

**GENETIC AND ENVIRONMENTAL VARIATION IN VITEX AND COST ANALYSIS  
OF A WOODY ORNAMENTAL BREEDING PROGRAM**

**by**

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**(Under the Direction of David Knauff)**

**ABSTRACT**

Segregating populations of *Vitex* and their parents were cloned and grown in containers and in the ground. Traits evaluated included *Cercospora* leaf spot resistance, first flower date, last flower date, flower duration, total weeks of flowering, average inflorescence number, average inflorescence length, average flower rating, plant height, and plant width. Overall, plants grown in ground were taller and wider than their in container counterparts. In ground plants also had a later flower date, longer flower duration, greater total weeks flowering, longer average inflorescence length, larger average inflorescence number, and more flowers on the inflorescence. All flowering traits and most height and width measurements showed significant genotype by environment interaction. Therefore it is evident that genotypes react differently in each environment and selection should occur in one environment only and that environment is in ground. Those traits that exhibited entry X treatment interactions would suggest that by selecting those in containers would overlook optimal plants in ground and vice versa. Those plants that are among the top performers in ground may not be in containers, but could still perform better in containers than available cultivars in containers.

High correlations were present in both environments between average inflorescence number and total weeks flower and between last flower date and total weeks flowering, First flower date and height measurements taken 33 weeks after planting, and average inflorescence number and last flower date were only correlated in ground, while total weeks flowering and flower duration were only correlated in containers. A breeder should be conscious of this as selection for one trait may also select for another.

Costs per plant in ground were greater for both materials and labor. However, water usage was greatly reduced in the in ground trial. As cost analysis revealed higher costs in ground than in containers, it also revealed an extreme water use differential in which the container treatment received the most water. Many hours and materials costs were due to mulch which was received *gratis* through the university. It is not known how the lack of mulch application would affect overall water usage in ground. This additional cost for field growing plant material may become necessary as water use restrictions continue to be imposed.

INDEX WORDS: *Vitex agnus-castus* L., *Vitex rotundifolia* L.f., G X E, Trait correlation, Cost analysis

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

### Botany of *Vitex*

*Vitex* L. is a genus of about 250 species distributed throughout the world. *Vitex* has recently been moved from Verbenaceae to Lamiaceae in an effort to make Lamiaceae monophyletic. In doing this, over half of the genera previously included in Verbenaceae were moved to the Lamiaceae (Judd et al., 2002). There is still debate as to which family *Vitex* belongs. Most systematists have restricted Lamiaceae to those species with more or less gynobasic styles. These families have traditionally been distinguished on the basis of styler position with plants having terminal styles classified in the Verbenaceae and those with gynobasic styles in the Lamiaceae (Cantino, 1992). However, Lamiaceae contains both genera with gynobasic flowers and genera with terminal flowers (Royal Botanic Gardens, 2008).

*Vitex agnus-castus* L., native to the Mediterranean (Maloupa et al., 1999; Mehlhorn et al., 2005), has leaves that are opposite, palmate, and aromatic. It has five to seven narrow leaflets per leaf measuring five to 10 centimeters long. Inflorescences are racemes that typically measure from seven to 15 centimeters (Dirr, 1998). Plant height ranges from two to six meters with older plants having grey bark. There are at least nine cultivars of *V. agnus-castus* in the nursery trade, four of which were used in this study. *Vitex agnus-castus* ‘Blushing Spires’ has off-white flowers that age to blush. *V. agnus-castus* ‘Silver Spires’ has white flowers with smaller leaflets

(personal observation). *V. agnus-castus* ‘Abbeville Blue’ has lavender flowers. *V. agnus-castus* ‘Shoal Creek’ has lavender flowers and is said to have leafspot resistance (Dirr, 1998).

*Vitex rotundifolia* L.f., native to East Asia, Australia, Pacific Islands, and Hawaii (Wagner et al., 1999), has a prostrate habit growing 0.3 to 0.6 meters high and spreading indefinitely. Leaves are rounded and bluish-green in color that measure five centimeters long by four centimeters wide, growing in zone seven to 10 (Dirr, 1998). Plants reproduce by seed and vegetatively, with low-growing stems rooting in the soil. Lavender flowers bloom in short inflorescences approximately eight centimeters out of the leaf axils (personal observation).

### **History of *Vitex***

The use of *Vitex* species was well known during ancient times. As mentioned in Homer’s 6<sup>th</sup> century BCE work, the *Iliad*, it was described as having the ability to ward off evil and was a symbol of chastity (Chevallier, 1996). Ancient Mediterranean countries used it for a variety of health problems. Hippocrates recommended *V. agnus-castus* for inflammation in the 4<sup>th</sup> century BCE. In the 1<sup>st</sup> century CE Dioscorides recommended *V. agnus-castus* specifically for inflammation of the womb and for lactation (Brown, 1994; Hobbs, 1991). The use of *Vitex* continued with medieval monks chewing the bark to maintain their celibacy. They also put the fruit in their robe pockets for this same purpose. For this reason, *V. agnus-castus* is synonymous with Monk’s Pepper and Chaste Tree (DuMee, 1993). Its pharmacological use has steadily gained popularity in the United States since the mid-20<sup>th</sup> century (Hobbs, 1991). The plant part traditionally used has been the fruit, which is orally ingested (DuMee, 1993). This has been most commonly used for symptoms related to female reproduction. It has been reported that essential oils present in the fruit of *V. agnus-castus* bind to estrogen receptors in human breast

adenocarcinoma cells. Other uses include treatment of fever, rheumatic conditions, colds, eye pain, and promoting urination. *V. agnus-castus* also has been used topically for insect bites and stings (Jellin, 2008). Introduced to the United States in 1570, *V. agnus-castus* is used ornamentally as a shrub border or as a specimen planting, and it has been noted to attract bees (Gilman and Watson, 1994).

Fruit of *Vitex rotundifolia* L.f. has been traditionally used for colds, headaches, migraine headaches, sore eyes, and muscle pain in Asian countries (But and Chang, 1996). In Korea the fruit has also been used for the relief of headaches caused by upper respiratory infection. The fruit of *V. rotundifolia* contains compounds that inhibit lung and colon cancer cells (Westbrooks and Brabson, 2007). Dried fruits of *V. rotundifolia* have been known to show strong estrogenic activity in the same manner as *V. agnus-castus*. Research has been conducted on the effect of components of the essential oil from the fruit on human breast adenocarcinoma cells (Hu et al., 2007). Current research includes its effect on leukemia as well (Ko et al., 2001). *V. rotundifolia* was originally introduced to the United State by the J.C. Raulston Arboretum at North Carolina State University in the 1980s for dune stabilization and ornamental use.

Research has also been conducted on the use of *V. negundo* L. as a neutralizer of snake venom. It was observed that the methanolic root extracts of *V. negundo* possessed the ability to reduce the venom-induced hemorrhage and inflammatory activity of the viper, *Vipera russellii* Gray (Alam and Gomes, 2003). This species is also used for biomass and fuel wood production in tropical and subtropical areas (Misra and Verma, 1989). This species is also grown ornamentally as a deciduous shrub sharing a common name with *V. agnus-castus* of Chastetree. It is similar to *V. agnus-castus* in shape and inflorescence type, but is considered ornamentally

different in that it has a shorter bloom time and fewer inflorescences. It is, however, considered hardier than *V. agnus-castus* and grows in USDA hardiness zones 6-9. (Dirr, 1998)

### **Environmental variation**

Phenotypic plasticity, or environmentally dependent phenotypic expression, has been of increasing interest since Bradshaw's 1965 classic review (Bradshaw, 1965). The ability of plants to modify phenotypes in different environments is especially important for plants as they lack the ability to move quickly from place to place (Schlichting, 1986). Environmental effects on plants were formerly thought of as useless "noise" blocking the "true expression of the phenotype" (Allen, 1979; Schlichting and Pigliucci, 1998). Research has been conducted on the effects of various environments on the phenotypes of horticultural plants including density (Shaw, 1986), light (Avramov et al., 2007; Barisic et al., 2006; Pigliucci and Schlichting, 1995; Smith and Whitelam, 1997; Valladares et al., 2006), water (Caruso, 2006; Chun et al., 2007; Elle and Hare, 2002; Frazee and Marquis, 1994), nutrient availability (Chun et al., 2007; Dogan and Huseyin, 1998; Lehmann and Rebele, 2005; Mony et al., 2007; Pigliucci and Schlichting, 1998), defoliation (Marshall et al., 2005), temperature (Medek et al., 2007), and biotic factors (Elle and Hare, 2002; Sultan, 2004).

Various environmental effects have been determined for different plant traits. Recently, studies have shown plants exhibit plasticity for many traits ranging from morphology, anatomy, and physiology. It has also been shown for developmental patterns, reproductive timing, and breeding systems (Sultan, 2000). A particular stress may initiate a series of changes involving several observable aspects of development such as "shade avoidance syndrome", which includes altered allocation, stem elongation, suppressed branching, and accelerated reproduction (Smith and Whitelam, 1997). A plant can respond to environmental changes simply by altering its

growth rate without affecting the distribution of resources or plant morphology (Schlichting, 1986).

Environmentally plastic vegetative traits have been extensively researched in ornamentals. In *Iris pumila* L. the morphology, anatomy, and physiology of leaf components were influenced by light conditions (Avramov et al., 2007). In *Polygonum* the greatest biomass was allocated to leaf tissues in low light (Sultan, 2003). Total biomass increased with greater soil fertility in *Calamagrostis* (Lehmann and Rebele, 2005) and with higher light availability in *Pinus* and *Quercus* (Valladares et al., 2006). Higher density resulted in smaller plants and leaves in three *Lamium* species (Barisic et al., 2006) and *Salvia lyrata* L. (Shaw, 1986). An increase in temperature in grass species caused an increase in relative growth rate (Medek et al., 2007). Environmentally variable reproductive traits in ornamentals also have been well documented (Carroll et al., 2001; Dogan and Huseyin, 1998; Dorken and Barrett, 2004; Gilman and Watson, 1994; Vogler et al., 1999; Weinig, 2002). Levels of invasiveness were found to be closely associated with the interaction of high levels of soil nutrients and flooding in *Lythrum* species (Chun et al., 2007). It has been found that inflorescence traits are, in general, less plastic than vegetative traits (Bradshaw, 1965; Frazee and Marquis, 1994). Traits expressed in individual flowers, such as stigma length or petal size, have been found to be less plastic than plant-level traits such as flower number and date of first flower (Dorken and Barrett, 2004; Wolfe and Mazer, 2005). It has been suggested that earlier flowering is a response to stress in plants (Westerman and Lawrence, 1970). Environmentally induced changes may be advantageous in some species as is the case with *Mimulus guttatus* DC. These plants flowered earlier when stressed whereas plants in optimal conditions allocated more biomass to vegetative growth prior to flowering (Galloway, 1995). Water stress can decrease flower production in *Clarkia*

*unguiculata* Lindl. (Smith-Heurta and Vasek, 1987), *Lavandula stoechas* L. (Herrera, 1991), and species of *Phlox* (Schlichting, 1986). In the case of *Ranunculus peltatus* Schrank researchers found the presence of sexual organs increased in the presence of lower concentrations of phosphorus in water (Mony et al., 2007). The length of the inflorescence decreased under drought conditions in *Lobelia siphilitica* L. (Caruso, 2006). Inflorescence weight decreased in *Arabidopsis thaliana* L. in response to low light (Pigliucci et al., 1995).

### **Genetic X Environmental Interaction**

Genotypic variation in a population's phenotypic response across environments is referred to as G X E interaction (Fry, 1992). G X E interactions are therefore characteristic of populations of differing genotypes (Via et al., 1995). Although many studies assume that intraspecific variation is either infrequent or insignificant (Bolnick et al., 2003), variation among populations is evident in all natural systems (Pachepsky et al., 2007) and is a fundamental component of diversity (Mayr, 1996). The observation of G X E interaction means that the effects of genotype and environment are not independent (Via, 1984). The majority of research on G X E interactions has been conducted on agronomic plants and focus on seed or biomass production (Chahal and Gosal, 2002). While the methodologies for evaluation of G X E interactions are relevant for ornamental breeding, the characteristics of interest are different for ornamental breeding. Some of the ways in which G X E has been evaluated are the use of stability analysis (using regression analysis and method of means to determine which genotypes are stable across environments), reaction norms (graphically depicting population differences in separate environments), and trait correlations in separate environments. Additional ways to analyze G X E interactions include non-parametric methods such as principal component



analysis, factor analysis, and the additive mean effects multiplicative interaction (Acquaah, 2007).

It has been shown that closely related species can substantially differ in the amount, direction, and timing of plastic responses. Plasticity of growth trait response to temperature depends on the developmental and evolutionary background of the species (Schlichting, 1986). The ability to alter reproduction time in response to stress can differ among related species in *Polygonum* (Sultan, 2003). In *Lobelia siphilitica* L. flowering time in response to drought varied by population (Caruso, 2006). In some *Poa* species there is no difference in the plasticity of growth response between species in response to temperature (Medek et al., 2007). There was a significant population X substrate interaction for biomass in *Calamagrostis epigejos* L. (Lehmann and Rebele, 2005). Components of reproduction in *Sesbania* may vary within species in response to defoliation (Marshall et al., 2005). In a study comparing native and invasive *Lythrum salicaria* L., native *L. salicaria* plants flowered earlier. Invasive plants exhibited an extended period of vegetative growth before flowering to increase height and allocation to clonal reproduction (Chun et al., 2007). Studies also use rank to determine the presence of G X E interactions. *Liriomyza sativae* Blanchard is a dipteran pest of vegetables. Larval size of 11 populations of *L. sativae* were ranked on two plant hosts, tomato and pea and no correlation was found in rank between the two plant hosts (Via, 1984).

### **Trait correlations**

Breeders generally attempt to improve a number of traits simultaneously. If these traits are positively correlated, the response to selection will be more rapid than for characteristics selected separately. Conversely, attempting to select for traits with negative genetic correlations can slow their rate of simultaneous improvement (Antonovics, 1976; Lande, 1982). Trait

incorporation may even be delayed for many generations if several important traits are negatively correlated (Lande, 1980). Most leaf traits in *Iris pumila* L. are not correlated, suggesting that they can be independently selected (Avramov et al., 2007). In *Raphanus sativus* L. low correlations between 13 floral traits suggest that most are independent, indicating the potential of developing lines with new floral morphologies by selecting for these genetically independent traits (Kobayashi et al., 2007). This was not the case in *Penstemon* where some floral traits were highly correlated and therefore not independent from each other. Examples include high positive correlation between gynoecium length and corolla width. High correlation was also present with nectar volume and nectar sugar. High negative correlation between nectar volume and flowering date was present as was correlation with corolla length and stigma exertion (Mitchell and Shaw, 1993).

In some cases environmental change can alter genetic correlation values as was the case with *Townsendia annua* Beaman. At higher nitrogen availability there were positive genetic correlations between water use efficiency and nitrogen use efficiency but at lower nitrogen availability there was a negative correlation between these traits (Evans, 1998) as cited in (Ackerly et al., 2000). On the other hand, a study did not find any significant differences in the genetic correlations among floral traits of *Raphanus sativus* when the environment was altered (Young et al., 1994).

### **Cost analysis**

Research has been conducted on the trends in the number of plant breeders in the public and private sectors. These studies use the term Science Person Year (SY), defined as work done by a person who has responsibility for designing, planning, managing, and conducting plant breeding research and related tasks in one year (2,080 hours), not including technicians, farm and

clerical workers, computer specialists, post doc, grad student, etc. (Traxler et al., 2005). Of the 2241 SYs in U.S. plant breeding in 1994, 1499 (67%) were employed by the private sector, 529 (24%) by State Agricultural Experiment Stations and 213 (9%) by USDA. In the public sector, horticulture plant breeding, which includes vegetable, fruit, and ornamental breeding, totaled 35-37% of the overall research effort. The private sector had 25% of the research devoted to horticulture plant breeding. It was found in a 1994 plant breeding study that the number of SYs devoted to ornamentals was 87, or 3.95% of total research, with 18 SYs from SAES, 5 SYs from ARS/USDA, and 64 SYs from private industry (Frey, 1996). A study concerning the plant breeding resources for 2001 (Traxler et al., 2005) revealed that an increase in the number of plant breeders occurred from data collected in 1994 (Frey, 1996) at SAES (21%) and at the USDA/Agricultural Research Service (23%). Ornamental breeding was the only category with significant growth, with 20 SYs added in the seven years between the two studies.

Few analyses have been conducted to compare costs of plant breeding methods. Cost analysis was conducted for 50 wholesale ornamental plant nurseries in Florida in 1995. Information was presented on sales, production, costs, assets and liabilities, and efficiency indicators. This includes information on container and field grown woody ornamentals. There were 23 nurseries that grew woody ornamentals and only 2 of those had plants growing both in containers and in the field. Expenses were grouped into the categories of management's compensation, employees' wages and benefits, materials, facility and equipment, administrative overhead, depreciation, and interest. Employee wages and benefits averaged 33.8% of costs for all firms. These include benefits and also consider employment on a full time equivalent basis. Other costs include 4.5% for management, , 32.0% for materials, 4.9% for equipment/facilities, 16.0% for overhead, 3.8% for depreciation, and 4.4% for interest. (Hodges et al., 1995).

Cost analyses have been conducted to compare traditional breeding programs with marker-assisted selection in wheat breeding (Brennan and Martin, 2007). In a hypothetical breeding program, the costs of molecular markers ranged from \$2.59 to \$16.28 depending on the form of analysis and the degree of multipooling and multiplexing employed in the marker analysis while the costs for phenotypic selection for rust ranged from \$1.48 for field screening to \$5.18 for glasshouse screening. With this information it can be determined that replacing field screening with marker-assisted selection would cause an increase in costs while replacing glasshouse screening with marker-assisted selection may reduce costs, depending on the type of marker selection utilized. However, in other studies, markers were generally less expensive than other phenotypic evaluations (Brennan and Martin, 2006) as cited in (Brennan and Martin, 2007). The 2007 study showed that the net present value per hectare of wheat was \$6.93 more than work without this new technology. Therefore new breeding technologies can bring improved revenue to wheat producers (Brennan and Martin, 2007). Previous research involving cost analysis of plant breeding has been limited and this is more so concerning ornamental plant breeding.

Related research includes comparing costs of growing in field, pot-in-pot, or in containers. Fixed and production costs were obtained for a hypothetical nursery using a 10 acre production area to grow crape myrtle (*Lagerstroemia indica* L.) for three years. Fixed costs were found to be similar for in-field and above ground containers with a cost of \$350,000. Fixed costs for pot-in-pot were found to be \$25,000 higher. Total production costs for three years were found to be similar for above ground container and pot-in-pot at \$500,000 and in field was found to be \$50,000 less than this. Per harvested plant total costs were lowest in pot-in-pot (\$21.52) and similar in field (\$23.73) and above ground container (\$23.17). When analyzing by only variable

costs the costs per plant were \$5.15 for in field, \$7.36 for above ground containers and \$5.47 for pot-in-pot (Adrian et al, 1998).

### **Research objectives**

New ornamental cultivars must display horticultural superiority when grown both in containers and in the ground. However, initial screening of individual plants can only be done in a single environment. It is important to determine which environment should be used to select superior plant material. A plant with suboptimal performance in containers may potentially be one of the highest performers in ground and vice versa. This is important as selection in containers may overlook an optimal plant in ground. An ideal plant would perform consistently well in both environments. Assessing G X E interactions allows the breeder to determine the nature of these interactions and can inform decisions on selection strategy. Evaluating rank of each entry will display those plants that are either highly ranked in both environments or only in one. This allows a breeder to more accurately select a plant based on this ranking. Calculation of trait correlations will provide information that can be used by the breeder to develop selection strategies for selection of multiple traits. Because environmental differences can alter trait correlations, their calculation in separate environments is useful. The objectives of the first portion of the study are to determine whether quantitative traits of breeding interest are expressed similarly in the two environments, to determine which environment is most appropriate for initial selection of characteristic expression, assess trait correlations to determine whether each trait is independent of other traits or if selection for trait will simultaneously select for another trait, and to combine this information to assess the optimal environment in which to do initial selection

The decision of the initial environment (containers or in ground) in which to select plants is based partially on an understanding of the G X E interaction for traits of interest and trait

correlations, but also on the cost differential of the two environments. The objectives of the second portion of the study were to elucidate costs of a breeding program for use in other studies, determine appropriate environment in which to initially select plants based on costs and resource use, and utilize this information with that of first study to determine optimal environment for selection of plants.

### **Literature cited**

- Ackerly, D.D., S.A. Dudley, S.E. Sultan, J. Schmitt, J.S. Coleman, C.R. Linder, D.R. Sandquist, G. M.A., A.S. Evans, T.E. Dawson, and M.J. Lechowicz. 2000. The Evolution of Plant Ecophysiological Traits: Recent Advances and Future Directions. *BioScience*. 50: 979-995.
- Acquaah, G. 2007. Principles of Plant Genetics and Breeding. 422-428.
- Adrian, J.L., Montgomery, C.C., Behe, B.K., Duffy, P.A., Tilt, K.M. 1998. Cost Comparisons for Infield, Above Ground Container and Pot-in-Pot Production Systems. *J. Environ. Hort.* 16: 65-68.
- Alam, M.I. and A. Gomes. 2003. Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts. *Journal of Ethnopharmacology*. 86: 75-80.
- Allen, G.E. 1979. Naturalists and experimentalists: the genotype and the phenotype. *Stud Hist Biol.* 3: 179-209.
- Antonovics, J. 1976. The nature of limits to natural selection. *Annals of the Missouri Botanical Garden*.
- Avramov, S., D. Pemac, and B. Tucic. 2007. Phenotypic plasticity in response to an irradiance gradient in *Iris pumila*: adaptive value and evolutionary constraints. *Plant Ecology*. 190: 275-290.

- Barisic, N., B. Stojkovic, and A. Tarasjev. 2006. Plastic responses to light intensity and planting density in three *Lamium* species. *Plant Systematics and Evolution*. 262: 25-36.
- Bolnick, D.I., R. Svanbaeck, J.A. Fordyce, L. Yang, J.M. Davis, C.D. Hulsey, and M.L. Forister. 2003. The Ecology of Individuals: Incidence and Implications of Individual Specialization. *American Naturalist*. 161: 1-28.
- Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.*: 115-155.
- Brennan, J.P. and P.J. Martin. 2006. Developing cost functions for a wheat breeding program. Contributed paper presented to the 50th Annual Conference of the Australian Agricultural and Resource Economics Society, Manly, Australia.
- Brennan, J.P. and P.J. Martin. 2007. Returns to investment in new breeding technologies. *Euphytica*. 157: 337-349.
- Brown, D. 1994. Herbal Research Review: *Vitex agnus-castus*, Clinical Monograph. *Q Rev Nat Med*. 2:111-121.
- But, P. and C. Chang. 1996. Chinese herbal medicine in the treatment of asthma and allergies. *Clinical Reviews in Allergy and Immunology*. 14: 253-269.
- Cantino, P.D. 1992. Evidence for a Polyphyletic Origin of the Labiatae. *Annals of the Missouri Botanical Garden*. 79: 361-379.
- Carroll, A.B., S.G. Pallardy, and C. Galen. 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany*. 88: 438-446.
- Caruso, C.M. 2006. Plasticity of inflorescence traits in *Lobelia siphilitica* (Lobeliaceae) in response to soil water availability. *American Journal of Botany*. 93: 531-538.

- Chahal, G.S. and S.S. Gosal. 2002. Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches. 153.
- Chevallier, A. 1996. The Encyclopedia of Medicinal Plants.
- Chun, Y.J., M.L. Collyer, K.A. Moloney, and J.D. Nason. 2007. Phenotypic plasticity of native vs. invasive purple loosestrife: A two-state multivariate approach. *Ecology*. 88: 1499-1512.
- Diggle, P.K. 1994. The expression of andromonoecy in *Solanum hirtum* (Solanaceae): phenotypic plasticity and ontogenetic contingency. *American Journal of Botany*. 81: 1354-1365.
- Dirr, M. 1998. Manual of Woody Landscape Plants. 1092, 1093.
- Dogan, Y. and H. Huseyin. 1998. An Autecological Study on the *Vitex agnus-castus* L. (Verbenaceae) Distributed in West Anatolia. *Turkish Journal of Botany*. 22: 327-334.
- Dorken, M.E. and S.C.H. Barrett. 2004. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology*. 92: 32-44.
- DuMee, C. 1993. Medicinal Plant Review: *Vitex agnus-castus*. *Australian Journal of Medicinal Herbalism*. 5: 63-65.
- Elle, E. and J.D. Hare. 2002. Environmentally induced variation in floral traits affects the mating system in *Datura wrightii*. *Functional Ecology*. 16: 79.
- Evans, A.S. 1998. How do tradeoffs in resource use efficiencies arise? *Ecological Society of America*, 83rd Annual Meeting, Abstracts: 9.
- Frazee, J.E. and R.J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae: Caesalpinoideae). *American Journal of Botany*. 81: 206.
- Frey, K.J. 1996. National Plant Breeding Study-I. Special Report 98. Iowa State University, IA.



- Fry, J.D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. *Evolution*. 46: 540-550.
- Galloway, L.F. 1995. Response to natural environmental heterogeneity: Maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. *Evolution*. 49: 1095-1107.
- Gilman, E.F. and D.G. Watson. 1994. *Vitex agnus-castus*: Chastetree. Forest Service: USDA.
- Herrera, C.M. 1991. Dissecting Factors Responsible For Individual Variation in Plant Fecundity. *Ecology*. 72: 1436-1448.
- Hobbs, C. 1991. The Chaste Tree: *Vitex agnus-castus*. *Pharmacy in History*. 33: 19-24.
- Hodges, A.W., L. Satterthwaite, and J.J. Haydu. 1995. Business Analysis of Ornamental Plant Nurseries in Florida.
- Hu, Y., T.T. Hou, H.L. Xin, Q.Y. Zhang, H.C. Zheng, and K. Rahman. 2007. Estrogen-like activity of volatile components from *Vitex rotundifolia* L. *Indian J Med Res*. 126: 68-72.
- Jellin, J. 2008. Natural Medicines Comprehensive Database. Therapeutic Research Facility.
- Judd, W.S., C.S. Campbell, E.A. Kellog, P.F. Stevens, and M.J. Donoghue. 2002. *Plant Systematics: A Phylogenetic Approach*. 466, 467, 468.
- Ko, W.G., T.H. Kang, S.G. Lee, Y.C. Kim, and B.H. Lee. 2001. Rotundifuran, a Labdane Type Diterpene from *Vitex rotundifolia*, Induces Apoptosis in Human Myeloid leukemia cells. *Phytother. Res*. 15: 535-537.
- Kobayashi, K., H. Atsushi, N. Satoshi, and O. Ryo. 2007. Diallel analysis of floral morphology in radish (*Raphanus sativus* L.). *Euphytica*. 158: 153-165.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*. 34: 292-305.

- Lande, R. 1982. A Quantitative Genetic Theory of Life History Evolution. *Ecology*. 63: 607-615.
- Lehmann, C. and F. Rebele. 2005. Phenotypic plasticity in *Calamagrostis epigejos* (Poaceae): response capacities of genotypes from different populations of contrasting habitats to a range of soil fertility. *Acta Oecologica-International Journal of Ecology*. 28: 127-140.
- Maloupa, E., D. Gerasopoulos, A. Marnasidis, and D. Zervaki. 1999. Paclobutrazol and Pinching Affects Visual Quality Characteristics of potted *Vitex agnus-castus* plants. IV International Symposium on New Floricultural Crops 541.
- Marshall, D.L., N.J. Abrahamson, J.J. Avritt, P.M. Hall, J.S. Medeiros, J. Reynolds, M.G.M. Shaner, H.L. Simpson, A.N. Trafton, A.P. Tyler, and S. Walsh. 2005. Differences in plastic responses to defoliation due to variation in the timing of treatments for two species of *Sesbania* (Fabaceae). *Annals of Botany*. 95: 1049-1058.
- Mayr, E. 1996. What Is a Species, and What Is Not? *Philosophy of Science*. 63: 262-277.
- Medek, D.E., M.C. Ball, and M. Schortemeyer. 2007. Relative contributions of leaf area ratio and net assimilation rate to change in growth rate depend on growth temperature: comparative analysis of subantarctic and alpine grasses. *New Phytologist*. 175: 290-300.
- Mehlhorn, H., G. Schmahl, and J. Schmidt. 2005. Extract of the seeds of the plant *Vitex agnus-castus* proven to be highly efficacious as a repellent against ticks, fleas, mosquitoes and biting flies. *Parasitology Research*. 95: 363-365.
- Misra, P.N. and S.C. Verma. 1989. Biomass and energy production in coppice stands of *Vitex negundo* L. in high density plantations on marginal lands. *Biomass*. 19: 189-194.
- Mitchell, R.J. and R.G. Shaw. 1993. Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): clones and crosses. *Heredity* 71: 185-192.

- Mony, C., G. Thiebaut, and S. Muller. 2007. Changes in morphological and physiological traits of the freshwater plant *Ranunculus peltatus* with the phosphorus bioavailability. *Plant Ecology*. 191: 109-118.
- Pachepsky, E., J.L. Bown, A. Eberst, U. Bausenwein, P. Millard, G.R. Squire, and J.W. Crawford. 2007. Consequences of intraspecific variation for the structure and function of ecological communities Part 2: Linking diversity and function. *Ecological Modelling*. 207: 277-285.
- Pigliucci, M. and C.D. Schlichting. 1995. Ontogenetic Reaction Norms in *Lobelia siphilitica* (Lobeliaceae): Response to Shading. *Ecology*. 76: 2134-2144.
- Pigliucci, M. and C.D. Schlichting. 1998. Reaction norms of Arabidopsis. V. Flowering time controls phenotypic architecture in response to nutrient stress. *Journal of Evolutionary Biology*. 11: 285-301.
- Pigliucci, M., C.D. Schlichting, and J. Whitton. 1995. Reaction Norms of Arabidopsis. II. Response to Stress and Unordered Environmental Variation. *Functional Ecology*. 9: 537-547.
- Royal Botanic Gardens, K. 2008. Major Groups, Families, and Genera.
- Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*. 17: 667-693.
- Schlichting, C.D. and M.S. Pigliucci. 1998. Phenotypic evolution: A reaction norm perspective. Sinauer Associates.
- Shaw, R.G. 1986. Response to Density in a Wild Population of the Perennial Herb *Salvia lyrata*: Variation Among Families. *Evolution*. 40: 492-505.
- Smith-Heurta, N.I. and F.C. Vasek. 1987. Effects of environmental stress on components of reproduction in *Clarkia unguiculata*. *American Journal of Botany*. 74: 1-8.

- Smith, H. and G.C. Whitelam. 1997. The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. *Plant, Cell & Environment*. 20: 840-844.
- Sultan, S.E. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*. 5: 537-542.
- Sultan, S.E. 2003. Phenotypic plasticity in plants: a case study in ecological development. *Evolution & Development*. 5: 25-33.
- Sultan, S.E. 2004. Promising directions in plant phenotypic plasticity. *Perspectives in Plant Ecology Evolution and Systematics*. 6: 227-233.
- Traxler, G., A.K.A. Acquaye, K. Frey, and A.M. Thro. 2005. Public Sector Plant Breeding Resources in the US: Study Results for the year 2001.
- Valladares, F., D. Sanchez-Gomez, and M.A. Zavala. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology*. 94: 1103-1116.
- Via, S. 1984. The Quantitative Genetic of Polyphagy in an Insect Herbivore .1. Genotype-Environment Interaction in Larval Performance on Different Host Plant Species. *Evolution*. 38: 881-895.
- Via, S., R. Gomulkiewicz, G. Dejong, S.M. Scheiner, C.D. Schlichting, and P.H. Vantienderen. 1995. Adaptive phenotypic plasticity - Consensus and controversy. *Trends in Ecology & Evolution*. 10: 212-217.
- Vogler, D.W., S. Peretz, and A.G. Stephenson. 1999. Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment, and ontogeny in *Campanula rapunculoides* (Campanulaceae). *American Journal of Botany*. 86: 482-494.

- Wagner, L.W., D.R. Herbst, and S.H. Sohmer. 1999. Manual of the Flowering Plants of Hawaii Vol. II. University of Hawaii Press, Honolulu, HI.
- Weinig, C. 2002. Phytochrome photoreceptors mediate plasticity to light quality in flowers of the Brassicaceae 1. American Journal of Botany. 89: 230-235.
- Westbrooks, R.G. and E.C. Brabson. 2007. Weed Risk Assessment for Beach *Vitex* in the United States. United States Geological Survey-Carolinas Beach *Vitex* Task Force.
- Westerman, J.M. and M.J. Lawrence. 1970. Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana*. I. Inbred lines; description. Heredity. 26: 373-382.
- Wolfe, L.M. and S.J. Mazer. 2005. Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus*: Brassicaceae). International Journal of Plant Sciences. 166: 631-640.
- Young, H.J., S. M.L., N.C. Ellstrand, and J.M. Clegg. 1994. Temporal and spatial variation in heritability and genetic correlations among floral traits in *Raphanus sativus*, wild radish. Heredity. 73: 298-308.

**CHAPTER 2**  
**GENETIC AND ENVIRONMENTAL VARIATION IN *VITEX***

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## Abstract

Horticulturally important traits were evaluated in ground and in containers for parents and segregating populations of *Vitex* to assist in determining the most appropriate environment for initial plant selection. These traits include a rating of *Cercospora* leaf spot resistance, height and width measured three times over the course of the study, and weekly evaluation for first flower date, last flower date, average inflorescence number, average inflorescence length, and rating for average number of flowers on the inflorescence. Flower duration and total weeks of flowering were calculated from the data. Overall plants grown in ground were taller, wider, had longer flower duration, more total weeks flowering, greater average inflorescence length, more inflorescences and more flowers per inflorescence when compared to the same plants grown in containers.

There was significant entry X treatment effects for *Cercospora* leaf spot, second and final height and width measurements, first flower date, average inflorescence number, and rating for average number of flowers on the inflorescence. No entry X treatment effects were present for first height and width measurements, last flower date, total weeks flower, and average inflorescence length. Most trait correlations were low to moderate in both environments. Those that exhibited high correlations were between first flower date and height measurements taken 33 weeks after planting in ground and in containers. Also, last flower date and total weeks flower in both environments, flower duration and total weeks of flower in containers and in ground, last flower date and average inflorescence number in ground and in containers, and total weeks flower and average inflorescence number in both environments. A breeder should be conscious of this as selection for one trait may also select for another.

High correlations with traits having entry X treatment interaction include second width measurement and final width measurement in both environments, final height measurements and first flower date was high in ground and moderate in containers, and average inflorescence number and total weeks flower was high in both environments. These interactions suggest that one environment should be utilized for initial selection. The traits with entry X treatment interactions also have high correlations with traits that do not. Some traits that exhibit G X E interactions have also been found to be correlated with traits of horticultural significance. Therefore a breeder must be aware that by selecting for traits without G X E interactions may also select for another trait with which it is highly correlated.

Those traits that exhibited entry X treatment interactions would suggest that by selecting those in containers would overlook optimal plants in ground and vice versa. Those plants that are among the top performers in ground may not be in containers, but could still perform better in containers than available cultivars in containers.

## **Introduction**

*Vitex* L. is a genus of about 250 species distributed throughout the world. *Vitex* has recently been moved from Verbenaceae to Lamiaceae (Judd et al., 2002). The use of *Vitex* species was well known during ancient times. Ancient Mediterranean countries used it for a variety of health problems. The use of *Vitex agnus-castus*, native to the Mediterranean (Maloupa et al., 1999; Mehlhorn et al., 2005), continued with medieval monks chewing the bark to maintain their celibacy. They also put the fruit in their robe pockets for this same purpose. For this reason, *V. agnus-castus* is synonymous with Monk's Pepper and Chaste Tree (DuMee, 1993). Its pharmacological use has steadily gained popularity in the United States since the mid-20<sup>th</sup> century (Hobbs, 1991). Introduced to the United States in 1570, *V. agnus-castus* is used



ornamentally as a shrub border or as a specimen planting (Gilman and Watson, 1994). *Vitex rotundifolia* L.f., native to East Asia, Australia, Pacific Islands, and Hawaii (Wagner et al., 1999), was originally introduced to the United State for dune stabilization and ornamental use from the J.C. Raulston Arboretum at North Carolina State University in the 1980s.

Phenotypic plasticity, or environmentally dependent phenotypic expression, has been of increasing interest since Bradshaw's 1965 classic review (Bradshaw, 1965). Environmental effects on plants were formerly thought of as useless "noise" blocking the "true expression of the phenotype" (Allen, 1979; Schlichting and Pigliucci, 1998). Developmental patterns, reproductive timing, and breeding systems are all affected by environmental effects (Sultan, 2000). Little research has been conducted on environmental variation in *Vitex*. One study observed the variation in plant nutrients in different soil types with varying nutrient levels. The major finding was that an increase in the  $\text{CaCO}_3$  content in the soil resulted in a decrease in the growth of *V. agnus-castus* (Dogan and Huseyin, 1998). Environmentally plastic vegetative traits have been extensively researched in other ornamentals. Higher density resulted in smaller plants and leaves in three *Lamium* species (Barisic et al., 2006) and *Salvia lyrata* L. (Shaw, 1986). It has been found that inflorescence traits are, in general, less plastic than vegetative traits (Bradshaw, 1965; Frazee and Marquis, 1994). Traits expressed in individual flowers, such as stigma length or petal size, have been found to be less plastic than plant-level traits such as flower number and date of first flower (Dorken and Barrett, 2004; Wolfe and Mazer, 2005). It has been suggested that earlier flowering is a response to stress in plants (Westerman and Lawrence, 1970). Water stress can decrease flower production in *Clarkia unguiculata* Lindl. (Smith-Heurta and Vasek, 1987), *Lavandula stoechas* L. (Herrera, 1991), and species of *Phlox* (Schlichting, 1986). The length of the inflorescence decreased under drought conditions in *Lobelia siphilitica* L. (Caruso, 2006).

Genotypic variation in a population's phenotypic response across environments is referred to as G X E interaction (Fry, 1992). G X E interactions are therefore characteristic of populations of differing genotypes (Via et al., 1995). Although many studies assume that intraspecific variation is either infrequent or insignificant (Bolnick et al., 2003), variation among populations is evident in all natural systems (Pachepsky et al., 2007). It has been shown that closely related species can substantially differ in the amount, direction, and timing of plastic responses. (Schlichting, 1986). The ability to alter reproduction time in response to stress can differ among related species in *Polygonum* (Sultan, 2003). In *Lobelia siphilitica* L. flowering time in response to drought varied by population (Caruso, 2006). Components of reproduction in *Sesbania* may vary within species in response to defoliation (Marshall et al., 2005). Studies also use rank to determine the presence of G X E interactions. *Liriomyza sativae* Blanchard is a dipteran pest of vegetables. Larval size of 11 populations of *L. sativae* were ranked on two plant hosts, tomato and pea and no correlation was found in rank between the two plant hosts (Via, 1984).

Breeders generally attempt to improve a number of traits simultaneously. If these traits are positively correlated, the response to selection will be more rapid than for characteristics selected separately. Conversely, attempting to select for traits with negative genetic correlations can slow their rate of simultaneous improvement (Antonovics, 1976; Lande, 1982). Trait incorporation may even be delayed for many generations if several important traits are negatively correlated (Lande, 1980). In *Raphanus sativus* L. low correlations between 13 floral traits suggest that most are independent, indicating the potential of developing lines with new floral morphologies by selecting for these genetically independent traits (Kobayashi et al., 2007). This was not the case in *Penstemon* where some floral traits were highly correlated and therefore

not independent from each other. Examples include high positive correlation between gynoecium length and corolla width along with nectar volume and nectar sugar, high negative correlation between nectar volume and flowering date as well as corolla length and stigma exertion (Mitchell and Shaw, 1993) In some cases environmental change can alter genetic correlation values as was the case with *Townsendia annua* Beaman. At higher nitrogen availability there were positive genetic correlations between water use efficiency and nitrogen use efficiency but at lower nitrogen availability there was a negative correlation between these traits (Evans, 1998) as cited in (Ackerly et al., 2000). On the other hand, a study did not find any significant differences in the genetic correlations among floral traits of *Raphanus sativus* when the environment was altered (Young et al., 1994).

Limited research has been conducted on the most effective environment for initial plant selection in ornamental plant breeding programs. Our objectives were to evaluate parents and segregating populations of *Vitex* in both containers and in ground i) to determine whether quantitative traits of breeding interest were expressed similarly in the two environments, ii) to determine trait correlations in each environment, and iii) to combine this information to determine the most appropriate environment in which to do initial selection.

## **Materials and Methods**

As part of the *Vitex* breeding program at the University of Georgia, seven crosses were made in the summer of 2005. Single parent plants were placed in bee cages and honeybee (*Apis mellifera*) hives were introduced to enhance pollination. No attempt was made to force cross-pollination, and seeds collected from each female parent likely originated from both self- and cross-pollination. Progeny with desirable horticultural attributes were selected for further evaluation (Table 2.1). Each of the parents and all segregating offspring were clonally

propagated 16 Aug. 2006 from stem cuttings using a 1% K-IBA solution and placed under mist in 16-cell packs containing 3:1 Fafard 3B and perlite for eight weeks. All plants were potted in trade 1 containers holding 0.00382 m<sup>3</sup> of potting material with Fafard 3B on 14 Nov 2006. All plants received similar watering and fertilizing treatments using a 100 ppm solution of Peter's 20N-10P-20K at each watering. On 23 Apr. 2007, all plants were transplanted at the University of Georgia Horticulture Farm in Watkinsville, GA at latitude 33° 53' 17.6028" and longitude - 83° 24' 59.0436" in soil that ranged from a sandy loam to a sandy clay loam. Plants in ground were placed 1.5 m apart in 122.92 x 1.22 m beds with drip irrigation. Plants in containers were placed in trade 3 containers with a holding capacity of 0.011 m<sup>3</sup> with Fafard 3B on an overhead irrigated growing pad with edge of containers spaced 30 cm apart. Both treatments contained two replicates of the segregating species and eight replicates of each parent arranged in a randomized complete block design. Both treatments received similar fertilizer regimes using Osmocote® Classic 14N-14P-14K on 4 June 2007. The ground treatment was given 15.12 kg per 121.92 x 1.22 m bed and the container treatment received 44 g of fertilizer per container. Ten cm-deep hardwood mulch was applied to the ground treatment two weeks after planting. Plants were watered as needed using an overhead watering system for the containers and a drip system for the in ground material. Data collection was taken throughout the growing season, evaluating characteristics used in the selection of potential *Vitex* cultivars. Height and width were measured three times over the course of the study. First height and width measurements were taken 16 May 2007. Subsequent measurements were taken 23 Sept 2007 and 8 Dec 2007, with the last date following first frost and subsequent cessation of plant growth.

Table 2.1 *Vitex* crosses and number of progeny used in this study

<b>Cross number</b>	<b>Parents</b>	<b>N</b>
<b>V0502</b>	<i>Vitex agnus-castus</i> ‘Shoal Creek’ x <i>V. rotundifolia</i>	19
<b>V0504A</b>	<i>V. agnus-castus</i> ‘Silver Spires’ x <i>V. agnus-castus</i> ‘Shoal Creek’	1
<b>V0504B</b>	<i>V. agnus-castus</i> ‘Shoal Creek’ x <i>V. agnus-castus</i> ‘Silver Spires’	32
<b>V0506A</b>	<i>V. agnus-castus</i> ‘Shoal Creek’ x <i>V. agnus-castus</i> ‘Blushing Spires’	52
<b>V0506B</b>	<i>V. agnus-castus</i> ‘Blushing Spires’ x <i>V. agnus-castus</i> ‘Shoal Creek’	11
<b>V0509A</b>	<i>V. agnus-castus</i> ‘Abbeville Blue’ x <i>V. agnus-castus</i> ‘Silver Spires’	16
<b>V0509B</b>	<i>V. agnus-castus</i> ‘Silver Spires’ x <i>V. agnus-castus</i> ‘Abbeville Blue’	11

*A and B denote reciprocal crosses with the female parent being listed first*

Reproductive traits, which were measured weekly included first flower date, flowering duration, average inflorescence number and length, total recorded weeks of flowering, and average quantity of flowers on each inflorescence. Average quantity of flowers on each inflorescence was rated on a scale of one to five. A value of one denoted that flowers covered up to 20%, two with 20-40%, three with 40-60%, four with 60-80%, and five with 80-100% of the

inflorescences. Flowering data was taken weekly beginning 15 May 2007 and ending 8 Nov 2007, the date of the first frost. First date of flower was defined as first appearance of petal color, and last date of flower was defined as the date when no petal color was detected. Flower duration, and total weeks of bloom were calculated from these data. The latter two numbers differed on occasion when plants stopped flowering and then began re-flowering. Flower rating for average number of flowers on the inflorescence, number of inflorescences, and inflorescence length were averaged over all weeks. Presence of *Cercospora* leaf spot was measured on a scale of zero to five. Zero was defined as no leaf spot present, one as presence of the precursor for the disease with white areas on the leaf surface, two with 20-40% of the leaf area covered with leaf spot, three with 40-60% leaf area covered with leaf spot, four with 60-80% leaf area covered with leaf spot, five with 80-100% leaf area covered with leaf spot.

### **Data analysis**

Data was analyzed with a two-way ANOVA using the SAS General Linear Model procedure (SAS Institute, 2003). The following model was used for each of the characters investigated: treatment effect (ground vs. container), entry effect (differences among each clone), cross number (differences among crosses), and parent effect (differences among each parent), entry X treatment interaction, cross number X treatment interaction, and parent number\*treatment interaction. Entries were defined as all genetically distinct plants in the study, whether parent or offspring. Cross numbers were separated by each distinct cross. Analysis using cross number did not include cross V0504A as there was only one representative. Parent number included only parents and separated them by cultivar or species as was the case with *V. rotundifolia*. Differences among parents and crosses were separated using the Tukey mean separation test. Ranking from highest to lowest value for each trait observed was made for

treatments, entries, crosses, and parent. Pearson correlation coefficients between traits were estimated for all populations. Traits that exhibited G X E interaction were further evaluated to assess whether these traits also had high correlations with those that did not have G X E interaction.

## **Results and Discussion**

### **Treatment effects**

Of the 14 traits measured, all but two were expressed differently in containers and in ground (Table 2.2). There was no significant treatment effect for *Cercospora* leaf spot rating or width measurements taken three weeks after planting. Overall plants were taller and wider in ground than in containers except width measurements taken three weeks after planting. This is expected since *Vitex* is fast growing and plants in ground have a much larger area for root growth in an environment cooler than black plastic containers and thus are able to devote more of their resources to vegetative growth.

All reproductive traits demonstrated significant treatment effects. Plants in containers flowered earlier than in ground (Table 2.2). This is expected as plants may flower earlier in response to stress (Galloway, 1995). The ground treatment had a later last flower date than the container treatment. Flower duration and total weeks flower were longer in ground than in containers. Average inflorescence number, average inflorescence length, and rating for average number of flowers on the inflorescence were all significantly smaller in-container than in ground (Table 2.2). These results are similar to those found by (Dorken and Barrett, 2004) who found that nutrient levels affected days to flower in some populations of *Sagittaria latifolia* and work by (Wolfe and Mazer, 2005) that showed that an increase in density from low to medium could shorten the days to flower in *Raphanus sativus*. The smaller root volume occupied by container-

grown plants compared to field-grown may have resulted in plant stress, including water stress. Previous research has shown that earlier flowering (Westerman and Lawrence, 1970), a decrease flower production (Herrera, 1991; Schlichting, 1986; Shaw, 1986; Smith-Heurta and Vasek, 1987), and decrease in the length of the inflorescence (Caruso, 2006) are all indicators of water stress in plants.

### **Significant treatment interactions**

*Cercospora* leaf spot rating exhibited significant treatment interactions with entry, cross, and parent (Table 2.2). This indicates that those entries, crosses, or parents with the best leaf spot resistance in one environment may not be the best in the other. Parents used in this study had similar leaf spot ratings, with the exception of ‘Silver Spires’ that was more susceptible than other parents in containers (Table 2.3B). This may be due to the increased moisture levels in containers with overhead watering and closer proximity to other plants that increased the level of *Cercospora* leafspot inoculum to a level that allowed growth on this cultivar.

On average, progeny from the V0504B cross were more resistant than progeny from other crosses when measured in ground. In containers, progeny from V0509B had a higher rating than other crosses (Table 2.4B). This finding is not unexpected as the more susceptible ‘Silver Spires’ is the female parent in V0509B. The differential expression of leaf spot resistance in the two environments suggests that selection may need to be made both in ground and in containers.

Of the three height measurements, entries responded differently in the treatments at 19 and 33 weeks after planting. Significant cross number X treatment interactions were only present for height taken at 33 weeks after planting (Table 2.2). Choosing either tall or short plants on an individual or cross basis in one environment would not necessarily identify similar



types of plants in the second environment. Observing rankings of entry and cross number over both environments would be necessary in determining selection of plant material in a breeding program. There was no significant parent number X treatment interactions for height measurements taken at any of the three dates. Therefore selection of parents could have been done in either environment.

In ground, progeny from cross V0502 were, on average, shorter than progeny from other crosses, although they were not significantly different from progeny from cross V0504B. Average performance of progeny from other crosses was not different. In containers, the progeny from the cross V0502 were the shortest although the value was not different from that of V0509B. Therefore, V0502 was the shortest in both environments and selection for smaller plants could be done in either environment (Table 2.4A).

Entries were ranked by height in each environment. Values that tied were considered the same rank. Entry V0502-4 was the shortest in both environments and six other entries, V0506A-60, V0502-19, V0506A-8, V0502-31, V0506A-72, and V0506A-67 were all among the 10 shortest entries in both environments. As in the case of identifying crosses with the shortest average progeny, individual plant selection in either environment would identify the same small plants (Table 2.4A).

Only three entries, V0502-17, V0502-14, and V0506A-19 were common to the tallest 10 plants in both environments (Table 2.5A), indicating that it would be more difficult to use a single environment to identify tall plants. In the University of Georgia breeding program, primary emphasis is placed on selection of compact rather than tall plants.

Of the three width measurements, entry X treatment interactions were significant at 19 and 33 weeks after planting and not at three weeks after planting. Significant cross number X

treatment interaction was present only in width measurements taken at 33 weeks after planting. Significant parent number X treatment effects existed only for measurements taken 33 weeks after planting (Table 2.2). Therefore selection of entry, cross number, or parent number for plant width at the end of the growing season would need to be made in both environments. In ground and in containers the widest parent was *V. rotundifolia*. This is explained by the prostrate growth habit of *V. rotundifolia* unlike the other parents (Table 2.3A).

In ground, there were no differences in width among the four parents when removing *V. rotundifolia* from analysis. In containers, the widest plants were ‘Shoal Creek’, ‘Abbeville Blue’, and ‘Blushing Spires’. ‘Abbeville Blue’ and ‘Blushing Spires’ were not different from ‘Silver Spires’. Therefore selection of ‘Silver Spires’ for narrow growth form, although not different from ‘Abbeville Blue’ and ‘Blushing Spires’, may have occurred in containers as this cultivar is not different from any parent in ground.

In ground, the narrowest plants came from progeny of cross V0502. In containers there was no difference in width among crosses although progeny from cross V0502 ranked as the narrowest (Table 2.4A). Therefore progeny from V0502 are consistently narrow in either environment. This consistent performance in both environments was also evident when evaluating individual plants. Eight of the 12 entries ranked as the most narrow growth habit in ground were also the narrowest in containers (Table 2.5A). Entries that were consistently ranked as widest in both environments were *V. rotundifolia*, V0502-7, V0506A-37, and V0509A-5. (Table 2.5 A) Therefore, selection could be done in either environment for the above entries. Width (either widest or most narrow) could be identified in either environment.

Treatment interactions for first flower date were significant only for entries. This has been previously shown in *Polygonum* where reproduction time differed among related species in

response to stress (Sultan, 2003). There was so little variability in the expression of this trait among entries in this study that selection for earlier flowering is unlikely to be successful.

Last flower date had no significant interactions in any category. (Table 2.2) While it was anticipated that plants would stop flowering later in the season, in this experiment most plants were still flowering when the first frost occurred on 6 Nov. 2007. This prevented meaningful separation of plants based on this trait. Flowering was measured if there was any color present on the plant. While most plants are almost done flowering, there are still occasional inflorescences that are differentiated throughout the remainder of the season.

Parent number and treatment have significant interaction for flower duration. Selection could therefore be done in either environment when observing at the entry and cross number (Table 2.2). This is in contrast to a study done that determined flowering time in response to drought varied by population with *Lobelia siphilitica*

(Caruso, 2006). *V. rotundifolia* had the shortest flower duration in both environments, with few differences among the *V. agnus-castus* parents. While the total number of weeks flowering was lower in containers than in- ground, there were no treatment interactions with entries or cross. There was a significant interaction between treatment and parent number for total weeks flowering. Therefore selection for either crosses or individual entries with long flowering duration could be done in either environment (Table 2.2).

Entry number, cross number, and parent number all exhibited significant interactions with treatment for average inflorescence number, indicating that selection for this trait may need to be done in each environment depending on rankings. In both environments, ‘Blushing Spires’ had more inflorescences than the other parents, although in containers *V. rotundifolia* was not significantly different (Table 2.3A).

In ground, the crosses with the highest average inflorescence numbers were V0506B, V0504B, V0509A, V0509B, and V0506A. In containers, the cross with the highest value for average inflorescence number was V0509B. This value was significantly different from all other crosses. Thus while selection for crosses with large average numbers of inflorescences in containers would identify the best performing cross in ground, the reverse is not necessarily true. Progeny from cross V0502 had the fewest inflorescences in both environments and would therefore have been identified in either environment. Crosses V0504B and V0506A, with progeny averaging large numbers of inflorescences in ground, did not have high values for this trait in containers (Table 2.4A). For these crosses selection environment would have a confounding effect on selection efficiency. There were many entries with identical average inflorescence number, making separation of entries difficult.

Average inflorescence length had no significant interactions. Selection for long inflorescences in either environment would identify the same genotypes.

Rating for average number of flowers on the inflorescence had significant treatment interaction by entry number and cross number. No significant treatment interaction occurred with parent number (Table 2.2). It would then be necessary to rank by entry and cross to determine appropriate plant material for this trait. Parents could be selected in either environment.

In ground, progeny from crosses V0504B, V0506B, and V0509A had the most flowers on the inflorescence. V0506B and V0509A were not significantly different from V0506A and V0509B. Progeny from the cross V0502 had the lowest average rating for number of flowers on the inflorescence. This value was not significantly different from V0506A and V0509B. In containers, the cross with the highest value for average rating for number of flowers on the inflorescence was V0509A. This value was significantly different from that of all other crosses.

The cross with the lowest value was V0502. This value was significantly different from that of all other crosses. (Table 2.4B) Selection for large numbers of flowers on the inflorescence could eliminate cross V0502 based on performance in either environment. Progeny from cross V0509A had among the highest average flower number in either environment. Average number of flowers was not ranked by entry as this discrete variability resulted in many entries with the same value.

### **Trait correlations**

Trait correlations were calculated to inform decisions regarding simultaneous selection of multiple traits. Because of the large number of distinct plants in this study, correlations between traits were often statistically significant, yet the correlation coefficient was sufficiently low that the traits could be selected independently. Values for trait correlations were designated as low ( $r \leq 0.49$ ), moderate ( $0.50 \leq r \leq 0.69$ ), and high ( $r \geq 0.70$ ). Many trait correlations were low. As expected the last two width measurements were correlated with each other. The high correlation between the last two measurements suggests that selection for wide or narrow plants could be done at either 19 or 33 weeks after planting (Table 2.6A) Correlation between height measurements taken 33 weeks after planting and first flower date was high in ground ( $r=0.88$ ) and moderate in containers ( $r=0.63$ ) (Table 2.6B). Width measurements taken 33 weeks after planting and first flower date for plants was moderate in the ground ( $r=0.51$ ), but only weakly correlated in containers ( $r=0.21$ ). Width measurements taken 33 weeks after planting and average rating for number of flowers on the inflorescence were moderate both in- ground ( $r=0.55$ ) and in containers ( $r=0.49$ ) indicate that the breeder should be conscious of selecting narrow plants with fewer flowers (Table 2.6 B).

First flower date and flower duration were expected to be correlated since plants that flowered for a long time would, of necessity, need to flower relatively early. In this study the

correlation between the two traits had low  $r$  values in both environments suggesting that this is not the case in this study. Correlation between last flower date and total weeks flower was high in both environments, as is expected since last flower date is a component of the total weeks flowering. While first flower date is also a component there was little variability with this trait and this could explain the lack of correlation between this and total weeks flower.

The correlation between last flower date and average inflorescence number was high and negative in ground, but moderate and negative in containers. The selection of high average inflorescence number would tend to also select plants with an earlier last flower date, possibly due to the overall expenditure of the plant to produce many flowers. (Table 2.6 B). Correlation between flower duration and average inflorescence number in both environments were moderate, with  $r=0.63$  in ground and  $r=0.67$  in containers. Moderate correlation was present with flower duration and total weeks flower in ground and was high with the same trait in containers (Table 2.6B). Total weeks flowering was highly correlated in both environments with average inflorescence number ( $r=0.92$  in- ground and  $r=0.88$  in containers). This may exist due to increase average inflorescence number and total weeks flowering would make the plant more reproductively successful. These plants would therefore be more likely to have high reproduction rates as this is a mechanism used by plants for survival of species. A moderate correlation existed between average inflorescence number and average inflorescence length in ground ( $r=0.59$ ), but not in containers ( $r=0.34$ ) (Table 2.6 B).

Correlation values frequently differed in the two environments in this study. Most differing correlations were higher in ground than in container and those values were still low enough to exhibit independence of other traits. The higher correlations present in ground may be due to the fact that this environment is such that higher correlations exist. Plants may allocate

their resources differently in this environment as availability of nutrients, space, and water are different from that of the container treatment.

This is consistent with work done by (Evans, 1998) as cited in (Ackerly et al., 2000) in which environmental change altered genetic correlation values in *Townsendia annua* Beaman. At higher nitrogen availability there were positive genetic correlations between water use efficiency and nitrogen use efficiency but at lower nitrogen availability there was a negative correlation between these traits.

### **Combining trait correlations with traits with entry X treatment interactions**

Significant entry X treatment effects existed for *Cercospora* leaf spot, second and third height and width measurements, first flower date, and average inflorescence number. It is necessary to view trait correlations with these traits to assess appropriate breeding protocol. High and moderate correlations with traits having entry X treatment interaction included high correlation between width measurements taken 19 weeks after planting and width measurements taken 33 weeks after planting in both environments. Also, moderate correlation between width measurements taken 33 weeks after planting and average number of flowers on the inflorescence was moderate in both environments. Correlation between width measurements taken 33 weeks after planting and first flower date was moderate in ground and low in containers. Height measurements taken 33 weeks after planting and first flower date had correlation that was high in ground and moderate in containers. Average inflorescence number and flower duration exhibited moderate correlation in both environments. Average inflorescence number and total weeks flower had high correlation in both environments. Correlation between average inflorescence number and inflorescence length was moderate in ground and low in containers. For those traits have low correlation with the others in this study, selection of a trait could be made

independently of other traits. This would suggest that, for example, selecting for height would not select for most traits. Those high correlations such as between total weeks flower and average inflorescence number would ensure that selection for one would be beneficial in a breeding program as these are both desirable traits. Selection for these traits would then simultaneously select for the other trait. These interactions suggest that one environment should be utilized for initial selection. The traits with entry X treatment interactions also have high correlations with traits that do not. Therefore traits of horticultural significance would then also be affected by environment. Growing in ground for initial selection would be beneficial for an efficient breeding program. This is due to the eventuality that these plants will most likely end up in ground. Those traits that exhibited entry X treatment interactions would suggest that by selecting those in containers would overlook optimal plants in ground. Those traits that exhibited entry X treatment interactions would suggest that by selecting those in containers would overlook optimal plants in ground and vice versa. Those plants that are among the top performers in ground may not be in containers, but could still perform better in containers than available cultivars. Selection should occur in ground with selected plants being further evaluated in containers.

Quantitative traits of breeding interest were not expressed similarly in both environments. Trait correlations were assessed in each environment. Some of those correlations varied by environment, but for the most part those with differing correlations were still so low as to have independence from other traits. This information was combined to determine that initial selection should occur in ground due to differential expression of some traits depending on the genotype and environment and because some trait correlations were higher in ground than in containers.



## Literature cited

- Ackerly, D.D., S.A. Dudley, S.E. Sultan, J. Schmitt, J.S. Coleman, C.R. Linder, D.R. Sandquist, G. M.A., A.S. Evans, T.E. Dawson, and M.J. Lechowicz. 2000. The Evolution of Plant Ecophysiological Traits: Recent Advances and Future Directions. *BioScience*. 50: 979-995.
- Allen, G.E. 1979. Naturalists and experimentalists: the genotype and the phenotype. *Stud Hist Biol*. 3: 179-209.
- Antonovics, J. 1976. The nature of limits to natural selection. *Annals of the Missouri Botanical Garden*. Vol 63. 2:224-247
- Barisic, N., B. Stojkovic, and A. Tarasjev. 2006. Plastic responses to light intensity and planting density in three *Lamium* species. *Plant Systematics and Evolution*. 262: 25-36.
- Bolnick, D.I., R. Svanbaeck, J.A. Fordyce, L. Yang, J.M. Davis, C.D. Hulsey, and M.L. Forister. 2003. The Ecology of Individuals: Incidence and Implications of Individual Specialization. *American Naturalist*. 161: 1-28.
- Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.*: 115-155.
- Caruso, C.M. 2006. Plasticity of inflorescence traits in *Lobelia siphilitica* (Lobeliaceae) in response to soil water availability. *American Journal of Botany*. 93: 531-538.
- Dogan, Y. and H. Huseyin. 1998. An Autecological Study on the *Vitex agnus-castus* L. (Verbenaceae) Distributed in West Anatolia. *Turkish Journal of Botany*. 22: 327-334.
- Dorken, M.E. and S.C.H. Barrett. 2004. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology*. 92: 32-44.

- DuMee, C. 1993. Medicinal Plant Review: *Vitex agnus-castus*. Australian Journal of Medicinal Herbalism. 5: 63-65.
- Frazee, J.E. and R.J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae: Caesalpinoideae). American Journal of Botany. 81: 206.
- Fry, J.D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. Evolution. 46: 540-550.
- Gilman, E.F. and D.G. Watson. 1994. *Vitex agnus-castus*: Chastetree. Forest Service: USDA.
- Herrera, C.M. 1991. Dissecting Factors Responsible For Individual Variation in Plant Fecundity. Ecology. 72: 1436-1448.
- Hobbs, C. 1991. The Chaste Tree: *Vitex agnus-castus*. Pharmacy in History. 33: 19-24.
- Judd, W.S., C.S. Campbell, E.A. Kellog, P.F. Stevens, and M.J. Donoghue. 2002. Plant Systematics: A Phylogenetic Approach. 466-468.
- Kobayashi, K., H. Atsushi, N. Satoshi, and O. Ryo. 2007. Diallel analysis of floral morphology in radish (*Raphanus sativus* L.). Euphytica. 158: 153-165.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution. 34: 292-305.
- Lande, R. 1982. A Quantitative Genetic Theory of Life History Evolution. Ecology. 63: 607-615.
- Maloupa, E., D. Gerasopoulos, A. Marnasidis, and D. Zervaki. 1999. Paclobutrazol and Pinching Affects Visual Quality Characteristics of potted *Vitex agnus-castus* plants. IV International Symposium on New Floricultural Crops 541.
- Marshall, D.L., N.J. Abrahamson, J.J. Avritt, P.M. Hall, J.S. Medeiros, J. Reynolds, M.G.M. Shaner, H.L. Simpson, A.N. Trafton, A.P. Tyler, and S. Walsh. 2005. Differences in plastic

responses to defoliation due to variation in the timing of treatments for two species of *Sesbania* (Fabaceae). *Annals of Botany*. 95: 1049-1058.

Mehlhorn, H., G. Schmahl, and J. Schmidt. 2005. Extract of the seeds of the plant *Vitex agnus-castus* proven to be highly efficacious as a repellent against ticks, fleas, mosquitoes and biting flies. *Parasitology Research*. 95: 363-365.

Mitchell, R.J. and R.G. Shaw. 1993. Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): clones and crosses. *Heredity* 71: 185-192.

Pachepsky, E., J.L. Bown, A. Eberst, U. Bausenwein, P. Millard, G.R. Squire, and J.W. Crawford. 2007. Consequences of intraspecific variation for the structure and function of ecological communities Part 2: Linking diversity and function. *Ecological Modelling*. 207: 277-285.

Schlichting, C.D. and M.S. Pigliucci. 1998. Phenotypic evolution: A reaction norm perspective. Sinauer Associates.

Shaw, R.G. 1986. Response to Density in a Wild Population of the Perennial Herb *Salvia lyrata*: Variation Among Families. *Evolution*. 40: 492-505.

Smith-Heurta, N.I. and F.C. Vasek. 1987. Effects of environmental stress on components of reproduction in *Clarkia unguiculata*. *American Journal of Botany*. 74: 1-8.

Sultan, S.E. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*. 5: 537-542.

Sultan, S.E. 2003. Phenotypic plasticity in plants: a case study in ecological development. *Evolution & Development*. 5: 25-33.

- Via, S. 1984. The Quantitative Genetic of Polyphagy in an Insect Herbivore .1. Genotype-Environment Interaction in Larval Performance on Different Host Plant Species. *Evolution*. 38: 881-895.
- Via, S., R. Gomulkiewicz, G. Dejong, S.M. Scheiner, C.D. Schlichting, and P.H. Vantienderen. 1995. Adaptive phenotypic plasticity - Consensus and controversy. *Trends in Ecology & Evolution*. 10: 212-217.
- Wagner, L.W., D.R. Herbst, and S.H. Sohmer. 1999. Manual of the Flowering Plants of Hawaii Vol. II. University of Hawaii Press, Honolulu, HI.
- Westerman, J.M. and M.J. Lawrence. 1970. Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana*. I. Inbred lines; description. *Heredity*. 26: 373-382.
- Wolfe, L.M. and S.J. Mazer. 2005. Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus*: Brassicaceae). *International Journal of Plant Sciences*. 166: 631-640.
- Young, H.J., S. M.L., N.C. Ellstrand, and J.M. Clegg. 1994. Temporal and spatial variation in heritability and genetic correlations among floral traits in *Raphanus sativus*, wild radish. *Heredity*. 73: 298-308.

Table 2.2 Effects of environment on expression of various traits in *Vitex* with statistical significance of interactions with genotypes

	<b>Cerc. Rating</b>	<b>H 1 (cm)</b>	<b>W 1 (cm)</b>	<b>H 2 (cm)</b>	<b>W 2 (cm)</b>	<b>H 3 (cm)</b>	<b>W 3 (cm)</b>	<b>First Flw Date</b>	<b>Last Flw Date</b>	<b>Flw Dur</b>	<b>Total Wks Flw</b>	<b>Avg inf. Num</b>	<b>Avg. inf. lgth</b>	<b>Avg Flw Rating</b>
G	0.49a	32 a	33 a	100 a	117.5 a	112 a	121 a	9 July a	23 Oct.a	16 a	15 a	12 a	17.5a	2.6 a
C	0.45a	30 b	34 a	95 b	81 b	100 b	72 b	26 June b	1 Oct.b	14 b	13 b	6 b	15.5 b	2.4 b
Level of treat sig	NS	*	NS	**	***	***	***	***	***	***	***	***	***	*
E X T	**	NS	NS	*	**	*	***	*	NS	NS	NS	*	NS	*
Cross X T	***	NS	NS	NS	NS	*	**	NS	NS	NS	NS	**	NS	**
P X T	***	NS	NS	NS	NS	NS	*	NS	NS	*	NS	***	NS	NS

Means in columns followed by different letters are significantly different based on Tukey mean separation test at  $P \leq 0.05$ .

Interactions are NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively. G and C refer to ground and container, respectively, *Cerc.* = *Cercospora*; H=Height; W=Width; Flw=Flower; Wks=Weeks; Inf=Inflorescence; Num=number; Lgth=Length, Dur=Duration, H1, W1, H2, W2, H3, W3 refer to measurements taken three, 19, and 33 weeks after planting, respectively, E X T=entry X treatment, P X T=Parent X treatment

Table 2.3A Expression of various vegetative and floral traits in *Vitex* parental lines in container and ground.

	Ground		Container		Ground		Container	
	Final height	SD	Final height	SD	Final width	SD	Final width	SD
'Abbeville Blue'	126.5 a	5.66	122 a	5.48	144.5 b	18.03	71b	3.36
'Blushing Spires'	137 a	9.19	112 a	9.55	143 b	8.13	85b	7.07
'Shoal Creek'	128 a	3.71	111 a	9.55	150 b	9.72	75b	6.54
'Silver Spires'	113.5 b	24.93	96 a	9.55	124 b	17.32	84b	6.72
<i>V. rotundifolia</i>	64 b	22.63	44.5 b	9.55	349 a	74.07	173.5a	10.61
	Ground		Container		Ground		Container	
	First flw. Date	SD.	First flw. date	SD.	Last flw date	SD	Last flw date	SD.
'Abbeville Blue'	12 June b	0.88	12 June b	0.18	16 Oct a	0.71	25 Sept bc	1.41
'Blushing Spires'	12 June b	0.18	12 June b	0.18	30 Oct a	0.18	16 Oct ab	1.24
'Shoal Creek'	19 June b	1.41	12 June b	0.53	30 Oct a	0.71	18 Sept c	3.01
'Silver Spires'	26 June b	1.77	12 June b	0	23 Oct a	1.06	23 Oct a	0.53
<i>V. rotundifolia</i>	11 Sept. a	0.71	7 Aug a	1.83	30 Oct a	0	16 Oct abc	0.71
	Ground		Container		Ground		Container	
	Total weeks flower	SD	Total weeks flower	SD	Avg. inf. Num	SD	Avg. inf. Num	SD
'Abbeville Blue'	17 a	0.53	13 ab	1.06	8 b	0.27	4 d	0.098
'Blushing Spires'	21a	0	18 a	2.47	21 a	0.46	10 a	0.97
'Shoal Creek'	17 a	1.94	12 ab	2.47	8.5 b	2.55	4 cd	0.3
'Silver Spires'	18 a	0	17 a	1.24	11.5 b	1.11	7 bc	0.52
<i>V. rotundifolia</i>	9 b	0.71	9 b	2.89	10 b	1.92	9 ab	0.84

Values among parents for individual traits followed by different letters are different at  $P \leq 0.05$  based on Tukey mean separation. Inf=Inflorescence, Num=Number, Flw=Flower

Table 2.3B Expression of various vegetative and floral traits in *Vitex* parental lines in container and ground.

	Ground		Container		Ground		Container	
	Avg.flw. rating	SD	Avg.flw. rating	SD.	Avg.inf length	SD.	Avg. inf. length	SD
'Abbeville Blue'	2.8 a	0.16	2.47 a	0.16	22 a	2.98	18 a	0.79
'Blushing Spires'	2.7 a	0.12	2.38 a	0.036	19 ab	1.43	15 a	0.67
'Shoal Creek'	2.64 ab	0.078	2.59 a	0.18	22 a	1.12	18.5 a	1.9
'Silver Spires'	2.65 ab	0.045	2.49 a	0.11	21 a	3.03	18 a	0.87
V. <i>rotundifolia</i>	2.14 b	0.13	2.23 a	0.025	10 b	1.57	7 b	0.29
•	•	•	•	•	•	•	•	•
	Ground		Container		Ground		Container	
	<i>Cercospora</i> leaf Spot	SD	<i>Cercospora</i> leaf spot	SD.	Flower duration	SD	Flower duration	SD
'Abbeville Blue'	0.75a	0.35	0.375b	0.18	19 a	1.59	16 ab	1.59
'Blushing Spires'	0.5ab	0	0.375b	0.18	21 a	0	19 a	1.24
'Shoal Creek'	0.625ab	0.18	0.625b	0.18	19 a	2.12	14 bc	3.36
'Silver Spires'	0.25ab	0	2.625a	0.18	18 a	0.71	20 a	0.53
V. <i>rotundifolia</i>	0b	0	0b	0	8 b	1	13 c	2.53

Values among parents for individual traits followed by different letters are different based on Tukey mean separation. Avg=Average, Flw=Flower, Inf=inflorescence

Table 2.4A Expression of various vegetative and floral traits in progeny of *Vitex* crosses in container and ground.

Ranking	Ground treatment		Container treatment		Ground treatment		Container treatment	
Cross	Final height (cm)	SD	Final height (cm)	SD	Final width (cm)	SD	Final width (cm)	SD
V0502	91.5 c	6.17	87.5 b	12.52	88 b	8.72	65a	4.03
V0504B	104.5 bc	0.45	104.5 a	6.68	121.5a	1.59	71 a	1.38
V0506A	116 ab	8.52	103 a	8.86	123a	12.64	71 a	2.98
V0506B	130 a	0.39	101.5 a	14.71	133 a	3.34	70 a	1.34
V0509A	118 ab	0.88	107.5 a	8.67	127a	4.51	77 a	8.79
V0509B	117.5 ab	1.84	96.5 ab	9.20	120a	1.09	73 a	1.83
	Ground treatment		Container treatment		Ground treatment		Container treatment	
	First flw. date	SD	First flw. date	SD	Last flw date	SD	Last flw date	SD
V0502	24 July a	0.29	17 July a	0.33	30 Oct a	1.65	25 Sept a	0.14
V0504B	17 July b	0.19	3 July a	0.41	23 Oct a	0.47	9 Oct a	0.82
V0506A	10 July b	0.66	26 June a	0.47	23 Oct a	0.33	2 Oct a	0.48
V0506B	19 June c	0.19	19 June a	1.74	30 Oct a	0.26	9 Oct a	2.50
V0509A	10 July b	0.088	26 June a	0.14	23 Oct a	0.84	25 Sept a	0.12
V0509B	10 July b	0.19	3 July a	1.29	23 Oct a	0.90	9 Oct a	2.06
	Ground treatment		Container treatment		Ground treatment		Container treatment	
	Total weeks flower	SD	Total weeks flower	SD	Avg. inf. num	SD	Avg. inf. num	SD
V0502	12 c	0.79	9a	0.39	7 b	0.86	4 d	0.29
V0504B	16 b	0.87	13 a	0.35	12 a	1.72	5 c	0.03
V0506A	15 b	0.031	13 a	0.92	11 ab	0.60	6 c	0.20
V0506B	19 a	0.58	15 a	3.80	16 a	0.66	7 b	0.091
V0509A	16 ab	0.84	12 a	0.42	12 a	0.53	7 b	0.37
V0509B	16 b	1.092	14 a	0.78	13 a	0.67	9 a	0.16

Mean separation by Tukey mean separation test at  $P \leq 0.05$ . Flw=flower. Num=numb, Inf=Inflorescence



Table 2.4B Expression of various vegetative and floral traits in progeny of *Vitex* crosses in container and ground

	Ground treatment		Container treatment		Ground treatment		Container treatment	
	Avg. flw. on inf.	SD	Avg. flw. on inf	SD	Avg. inf length (cm)	SD	Avg inf. length (cm)	SD.
<b>V0502</b>	2.28c	0.02	2.18d	0.01	14.5b	0.44	13.5c	1.52
<b>V0504B</b>	2.61a	0.07	2.48b	0.02	17.5a	0.03	15bc	0.50
<b>V0506A</b>	2.42bc	0.03	2.41bc	0.05	18a	0.29	16.5 b	0.97
<b>V0506B</b>	2.47ab	0.01	2.35c	0.05	17a	0.98	15bc	0.38
<b>V0509A</b>	2.53ab	0.06	2.62a	0.00	19a	0.56	18a	0.03
<b>V0509B</b>	2.43bc	0.02	2.4bc	0.02	17a	0.02	15bc	0.28

  

	Ground treatment		Container treatment		Ground treatment		Container treatment	
	<i>Cercospora</i> leaf spot	SD.	<i>Cercospora</i> leaf Spot	SD	Flower duration	SD	Flower duration	SD
<b>V0502</b>	0.49ab	0.10	0.39b	0.10	12.5c	0.87	11a	0.27
<b>V0504B</b>	0.36b	0.04	0.44b	0.06	16bc	0.68	14.5a	0.22
<b>V0506A</b>	0.46ab	0.17	0.32b	0.19	16bc	0.39	15a	0.97
<b>V0506B</b>	0.64ab	0.26	0.4b	0.28	20a	0.45	17a	4.24
<b>V0509A</b>	0.72a	0.13	0.28b	0.14	17ab	0.93	14a	0.02
<b>V0509B</b>	0.47ab	0.10	1.22a	0.08	16b	1.09	15a	0.89

Mean separation by Tukey mean separation test at  $P \leq 0.05$ . Flw = flower, Inf. = inflorescence

Table 2.5A Individual *Vitex* entries with most extreme height and width as ranked in each environment.

Shortest of final height measurements				Tallest of final height measurements							
Rank	Ground	Height in cm	Rank	Container	Height in cm	Rank	Ground	Height in cm	Rank	Container	Height in cm
1	V0502-4	9	1	V0502-4	14	1	V0506B-11	174	1	V0506A-59	179
2	V0506A-60	17	2	V0506-67	25	2	V0509A-16	167	2	V0502-7	166.5
3	V0502-19	34	3	V0502-3	26	3	V0506A-69	164	3	V0506A-5	153
4	V0506A-8	34	4	V0506A-60	27	4	V0506A-43	161	4	V0504B-47	147
5	V0504B-27	36.5	5	V0502-35	29	5	V0506A-78	160	5	V0506A-19	145
6	V0506A-58	38.5	6	V0502-23	36	6	V0502-17	159	6	V0502-17	144
7	V0502-31	50	7	V0502-19	42.5	7	V0506A-5	156.5	7	V0504B-44	136
8	V0504B-8	54	8	V0506A-8	44.5	8	V0506A-52	153.5	8	V0509A-22	132.5
9	V0506A-72	54.5	9	V0502-31	56.5	9	V0506A-19	152.5	9	V0506A-80	132
10	V0506A-67	58.5	10	V0506A-72	59	10	V0506A-9	152	10	V0504B-28	131
			10	V0506A-62	59	11	V0502-14	151.5	11	V0502-14	130
Narrowest of final width measurements				Widest of width measurements							
Rank	Ground	Width in cm		Pot	Width in cm	Rank	Ground	Width in cm	Rank	Pot	Width in cm
1	V0506A-60	4	1	V0502-4	18	1	<i>V. rotundifolia</i>	348.88	1	<i>V. rotundifolia</i>	173.5
2	V0502-4	11	2	V0504B-47	30	2	V0502-7	224	2	V0502-7	137
3	V0504B-27	30	3	V0502-3	35	3	V0506A-69	193	3	V0506A-65	112
4	V0502-3	34	4	V0502-29	35.5	4	V0506B-9	183	4	V0509A-5	111
5	V0502-23	36.5	5	V0506A-67	40	5	V0506A-37	182	5	V0506A-72	109
6	V0502-29	38.5	6	V0502-23	42	6	V0504B-44	173	6	V0504B-32	102.5
6	V0502-19	38.5	7	V0506A-8	43	7	V0509A-16	169.5	7	V0506A-37	100
7	V0506A-8	54.5	8	V0506A-75	45	8	V0506A-48	169	8	V0504B-38	98.5
8	V0506A-72	60.5	9	V0506A-60	49	9	V0509A-5	168.5	9	V0502-13	98
9	V0506A-75	63	10	V0506A-79	50.5	10	V0504B-4	164.5	10	V0509A-7	96
10	V0502-32	63.5	10	V0502-19	50.5	11	V0506A-56	161.5	11	V0506A-7	92
10	V0504B-8	63.5									

Table 2.5B Individual *Vitex* entries with the highest flower duration as ranked in each environment

<i>Flower duration</i>					
Rank		Weeks	Rank		Weeks
	Ground			Container	
1	V0506B-2	24	1	V0506B-16	25
2	V0506A-69	22.5	2	V0506B-1	23
3	V0509A-7	22	3	V0506B-6	22
3	V0506B-1	22	4	V0506a-69	20.5
3	V0509A-3	22	4	V0504B-21	20.5
3	V0506B-7	22	4	V0506A-65	20.5
3	V0506A-73	22	5	V0504B-38	20
3	V0506A-76	22	5	V0506B-14	20
4	V0506A-42	21	5	V0509B-21	20
4	V0506B-9	21	6	V0506B-9	19.5
4	V0504A-2	21	6	V0502-33	19.5
5	V0502-33	20.5	6	'Silver Spires'	19.5
5	V0506A-7	20.5	6	V0506A-73	19.5
6	V0506A-78	20			
6	V0506B-15	20			
6	V0506A-19	20			
6	V0509A-16	20			
6	V0506A-23	20			
6	V0506A-14	20			

Table 2.5C Individual *Vitex* entries with the total weeks of flower as ranked in each environment.

Rank		Weeks	Rank		Weeks
	Ground			Containers	
1	V0506A-69	22.5	1	V0506B-16	23
2	V0509A-3	22	2	V0506B-6	21
2	V0506A-73	22	3	V0506B-1	20
3	V0506A-27	21.5	3	V0504B-38	20
4	V0506B-9	21	3	V0506B-14	20
4	V0506A-7	21	4	V0502-33	19.5
4	V0506B-7	21	5	V0506A-76	18.5
4	V0504A-2	21	6	V0506B-9	18
4	V0506A-42	21	6	V0509B-14	18
4	'Blushing Spires'	21	6	V0506A-73	18
5	V0506A-78	20.5			
6	V0506A-14	20			
6	V0509A-7	20			
6	V0506B-12	20			
6	V0506A-76	20			
6	V0506B-15	20			
6	V0509A-16	20			

Table 2.5D Individual *Vitex* entries with the highest inflorescence length and number as ranked in each environment.

Inflorescence length						Inflorescence number					
Rank	Ground		Rank	Container	cm	Rank	Ground		Rank	Container	Quantity
1	V0506A-59	43.5	1	V0506A-59	39	1	V0502-33	26	1	V0506A-5	15
2	V0506A-52	28	2	V0506A-80	31	2	V0504B-46	23	2	V0502-33	14
3	V0509A-19	26.5	3	V0506A-19	28.5	3	V0506A-49	21	3	V0506A-3	13
4	V0506A-11	26	4	V0506A-11	24.5	3	V0504A-2	21	4	V0509B-11	12
5	V0506A-43	25.5	4	V0509A-8	24.5	3	V0504B-19	21	4	V0509B-7	12
6	V0504B-41	25.5	5	V0509A-3	24	3	V0506A-65	21	5	V0509B-13	11
			6	V0504B-22	23	3	V0504B-38	21	5	V0504B-35	11
			6	V0509A-19	23	3	'Blushing Spires'	21	6	V0509A-25	10
			6	V0506A-76	23	4	V0506B-12	20	6	V0509A-11	10
						4	V0506A-48	20	6	V0506A-49	10
						5	V0506B-6	19	6	V0504A-2	10
						5	V0509A-11	19	6	V0509A-16	10
						5	V0506A-3	19	6	'Blushing Spires'	10
						5	V0504B-12	19			
						6	V0506A-14	18			
						6	V0506A-43	18			
						6	V0506B-9	18			
						6	V0504B-13	18			
						6	V0504B-11	18			
						6	V0504B-6	18			

Table 2.6A Correlation in two environments between traits in *Vitex* entries.

	<b>H 1</b>		<b>W 1</b>		<b>H 2</b>		<b>W 2</b>		<b>H 3</b>		<b>W 3</b>		<b>First flw date</b>	
	G	C	G	C	G	C	G	C	G	C	G	C	G	C
<b>Cerc spot</b>	0.13*	NS	NS	NS	-0.15*	NS	NS	0.32***	0.13*	NS	NS	0.3***	NS	NS
<b>H 1</b>			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>W 1</b>					0.36***	0.27***	0.51***	0.31***	0.28***	NS	0.48***	0.34***	0.3***	0.17**
<b>H 2</b>							0.24***	NS	0.3***	0.25***	0.27***	NS	0.32***	0.33***
<b>W2</b>									0.44***	NS	0.82***	0.89***	0.46***	NS
<b>H 3</b>											0.45***	NS	0.88***	0.63***
<b>W 3</b>													0.51***	0.21***

G and C refer to ground and container treatments, respectively. *Cerc*=*Cercospora*, H and W 1, 2, and 3 refer to height and width measurements taken 3 weeks, 19 weeks, and 33 weeks after planting respectively as does width measurements.

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively based on Pearson correlation coefficient.

Table 2.6B Correlation in two environments between traits in *Vitex* entries

	Last flw date		Flw dur		Total wk flw		Avg inf no		Inf length		Avg flw rating	
	G	C	G	C	G	C	G	C	G	C	G	C
<i>Cercospora</i> leaf spot	NS	NS	-0.14*	-0.18**	NS	NS	NS	NS	NS	NS	NS	0.16*
H 1	NS	NS	0.13*	0.17*	NS	NS	NS	NS	NS	NS	NS	NS
W 1	-0.45***	-0.38***	0.21***	0.16*	0.46***	0.36***	0.48***	0.36***	0.4*	0.3***	0.29***	0.24***
H 2	-0.27***	NS	0.14*	NS	0.29***	NS	0.28***	NS	0.27***	0.31***	NS	NS
W 2	-0.28***	NS	0.39***	NS	0.42***	NS	0.46***	NS	0.36***	NS	0.49***	0.42***
H 3	-0.22***	NS	0.38***	0.14*	0.36***	0.13*	0.42***	0.21**	0.46***	0.22***	0.21***	NS
W 3	-0.3***	NS	0.34***	0.16*	0.41***	0.16*	0.46***	0.15*	0.37***	NS	0.55***	0.49***
First flw date	-0.21***	NS	0.43***	0.2**	0.38***	0.2**	0.45***	0.25***	0.45***	0.16*	0.24***	NS
Last flw date			-0.13*	NS	-0.84***	-0.71***	-0.75***	-0.65***	-0.35***	-0.27***	-0.33***	-0.33***
Flw duration					0.64***	0.74***	0.63***	0.67***	0.4***	NS	0.21***	NS
Total wk flw							0.92***	0.88***	0.48***	0.27***	0.36***	0.23***
Avg inf no									0.59***	0.34***	0.42***	0.21**
Avg inf length											0.27***	0.26***

G and C refer to ground and container treatments, respectively. H and W 1, 2, and 3 refer to height and width measurements taken 3 weeks, 19 weeks, and 33 weeks after planting respectively as does width measurements. flw=Flower, inf=Inflorescence, no=number, wk=weeks, dur=duration. NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively, based on Pearson correlation coefficient

**CHAPTER 3**  
**COST ANALYSIS OF A WOODY ORNAMENTAL BREEDING PROGRAM**

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Hershberger, A.J. To be submitted to the *American Journal for Horticulture Science*



## **Abstract**

This study was done to determine differences in cost of production and selection between container-grown and field-grown plants in a *Vitex* breeding. Cost per plant was higher in ground for both material and labor. Initial labor for planting in ground, mulch material, and data collection contributed to the increase in cost for this treatment. Labor hours for planting were 65.5 more in ground than in containers. This was due to the increased space and organization need for the ground treatment, as well as the increased time required to place plants in ground. Mulch material was assigned a cost of \$1411.85, but when this cost was removed (mulch was received *gratis* from the university) the cost of materials in ground was less than that of containers. Labor hours for data collection was 26 hours more in ground than in containers. The increase spacing of plants in ground accounts for this. Water usage in ground ranged from nine to 13% of the water used in the container treatment.

## **Introduction**

Research has been conducted on the number of plant breeders and trends in these numbers in recent years. These studies use the term Science Person Year (SY) to designate work done by a person who has responsibility for designing, planning, managing, and conducting plant breeding research and related tasks in one year (2,080 hours) not including technicians, farm and clerical workers, computer specialists, post doc, grad student, etc. (Traxler et al., 2005). Of the 2241 SYs in U.S. plant breeding, 1499 (67%) are employed by the private sector, 529 (24%) by State Agricultural Experiment Stations and 213 (9%) by USDA. In the public sector horticulture plant breeding, which includes vegetable, fruit, and ornamental breeding, totaled 35-37% of the overall research effort. The private sector had 25% of the research devoted to horticulture plant breeding. It was found in a 1994 plant breeding study that the number of SYs devoted to

ornamentals was 87, or 3.95% of total research, with 18 SYs from SAES, 5 SYs from ARS/USDA, and 64 SYs from private industry (Frey, 1996). A study concerning the plant breeding resources for 2001 (Traxler et al., 2005) revealed that an increase in the number of plant breeders occurred from data collected in 1994 (Frey, 1996) at SAES (21%) and at the USDA/Agricultural Research Service (23%). Ornamental was the only crop category with significant growth. It was also in this category that 20 SYs were added to research.

Few cost analyses have been conducted to compare plant breeding methods. Cost analysis was conducted for 50 wholesale ornamental plant nurseries in Florida in 1995. Information was presented on sales, production, costs, assets and liabilities, and efficiency indicators. This includes information on container and field grown woody ornamentals. Nurseries that grew woody ornamentals totaled 23 and only 2 of those had plants growing both in containers and in the field. Expenses were grouped into the categories of management's compensation, employees' wages and benefits, materials, facility and equipment, administrative overhead, depreciation, and interest. Employee wages and benefits averaged 33.8% of value produced for all firms. These include benefits and also consider employment on a full time equivalent basis. Other costs include 4.5% for management, 33.8% for labor, 32.0% for materials, 4.9% for equipment/facilities, 16.0% for overhead, 3.8% for depreciation, and 4.4% for interest (Hodges et al., 1995).

Research has been conducted to determine the impact that new genetic technologies have on rates of return on the investment in wheat breeding. These new technologies, such as the use of molecular markers, are now used in many breeding programs for the reason of increasing profits (Brennan and Martin, 2007). The costs for each marker must be compared to the cost of phenotypic selection to determine if the use of the marker will be economical for a breeding

program. In a hypothetical breeding program, the costs of molecular markers to select plants for rust resistance ranged from \$2.59 to \$16.28 depending on the form of analysis and the degree of multipooling and multiplexing employed in the marker analysis. The costs for phenotypic selection for rust resistance ranged from \$1.48 for field screening to \$5.18 for glasshouse screening. With this information it can be determined that replacing field screening with the marker-based selection would cause an increase in costs while replacing glasshouse screening with marker-based selection when the marker was applied in the most efficient manner would lower the cost of screening. In most cases markers were generally lower cost than other phenotypic evaluations (Brennan and Martin, 2006) as cited in (Brennan and Martin, 2007). The 2007 study showed that the net present value per hectare of wheat was \$6.93 with the benefits of new technology. Therefore new breeding technologies can bring improved revenue to wheat producers (Brennan and Martin, 2007) Limited research has been published on the cost of breeding programs, particularly costs associated with different methods of breeding. Previous research involving cost analysis of plant breeding has been limited and this is more so concerning ornamental plant breeding. Related research includes comparing costs of growing in field, pot-in-pot, or in containers. Fixed and productions costs were obtained for a hypothetical nursery using a 10 acre production area to grow crapemyrtle (*Lagerstroemia indica* L.) for three years. Fixed costs were found to be similar for in field and above ground containers with a cost of \$350,000. Fixed costs for pot-in-pot were found to be \$25,000 higher. Total production costs for three years were found to be similar for above ground container and pot-in-pot at \$500,000 and in field was found to be \$50,000 less than this. Per harvested plant total cost were lowest in pot-in-pot (\$21.52) and similar in field (\$23.73) and above ground container (\$23.17). When analyzing by only variable costs the costs per plant were \$5.15 for in field, \$7.36 for above

ground containers and \$5.47 for pot-in-pot (Adrian et al, 1998). The objectives of this study were to i) elucidate costs of a breeding program for use in other studies, ii) determine appropriate environment in which to initially select plants based on costs and resource use, iii) use this information with that of first study to determine optimal environment for selection of plants.

## **Materials and Methods**

Six crosses were made in a *Vitex* breeding program. Parents and selections from segregating progeny were clonally propagated, and two replications of identical plants were grown in both containers and in ground. A total of 648 plants were used in the study. Labor hours and plant production and maintenance costs were recorded, beginning with the clonal propagation of selections, and continuing through planting in both environments, plant maintenance, data collection and data entry. Total costs per plant were also calculated, factoring in the common input costs at the greenhouse stage prior to planting each treatment and labor hours for data entry. Labor included tasks in the initial vegetative propagation stage of the study that was conducted in a greenhouse and was common to both environments. These included taking and sticking cuttings, filling containers with potting media, placing cuttings in containers, pesticide application, watering, unloading of plants, and greenhouse clean-up. The greenhouse stage began 16 Aug. 2006 and ended 23 Apr. 2007 when plants were transplanted to either containers or ground. In-field treatment labor costs included tilling, planting, mulching, fertilizing, herbicide application, hand weeding, and data collection. Container treatment labor costs included planting, fertilizing, data collection, and weekly upkeep and maintenance (such as weed control, container blow-over, etc.). Data collection was taken throughout the growing season, evaluating characteristics used in the selection of potential *Vitex* cultivars. Labor value

for each environment was calculated using the median hourly wage of \$7.33 for the state of Georgia (Occupation Profile-America's Career InfoNet, 2008).

Quantities of all inputs were recorded and costs were calculated using prices charged at the time of purchase by commercial sources of the inputs. These included plant material (various sources), containers (Progress Growers), potting media (Gro South), fertilizer (Scotts), pesticides (Hummert), herbicides (Athens Seed Co.), and mulch (Smith Garden Products). Water usage was recorded for each treatment, but not for the greenhouse stage that was common for both treatments. All water was obtained from a well source and therefore no cost was assessed. Differential costs and water usage were obtained between treatments. Costs did not include overhead and fixed costs including depreciation, interest, repairs, maintenance, taxes, insurance, and management. These costs were not assessed as the study was done in a university setting so to assign costs for these things is unfeasible because these costs are not paid through this government entity. These fixed costs could be obtained for each specific production company. Also, it was impractical to assess depreciation of equipment for this study as equipment was used for various components not involving this project. In a standard business setting the above costs would have been assessed.

## **Results and discussion**

Greenhouse costs that contributed to the total cost per plant in this study, but were common to the two environments are listed in Table 3.1. Labor associated with the daily hand-watering of plants constituted nearly half the total cost of production. The highest contributor the materials cost was the potting medium utilized for 800 plants (Table 3.1).

Costs associated with container production are listed in Table 3.2. The labor and materials in the container treatment that contributed the most to the cost of this treatment were data collection and potting media. This could be due to the ease of potting and placing plant material in a shorter space than in ground. The container portion of the study used a range of 342733.41 to 511639.30 liters more water than the in ground portion (Table 3.4). These values were obtained using a range of 1.8 to 2.54 cm per acre per hour for this container treatment. Primary contributors to cost in ground were labor and mulch material. A majority of the labor was used to apply mulch and record data. Data collection in ground took 26 hours longer than collecting the same data in containerized plants. This is due to the fact that the ground treatment were spaced 1.5 m apart whereas the container treatment were spaced 30 cm apart thereby adding to labor hours accumulated for this task. Mulch material cost was based on standard mulch available. Mulch total was \$1411.846 for 80.68 yd<sup>3</sup>. Price was \$15.95 yd<sup>3</sup> per truck load plus \$125 energy surcharge from Smith Garden products. However many research programs and nurseries may have less expensive or even *gratis* sources of mulch, thereby reducing the overall cost of the in ground treatment (Table 3.3). Without mulch the cost of materials in ground was \$143.44 less than in containers. As mulch was received gratis through the university this was a more accurate differential assessment. The combined cost differential was determined to be \$2569.48. The cost differential without mulch material was \$1157.63 (Table 3.4). This additional cost for field growing plant material may become necessary as water use restrictions continue to be imposed. While overall the cost of the ground treatment exceeded the container treatment, it did conserve a great deal of water. This is important as this resource is becoming increasingly limited and has recently been under strict use restrictions. Labor was a major contributor to cost, but there may be more efficient ways in which to do these tasks.

Total hours accumulated over the course of this study, including final data entry, were 711.5. The total cost for labor was \$5215.30. The total cost of materials was \$2879.52. The total cost for labor and materials was \$8094.82 (Table 3.3). This assists in determining requirements for a breeding program. Labor hours for data collection totaled 182 with a cost of \$1334.06. Costs for this task were \$571.74 in containers and \$762.32 in ground. Labor hours for data entry totaled 90 and costs for this were \$659.70. This, based in research in this study, would be the cost associated with evaluation of material within a nursery operation for screening plants that have potential for cultivar release.

Other studies have obtained variable costs (Adrian et al, 1998) for production of plants; however, these studies do not include an itemized list of tasks and materials. Also, costs in this study involve labor hours due to data collection and entry which would not be done in a standard production setting. This is important as these itemized input costs can now be viewed to assess the approximate cost of a breeding program. The study included comparing costs of growing in field, pot-in-pot, or in containers. Fixed and productions costs were obtained to grow crapemyrtle (*Lagerstroemia indica* L.) for three years. Per harvested plant total cost were lowest in pot-in-pot (\$21.52) and similar in field (\$23.73) and above ground container (\$23.17). When analyzing by only variable costs the costs per plant were \$5.15 for in field, \$7.36 for above ground containers and \$5.47 for pot-in-pot (Adrian et al, 1998). In this study, costs were obtained for one year and did not include an evaluation of pot-in-pot plants. Per plant costs were \$3.88 in containers (Table 3.2) and \$7.45 in ground (Table 3.3). Cost per plant for data entry alone was \$1.02. Data collection costs in ground were \$1.76 in containers and \$2.35 in ground. These values could assist a breeder in determining the appropriate environment in which to evaluate plants based on this per plant cost. This study differs from the aforementioned study in that this study shows that

cost per plant was higher in ground than in containers. This is likely due to the duration of that study being three years. Costs per plant in ground would therefore decrease if the study were to occur over three years as well.

Again, these costs did not include overhead and fixed costs including depreciation, interest, repairs, maintenance, taxes, insurance, and management. These costs could be assessed in any production system by obtaining costs specific to a company. The overhead cost would vary from business to business. Taxes and insurance would be specific to certain areas, but could be obtained by viewing the area of the business and creating individual costs for these. Also, water costs were not obtained as the source was a well in this study. These costs could simply be obtained by an individual's municipal water district.

Selection for potential *Vitex* cultivars should be done in ground. While ground costs exceeded that of the container treatment the increase water use in containers is far too substantial to recommend selection in containers. The labor utilized for this study may be decreased in future work once more efficient ways in which to do these tasks are obtained.

### **Literature cited**

Adrian, J.L., Montgomery, C.C., Behe, B.K., Duffy, P.A., Tilt, K.M. 1998. Cost Comparisons for Infield, Above Ground Container and Pot-in-Pot Production Systems. *J. Environ. Hort.* 16: 65-68.

Brennan, J.P. and P.J. Martin. 2006. Developing cost functions for a wheat breeding program. Contributed paper presented to the 50th Annual Conference of the Australian Agricultural and Resource Economics Society, Manly, Australia.

Brennan, J.P. and P.J. Martin. 2007. Returns to investment in new breeding technologies. *Euphytica*. 157: 337-349.



Frey, K.J. 1996. National Plant Breeding Study-I. Special Report 98. Iowa State University, IA.

Traxler, G., A.K.A. Acquaye, K. Frey, and A.M. Thro. 2005. Public Sector Plant Breeding Resources in the US: Study Results for the year 2001

Table 3.1 Cost analysis of labor and materials at greenhouse stage

<i><b>Labor</b></i>	<i><b>Hours</b></i>	<i><b>Cost</b></i>
Cuttings	56	\$410.48
Potting	29	\$212.57
Pesticide spray	3	\$21.99
Greenhouse cleanup	5	\$36.65
Unloading	4	\$29.32
Watering	126	\$923.58
Labor Total	223	\$1634.59
<i><b>Materials</b></i>	<i><b>Quantity</b></i>	<i><b>Cost</b></i>
Containers	800 pots	\$150
Potting medium	32.48 bags	\$338.77
Fertilizer	50.76 lbs	\$55.24
Rooting hormone	1 oz	\$3.04
Bifenazate (Florimite)	0.3 oz	\$2.63
Dinotefuran (Safari)	0.3 oz	\$2.23
Materials Total		\$551.91
<b>Total Greenhouse labor and materials</b>		\$2186.5
<b>Cost per 648 plants</b>		\$3.37

Table 3.2 Cost analysis of labor and materials in container treatment

<i><b>Labor</b></i>	<i><b>Hours</b></i>	<i><b>Cost</b></i>
Planting	<b>18</b>	<b>\$131.94</b>
Weekly upkeep	<b>13</b>	<b>\$95.29</b>
Fertilize	<b>1.5</b>	<b>\$11</b>
Data collection	<b>78</b>	<b>\$571.74</b>
Labor total	<b>110.5</b>	<b>\$809.97</b>
<i><b>Materials</b></i>	<i><b>Quantity</b></i>	<i><b>Cost</b></i>
Fertilizer	<b>34.29 lbs</b>	<b>\$45.91</b>
Container	<b>324 pots</b>	<b>\$60.75</b>
Potting material	<b>4.86 yd<sup>3</sup></b>	<b>\$340.47</b>
Water estimate	<b>394245-563151 liters</b>	<b>Well source</b>
Total		<b>\$447.13</b>
Combined total		<b>\$1257.10</b>
Cost per plant (324)		<b>\$3.88</b>

Table 3.3 Cost analysis of labor and materials in ground treatment

<i><b>Labor</b></i>	<i><b>Hours</b></i>	<i><b>Cost</b></i>
Till	3	\$21.99
Planting	83.5	\$612.055
Mulch	56	\$410.48
Fertilize	2.5	\$18.33
Herbicide	39	\$285.87
Data collection	104	\$762.32
Labor Total	288	\$2111.04
<i><b>Materials</b></i>	<i><b>Quantity</b></i>	<i><b>Cost</b></i>
Herbicide	416.052 fl. oz.	\$180.72
Fertilizer	137.76 lbs	\$122.97
Mulch	80.68 yd <sup>3</sup>	\$1411.85
Water estimate	51512 liters	Well source
Material total		\$1715.54
Material Total without mulch		\$303.69
Combined total		\$3826.58
Cost per plant (324)		\$11.81
Combined total without mulch material		\$2414.73
Cost per plant (324)		\$7.45

Table 3.4 Treatment differentials of labor, materials, and total cost-Ground treatment minus container treatment

Treatment differential	\$2569.48
Treatment labor hour differential	177.5
Treatment labor cost differential	\$1301.07
Treatment material differential	\$1268.41
Water use differential	-342733.41 to -51639.30 liters
Treatment differential without mulch	\$1157.63
Treatment material differential without mulch	\$-143.44

Table 3.5 Cost analysis of total costs of project including data entry

Data entry	<b>90 hours</b>
Data entry	\$659.7
Total labor hours	711.5
Total labor cost	\$5215.30
Total materials cost	\$2714.58
Overall total	\$7929.88
Cost per plant (648)	\$12.24

## CHAPTER 4

### CONCLUSIONS

This study addressed aspects of a woody ornamental breeding program in an effort to determine treatment differences, G X E interactions, trait correlations, and cost analysis to determine the appropriate environment to select potential cultivars of *Vitex agnus-castus*. Except for presence of *Cercospora* leaf spot and the first height measurement, all other traits exhibited treatment difference in ground and in containers. Overall plant were taller, wider, began flowering later, had longer average inflorescence length, total weeks flower and flower duration as well as a later flower date in ground. In ground plants also had a higher inflorescence number and rating for average number of flowers on the inflorescence.

There was no entry by treatment interaction for last flower date, total week flower, flower duration, and average inflorescence length. Therefore selection for these traits could be done in either environment based on this information. There was significant entry by treatment interaction with *Cercospora* leaf spot, first flower date, average inflorescence number, and rating for average number of flowers on the inflorescence. Selection for *Cercospora* resistance should be conducted in containers as increased moisture levels in containers with overhead watering and closer proximity to other plants will increase the level of *Cercospora* leafspot inoculum to a level that will allow determination of truly resistant selections. There was not enough variability for first flower date and rating for average number of flowers on the inflorescence to determine the appropriate selection environment for these traits. While there was a significant entry by treatment interaction for average inflorescence number, a large portion of those top ranked in

ground were also top ranked in containers. These entries could be selected for in either environment while the other would need to be further evaluated.

Many of the trait correlations were low or moderate. This suggests that most traits in this study could be selected independently of other traits. Some that were high include first flower date and height measurements taken at 33 weeks in ground, width measurements taken 19 weeks after planting and width measurements taken 33 weeks after planting, total weeks flower and last flower date in both environments, average inflorescence number and last flower date in ground, flower duration and total weeks flower in ground, and average inflorescence number and total weeks flower in both environments. Using this information it can be determined that final height could be determined by the second height measurement. In ground, taller plants flower later. The higher the total weeks flower the earlier cessation of bloom occurs in both environments. Selecting for a higher average inflorescence number would increase the total weeks flower in both environments and last flower date in ground. Plants that have a longer flower duration also have a higher total weeks flower meaning that these plants are most likely to continually flower as opposed to flowering intermittently which is highly desirable horticulturally. Also, by selecting for a higher total weeks flower will simultaneously select for higher numbers of inflorescences. It should also be noted that height and width measurement are largely independent of other traits except for with first flower date. This is important because a breeder may select for short or tall plants without negatively impacting other significant traits. Selection in ground for these traits would be appropriate as the higher correlation in ground designates garden performance.

As cost analysis revealed higher costs in ground than in containers, it also revealed an extreme water use differential in which the container treatment received the most water. Many



hours and materials costs were due to mulch which was received gratis through the university. It is not known how the lack of mulch application would affect overall water usage in ground. This additional cost for field growing plant material may become necessary as water use restrictions continue to be imposed.

Data from both studies suggest that initial breeding *Vitex* in ground would be the most beneficial to a breeding program. Those traits that exhibited entry X treatment interactions would suggest that by selecting those in containers would overlook optimal plants in ground (and vice versa). The traits with entry X treatment interactions also have high correlations with other traits that do not. Therefore many traits of horticultural significance would then also be affected by environment. Although costs were higher in ground, water usage was far less in ground than in containers. Water use has become more restricted and may reduce the ability of growers to conduct business under container production. Labor was a major contributor to cost, but there may be more efficient ways in which to do these tasks.