
A. engleriana (Graebn.) Rehd.	6	Griffiths, 1994
A. engleriana (Graebn.) Rehd.	6	Krussmann, 1985a
A. engleriana (Graebn.) Rehd.	5	Redher, 1937
A. floribunda Decne.	9	Bailey Hortorium, 1976
A. floribunda Decne.	8	Beckett & Beckett, 1983
A. floribunda Decne.	8	Dirr, 1998
A. floribunda Decne.	7	Griffiths, 1994
A. floribunda Decne	8	Krussmann, 1985a
A. graebneriana Rehd.	7	Bailey Hortorium, 1976
A. graebneriana Rehd.	5	Griffiths, 1994
A. graebneriana Rehd.	6	Krussmann, 1985a
A. × *grandiflora* (André) Rehd.	6	Bailey Hortorium, 1976
A. × *grandiflora* (André) Rehd.	7	Beckett & Beckett, 1983

Taxa	Zone	Source
A. × *grandiflora* (André) Rehd.	6	Dirr, 1998
A. × *grandiflora* (André) Rehd.	5	Griffiths, 1994
A. × *grandiflora* (André) Rehd.	5	Krussmann, 1985a
A. × *grandiflora* (André) Rehd.	5	Rehder, 1937
A. integrifolia G. Koidz.	8	Bailey Hortorium, 1976
A. integrifolia G. Koidz.	6	Griffiths, 1994
A. ionandra Hayata.	8	Griffiths, 1994
A. ionandra Hayata.	8	Krussmann, 1985a
A. schumannii (Graebn.) Rehd.	7	Bailey Hortorium, 1976
A. schumannii (Graebn.) Rehd.	6	Beckett & Beckett, 1983
A. schumannii (Graebn.) Rehd.	6	Griffiths, 1994
A. schumannii (Graebn.) Rehd.	7	Krussmann, 1985a
A. schumannii (Graebn.) Rehd.	8	Rehder, 1937
A. serrata Sieb. & Zucc.	8	Bailey Hortorium, 1976
A. serrata Sieb. & Zucc.	6	Griffiths, 1994
A. serrata Sieb. & Zucc.	6	Krussmann, 1985a
A. spathulata Sieb. & Zucc.	8	Bailey Hortorium, 1976
A. spathulata Sieb. & Zucc.	7	Krussmann, 1985a
A. triflora R. Br.	7	Bailey Hortorium, 1976
A. triflora R. Br.	6	Grifftiths, 1994
A. triflora R. Br.	6	Krussmann, 1985a
A. triflora R. Br.	7	Rehder, 1937
A. umbellata (Graebn. & Buchw.) Rehd.	7	Grifftiths, 1994
A. umbellata (Graebn. & Buchw.) Rehd.	8	Krussmann, 1985a
A. uniflora R. Br.	7	Bailey Hortorium, 1976
A. uniflora R. Br.	7	Griffiths, 1994
A. uniflora R. Br.	8	Krussmann, 1985a

Taxa	Zone	Source
A. zanderi (Graebn.) Rehd.	6	Bailey Hortorium, 1976
A. zanderi (Graebn.) Rehd.	6	Griffiths, 1994
A. zanderi (Graebn.) Rehd.	6	Krussmann, 1985a
A. zanderi (Graebn.) Rehd.	5	Rehder, 1937

Table A.3. Segregation data from 1999 for yellow and green foliage among seedlings from crosses involving *A. chinensis, A.* ×*grandiflora* 'Francis Mason' and backcross progeny.

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
A. chinensis × ['Francis Mason' × *A. chinensis* (green)]	$y_1y_1Y_2Y_2$	$y_1y_1Y_2$--	BC1	48	0	48	0:1	0.00	0.99
['Francis Mason' × *A. chinensis* (green)] × *A. chinensis*	$y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC2	31	0	31	0:1	0.00	0.99
A. chinensis × [*A. chinensis* × 'Francis Mason' (green)]	$y_1y_1Y_2Y_2$	$y_1y_1Y_2$--	BC3	2	0	2	0:1	0.00	0.99
[*A. chinensis* × 'Francis Mason' (green)] × *A. chinensis*	$y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC4	18	1	17	0:1	0.06	0.90-0.75
A. chinensis × ['Francis Mason' × *A. chinensis* (yellow)]	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2$--	BC5	19	8	11	1:1	0.47	0.50-0.25
['Francis Mason' × *A. chinensis* (yellow)] × *A. chinensis*	$Y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC6	10	8	2	1:1	3.60	0.10-0.05
A. chinensis × [*A. chinensis* × 'Francis Mason' (yellow)]	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2$--	BC7	16	7	9	1:1	0.25	0.75-0.50
[*A. chinensis* × 'Francis Mason' (yellow)] × *A. chinensis*	$Y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC8	11	3	8	1:1	2.27	0.25-0.10

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
'Francis Mason' × ['Francis Mason' × *A. chinensis* (green)]	$Y_1y_1Y_2y_2$	$y_1y_1Y_2y_2$	BC9	16	6	10	3:5	0.00	0.99
		$y_1y_1Y_2Y_2$					1:1	1.00	0.50-0.25
['Francis Mason' × *A. chinensis* (green)] × 'Francis Mason'	$y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC10	21	9	12	3:5	0.25	0.75-0.50
	$y_1y_1Y_2Y_2$						1:1	0.43	0.75-0.50
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (green)]	$Y_1y_1Y_2y_2$	$y_1y_1Y_2y_2$	BC11	6	2	4	3:5	0.04	0.90-0.75
		$y_1y_1Y_2Y_2$					1:1	0.67	0.50-0.25
[*A. chinensis* × 'Francis Mason' (green)] × 'Francis Mason'	$y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC12	3	1	2	3:5	0.01	0.90-0.75
	$y_1y_1Y_2Y_2$						1:1	0.33	0.75-0.50
'Francis Mason' × ['Francis Mason' × *A. chinensis* (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC13	1	1	0	9:7	0.67	0.50-0.25
		$Y_1y_1Y_2Y_2$					3:1	0.38	0.75-0.50
['Francis Mason' × *A. chinensis* (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC14	8	4	4	9:7	0.13	0.75-0.50
	$Y_1y_1Y_2Y_2$						3:1	2.67	0.25-0.10
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC15	12	4	8	9:7	2.56	0.25-0.10
		$Y_1y_1Y_2Y_2$					3:1	11.11	< 0.005

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
[*A. chinensis* ×'Francis Mason' (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC16	6	3	3	9:7	0.11	0.90-0.75
	$Y_1y_1Y_2Y_2$						3:1	2.00	0.25-0.10

Table A.4. Lethal dosage effect of Y_1Y_1 on backcross progeny of *A. chinensis* and *A.* ×*grandiflora* 'Francis Mason' from 1999.

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
'Francis Mason' × ['Francis Mason' × *A. chinensis* (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC13	1	1	0	1:1	1.00	0.50-0.25
		$Y_1y_1Y_2Y_2$					2:1	0.49	0.50-0.25
['Francis Mason' × *A. chinensis* (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC14	8	4	4	1:1	0.00	0.99
	$Y_1y_1Y_2Y_2$						2:1	0.94	0.50-0.25
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC15	12	4	8	1:1	1.33	0.25-0.10
		$Y_1y_1Y_2Y_2$					2:1	6.00	0.025-0.01
[*A. chinensis* × 'Francis Mason' (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC16	6	3	3	1:1	0.00	0.99
	$Y_1y_1Y_2Y_2$						2:1	0.75	0.50-0.25

Table 5. Segregation data from 2001 for yellow and green foliage among seedlings from crosses involving *A. chinensis, A.* × *grandiflora* 'Francis Mason' and backcross progeny.

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
A. chinensis × ['Francis Mason' × *A. chinensis* (green)]	$y_1y_1Y_2Y_2$	$y_1y_1Y_2$--	BC1	23	0	23	0:1	0.00	0.99
['Francis Mason' × *A. chinensis* (green)] × *A. chinensis*	$y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC2	___[z]	___[z]	___[z]	0:1	___[z]	___[z]
A. chinensis × [*A. chinensis* × 'Francis Mason' (green)]	$y_1y_1Y_2Y_2$	$y_1y_1Y_2$--	BC3	10	0	10	0:1	0.00	0.99
[*A. chinensis* × 'Francis Mason' (green)] × *A. chinensis*	$y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC4	___[z]	___[z]	___[z]	0:1	___[z]	___[z]
A. chinensis × ['Francis Mason' × *A. chinensis* (yellow)]	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2$--	BC5	22	11	11	1:1	0.00	0.99
['Francis Mason' × *A. chinensis* (yellow)] × *A. chinensis*	$Y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC6	11	5	6	1:1	0.09	0.90-0.75
A. chinensis × [*A. chinensis* × 'Francis Mason' (yellow)]	$y_1y_1Y_2Y_2$	$Y_1y_1Y_2$--	BC7	32	18	14	1:1	0.50	0.50-0.25
[*A. chinensis* × 'Francis Mason' (yellow)] × *A. chinensis*	$Y_1y_1Y_2$--	$y_1y_1Y_2Y_2$	BC8	15	8	7	1:1	0.07	0.90-0.75

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
'Francis Mason' × ['Francis Mason' × *A. chinensis* (green)]	$Y_1y_1Y_2y_2$	$y_1y_1Y_2y_2$ $y_1y_1Y_2Y_2$	BC9	8	3	5	3:5 1:1	0.00 0.50	0.99 0.50-0.25
['Francis Mason' × *A. chinensis* (green)] × 'Francis Mason'	$y_1y_1Y_2y_2$ $y_1y_1Y_2Y_2$	$Y_1y_1Y_2y_2$	BC10	7	1	6	3:5 1:1	1.57 3.57	0.25-0.10 0.10-0.05
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (green)]	$Y_1y_1Y_2y_2$	$y_1y_1Y_2y_2$ $y_1y_1Y_2Y_2$	BC11	16	10	6	3:5 1:1	4.27 1.00	0.05-0.025 0.50-0.25
[*A. chinensis* × 'Francis Mason' (green)] × 'Francis Mason'	$y_1y_1Y_2y_2$ $y_1y_1Y_2Y_2$	$Y_1y_1Y_2y_2$	BC12	9	3	6	3:5 1:1	0.08 1.00	0.90-0.75 0.50-0.25
'Francis Mason' × ['Francis Mason' × *A. chinensis* (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$ $Y_1y_1Y_2Y_2$	BC13	15	11	4	9:7 3:1	1.83 0.01	0.25-0.10 0.95-0.90
['Francis Mason' × *A. chinensis* (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$ $Y_1y_1Y_2Y_2$	$Y_1y_1Y_2y_2$	BC14	20	13	7	9:7 3:1	0.62 1.07	0.50-0.25 0.50-0.25
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$ $Y_1y_1Y_2Y_2$	BC15	18	6	12	9:7 3:1	3.79 16.67	0.05-0.025 < 0.005

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
[*A. chinensis* × 'Francis Mason' (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC16	10	3	7	9:7	2.74	0.10-0.05
	$Y_1y_1Y_2Y_2$						3:1	10.80	< 0.005

[z]Cross not repeated in 2001.

Table 6. Lethal dosage effect of Y_1Y_1 on backcross progeny of *A. chinensis* and *A.* ×*grandiflora* 'Francis Mason' from 2001.

Cross	Parental genotypes		Code	Total no. seedlings	No. yellow seedlings	No. green seedlings	Ratio tested	χ^2	*P*
	P1	P2							
'Francis Mason' × ['Francis Mason' × *A. chinensis* (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC13	15	11	4	1:1	3.27	0.10-0.05
		$Y_1y_1Y_2Y_2$					2:1	0.30	0.75-0.50
['Francis Mason' × *A. chinensis* (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC14	20	13	7	1:1	1.80	0.25-0.10
	$Y_1y_1Y_2Y_2$						2:1	0.02	0.90-0.75
'Francis Mason' × [*A. chinensis* × 'Francis Mason' (yellow)]	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC15	18	6	12	1:1	2.00	0.25-0.10
		$Y_1y_1Y_2Y_2$					2:1	9.00	< 0.005
[*A. chinensis* × 'Francis Mason' (yellow)] × 'Francis Mason'	$Y_1y_1Y_2y_2$	$Y_1y_1Y_2y_2$	BC16	10	3	7	1:1	1.60	0.25-0.10
	$Y_1y_1Y_2Y_2$						2:1	6.19	0.025-0.01

[z]Cross not repeated in 2001.