

# Expression of Ornamental Traits in Container versus Field Plants in a *Vitex* L. Breeding Program

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**Abstract.** New ornamental cultivars must display horticultural superiority when grown in containers or in the field. The objectives of this study were to determine whether container or field is most appropriate for initial selection of ornamental traits in a *Vitex* breeding program by determining whether quantitative traits of breeding interest were expressed similarly in the two environments and by determining trait correlations in each environment. Segregating populations of *Vitex* and their parents were cloned and grown in containers and in the field. Ornamentally significant traits evaluated included first flower date, last flower date, flowering period, total weeks of flowering, inflorescence number, inflorescence length, flower rating, plant height, plant width, and *Cercospora* leaf spot resistance. Overall, field-grown plants were taller and wider than plants grown in containers. Field-grown plants also had a later first flowering date, longer flowering period, greater total weeks flowering, longer inflorescence length, larger inflorescence number, and more flowers on the inflorescence. Significant genotype × environment interactions were found for height and width measurements taken 19 and 33 weeks after planting, first flower date, total weeks in flower, inflorescence number, flower rating, and *Cercospora* rating. Most trait correlations were either non-significant or so low so that selection of these traits would be independent of other traits. High correlations were present in both environments between height measurements taken at 19 weeks and 33 weeks after planting. High correlation in the field and moderate correlation in containers were found between width measurements taken 19 and 33 weeks after planting. Correlation was high between flowering period and first flower date in both the field and container. Correlation between last flower date and flowering period was high in containers and moderate in the field. High correlation was present in both environments between flowering period and total weeks of flowering. Containers were determined to be best for initial selection for most traits having significant genotype × environment effects.

*Vitex* L. is a genus of ≈250 species distributed throughout the world. *Vitex* has recently been moved taxonomically from the Verbenaceae to the Lamiaceae (Judd et al., 2002). Introduced to the United States in 1570, *Vitex 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 States by the J.C. Raulston Arboretum at North Carolina State University, Raleigh,

NC, in the 1980s. It has been used for dune stabilization and as an ornamental.

Phenotypic plasticity, or environmentally dependent phenotypic expression, is an important consideration in a plant breeding program because it can confound selection schemes and greatly reduce selection effectiveness. Developmental patterns, reproductive timing, and breeding systems are all affected by the environment (Sultan, 2000). Reproductive traits are, in general, less plastic than vegetative traits (Bradshaw, 1965; Frazee and Marquis, 1994); however, earlier flowering was a response to stress in *Arabidopsis* L. (Westerman and Lawrence, 1970), and water stress decreased flower production in *Clarkia unguiculata* Lindl. (Smith-Heurta and Vasek, 1987), *Lavandula stoechas* L. (Herrera, 1991), and species of *Phlox* (Schlichting, 1986).

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). Harding et al. (1981, 1987, 1990, 1991) have studied heritability and correlation of traits in the cut flower *Gerbera hybrida*. Traits with high heritability generally had high correlation values with each other. Traits having low heritability and low correlation values were typically inflorescence traits resulting in the lowest efficiency of selection (Harding and Huang, 1998; Huang et al., 1990).

New ornamental cultivars must display horticultural superiority when grown both in containers and in the field. However, limited research has been conducted to determine whether initial evaluations would be more effective in the container or field. Numerous studies involving agronomic crops have addressed the issue of trait stability in varying environments (EdSouza, 1993; Fukai and Cooper, 1995; Kang, 1997; Patil and Deshmukh, 2000), but very few have evaluated variation between field-grown and container-grown plant material. One such study (Booker et al., 2005) examined seed yield between field-grown and container-grown soybean plants. Researchers found that values for most reproductive traits as well as stem biomass were less in containers than in their field-grown counterparts. Increased variability in characteristics of container-grown plants may be the result of higher fluctuations in root zone temperatures when compared with field-grown plants (Fiscus et al., 2007; Ingram et al., 1993; Ruter, 1993).

Ornamental research that has compared field-grown with container-grown plant material has focused primarily on the differences in growth rate and rooting characteristics and not on the effect on ornamental traits. Ruter (1993) evaluated growth differences in above-ground container-grown and in-ground pot-in-pot production. The pot-in-pot treatment increased the root dry weight in *Lagerstroemia* and *Magnolia* and increased the root–shoot ratio of *Lagerstroemia*. In a similar study, *Magnolia grandiflora* ‘St. Mary’s’ plants were taller in the pot-in-pot treatment (Ruter, 1995). In *Myrtus communis* L. (common myrtle), however, Miralles et al. (2009) observed greater plant height and shoot dry weight in the above-ground container treatment compared with those grown in the pot-in-pot treatment.

Woody ornamentals are typically sold in containers for eventual use in the field. Container performance is important to growers and retailers, because growth and appearance in a container influence sales and profitability. Ultimately, however, field performance is critical to end-user satisfaction. Therefore, it

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is important to determine trait expression in both environments. No research has been conducted on field versus container variation in *Vitex*. The objectives of this study were to evaluate parents and segregating populations of *Vitex* in both containers and in the field to determine whether quantitative traits of breeding interest were expressed similarly in the two environments and to determine trait correlations in each environment.

### Materials and Methods

**Plant material.** As part of the *Vitex* breeding program at the University of Georgia in Athens, seven populations were developed in the summer of 2005. Five parent plants were placed in pairs in bee cages, and honeybee (*Apis mellifera* L.) hives were introduced to enhance pollination (Table 1). Parent plants were *V. rotundifolia* and four cultivars of *V. agnus-castus*: Abbeville Blue, Blushing Spires, Shoal Creek, and Silver Spires. Because no controlled cross-pollinations were made, seeds collected from each plant likely originated from both self- and cross-pollination. Initial populations were grown in the spring and summer of 2006, and horticulturally desirable plants were selected from each population. The number of selected progeny used in this study is listed in Table 1. Each initial parent and all selected offspring were clonally propagated 16 Aug. 2006. Stem cuttings were dipped into 1% K-IBA solution and then placed under mist in 16-cell packs containing 3:1 Fafard 3B (Conrad Fafard, Inc., Agawam, MA) and perlite for 8 weeks. Plants were potted in 0.00382-m<sup>3</sup> volume containers (trade #1) with Fafard 3B on 14 Nov. 2006. Plants were fertilized with a 100 ppm solution of 20N-4.4P-16.6K at each watering event.

**Treatment implementation and experimental design.** On 23 Apr. 2007, all liners were transplanted into containers or the field

at the University of Georgia Horticulture Farm in Watkinsville, GA, at lat. 33°53'17.6028" N and long. -83°24'59.0436" W. Both the container and field environments contained two blocks with two replicates of each progeny and eight replicates of each parent arranged in a randomized complete block design. Field soil ranged from a sandy loam to a sandy clay loam. Plants (324) in the field were placed on 1.5-m spacing in 123 × 1-m beds with drip irrigation. Plants in containers (324) were placed in 0.011-m<sup>3</sup> volume containers (trade #3) with Fafard 3B on an overhead-irrigated growing pad with edge of containers spaced 30 cm apart. Both the container and field received similar fertilizer regimes using Osmocote 14N-6.2P-11.6K (14-14-14) (3–4 month; The Scotts Co., Marysville, OH) on 4 June 2007. The field was given 15.1 kg per 123 × 1-m bed and the containers received 44 g of fertilizer per container. Ten-centimeter deep hardwood mulch was applied to the field treatment 2 weeks after planting.

**Plant measurements.** Height and width measurements were taken 16 May 2007, 23 Sept. 2007, and 8 Dec. 2007 with the last date after first frost and subsequent cessation of plant growth. Flowering data were taken weekly beginning 15 May 2007 (given a value of one for data analysis because it was the first week that any of the plants flowered) and ending 8 Nov. 2007 (given a value of 26 because it was 26 weeks after any of the plants began flowering), the date of the first frost. First date of flowering was defined as the first appearance of petal color on any flower of a given plant, and last date of flowering was defined as the date when no petal color was detected on a plant. Flowering period was calculated as the number of weeks from the date of the first flower to the date of the last flower. Total weeks of flowering was the actual number of weeks with open flowers. For example, a plant may have

8 total weeks of flowering but a flowering period of 10 weeks because of intermittent flowering. In addition, inflorescences per plant were counted each week, and the visually longest inflorescence on each plant was measured. A flower rating was assigned weekly that estimated the percentage of open flowers on each inflorescence averaged over all inflorescences on the plant on a scale of 1 to 5. A value of 1 denoted that opened flowers covered up to 20% of total inflorescences, 2 denoted that open flowers covered 20% to 40% of total inflorescences, 3 denoted 40% to 60% covered, 4 denoted 60% to 80% covered, and 5 denoted 80% to 100% of the inflorescences were covered. Presence of *Cercospora* leaf spot was measured on a scale of 0 to 5. Zero was defined as no leaf spot present, 1 as presence of the precursor for the disease with white areas on the leaf surface, 2 as 20% to 40% of the leaf area covered with leaf spot, 3 as 40% to 60% leaf area covered with leaf spot, 4 as 60% to 80% leaf area covered with leaf spot, and 5 as 80% to 100% leaf area covered with leaf spot.

**Data analysis.** Data were analyzed with a two-way analysis of variance using the SAS General Linear Model procedure (SAS System, 2008). Main effects of genotype (G), environment (E), and G × E interaction were calculated. Means were separated using Tukey's honestly significant differences test ( $P \leq 0.05$ ). Pearson correlation coefficients between traits were estimated for all genotypes and were separated by environment.

### Results and Discussion

**Genotype/environment effects.** All traits exhibited significant genotypic effects (Table 2). Of the 14 traits measured, all but two were significantly different in containers than in the field (Tables 2 and 3). Only *Cercospora* leaf spot rating and width measurements taken 3 weeks after planting were not significantly different. Overall, plants were taller and wider in the field than in containers. All reproductive traits demonstrated significant environmental effects. Plants in containers flowered earlier than in the field; however, the field plants had a later last flower date than those in containers. Flowering period and total weeks flowering were longer in the field than in containers. Average inflorescence number, average inflorescence length, and rating for average number of open flowers on the inflorescences were all significantly less in containers than in the field. The

Table 1. *Vitex* breeding pairings and number of progeny used in this study.

Parents	Number of progeny
<i>Vitex agnus-castus</i> 'Shoal Creek' × <i>V. rotundifolia</i>	19
<i>V. agnus-castus</i> 'Silver Spires' × <i>V. agnus-castus</i> 'Shoal Creek'	1
<i>V. agnus-castus</i> 'Shoal Creek' × <i>V. agnus-castus</i> 'Silver Spires'	32
<i>V. agnus-castus</i> 'Shoal Creek' × <i>V. agnus-castus</i> 'Blushing Spires'	52
<i>V. agnus-castus</i> 'Blushing Spires' × <i>V. agnus-castus</i> 'Shoal Creek'	11
<i>V. agnus-castus</i> 'Abbeville Blue' × <i>V. agnus-castus</i> 'Silver Spires'	16
<i>V. agnus-castus</i> 'Silver Spires' × <i>V. agnus-castus</i> 'Abbeville Blue'	11
Total	142

Table 2. Levels of significance for an analysis of variance (ANOVA) conducted as a two-factor general linear model design with genotype and environment as the main factors for various traits in *Vitex*.

	df	<i>Cerc.</i> rating	H1 (cm)	W1 (cm)	H2 (cm)	W2 (cm)	H3 (cm)	W3 (cm)	First flw date	Last flw date	Flw per	Total wks flw	Avg inf num	Avg inf lgth	Avg flw rating
G	143	***	***	***	***	***	***	***	***	***	***	***	***	***	***
E	1	NS	*	NS	**	***	***	***	***	***	***	***	***	***	*
G × E	143	**	NS	NS	*	***	*	***	*	NS	NS	*	**	NS	*

*Cerc.* = *Cercospora* leaf spot; H = height; W = width; flw = flower; wks = weeks; inf = inflorescence; num = number; lgth = length; per = period; G = genotype; E = environment; G × E = genotype × environment.

H1, W1, H2, W2, H3, W3 refer to measurements taken 3, 19, and 33 weeks after planting, respectively. NS, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

increased values for reproductive traits are consistent with work done by Booker et al. (2005) and Fiscus et al. (2007) that compared field- and container-grown soybeans. They found that most reproductive trait values were lower in container plants than in their field-grown clones.

**Treatment interactions.** *Cercospora* leaf spot rating exhibited a significant environmental interaction with genotype (Table 2), indicating that genotypes with the best leaf spot resistance in one environment may not be the best in the other. The differential expression of leaf spot resistance suggests that selection may need to be made both in the field and in containers.

Height and width of genotypes at 19 and 33 weeks after planting responded differently to environments. Choosing either tall or short plants in one environment would not necessarily identify similar types of plants in the

second environment. Plant size is an important trait in a landscape setting, so if initial selection for height is conducted in containers, larger populations should be selected and then field-tested.

Genotype × environment interaction was significant for first flower date, total weeks flowering, average inflorescence number, and average flower rating. Growers and retailers prefer early blooming plants, because early flowering boosts spring sales. As with early flowering, high inflorescence number and high flower rating in containers are important for consumer purchasing, so initial selection in containers, followed by field evaluation, would be the best strategy.

Neither last flower date nor flowering period had significant G × E interactions. This is likely the result of the truncated data that occurred because most plants were still flowering when the first frost occurred on

6 Nov. 2007. Frost damaged any blooming or budding inflorescences. In addition, no environmental interaction with genotype occurred for inflorescence length; therefore, selection for entries with long inflorescences could be done effectively in either environment.

**Trait correlations.** Because of the large number of plants in this study, correlations between traits were often statistically significant, yet the correlation coefficients were 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 (Tables 4 and 5). Height measurements at 19 and 33 weeks were highly correlated with each other in both environments, and width measurements at 19 and 33 weeks were highly correlated with each other in the field and moderately correlated in containers (Tables 4

Table 3. Mean values of various traits in *Vitex* grown in the field and containers.

	<i>Cerc.</i> rating	H1 (cm)	W1 (cm)	H2 (cm)	W2 (cm)	H3 (cm)	W3 (cm)	First flw date	Last flw date	Flw per	Total wks flw	Avg inf num	Avg inf lgth	Avg flw rating
Field	0.49 a <sup>2</sup>	30 a	33 a	100 a	117.5 a	112 a	121 a	9 July a	23 Oct. a	16 a	15 a	12 a	17.5 a	2.48 a
Container	0.45 a	32 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	2.42 b

<sup>2</sup>Means in columns followed by different letters are significantly different based on Tukey's mean separation test at  $P \leq 0.05$ . The total number of plants evaluated was 648 with 324 in each environment.

*Cerc.* = *Cercospora* leaf spot; H = height; W = width; flw = flower; wks = weeks; inf = inflorescence; num = number; lgth = length; per = period. H1, W1, H2, W2, H3, W3 refer to measurements taken 3, 19, and 33 weeks after planting, respectively.

Table 4. Correlation between traits in *Vitex* grown in the field.

	H1	W1	H2	W2	H3	W3	First flw date	Last flw date	Flw per	Total wks flw	Avg inf num	Avg inf lgth	Avg flw rating
<i>Cerc.</i> rating	NS	NS	NS	NS	NS	NS	NS	0.13*	NS	NS	NS	NS	NS
H1		0.36***	0.51***	0.28***	0.48***	0.3***	-0.45***	0.2***	0.46***	0.48***	0.4***	0.29***	0.21***
W1			0.24***	0.3***	0.27***	0.32***	-0.27***	0.14***	0.29***	0.29***	0.27***	NS	0.15*
H2				0.44***	0.82***	0.46***	-0.28***	0.38***	0.42***	0.46***	0.36***	0.49***	0.29***
W2					0.48***	0.88***	0.22***	0.38***	0.36***	0.42***	0.46***	0.21***	0.32***
H3						0.51***	-0.3***	0.33***	0.41***	0.46***	0.37***	0.55***	0.3***
W3							-0.21***	0.43***	0.38***	0.45***	0.45***	0.23***	0.33***
First flw date								-0.13*	-0.84***	-0.77***	-0.35***	-0.33***	-0.42***
Last flw date									0.64*	0.63***	0.4***	0.21***	-0.35***
Flw per										0.94***	0.48***	0.36***	0.51***
Total wks flw											0.58***	0.42***	0.55***
Avg inf num												0.27***	0.46***
Avg inf lgth													0.33***

*Cerc.* = *Cercospora* leaf spot; H = height; W = width; flw = flower; wks = weeks; inf = inflorescence; num = number; lgth = length; per = period. H1, W1, H2, W2, H3, W3 refer to measurements taken 3, 19, and 33 weeks after planting, respectively. NS, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

Table 5. Correlation between traits in *Vitex* grown in containers.

	H1	W1	H2	W2	H3	W3	First flw date	Last flw date	Flw per	Total wks flw	Avg inf num	Avg inf lgth	Avg flw rating
<i>Cerc.</i> rating	NS	NS	NS	NS***	NS	NS	NS	0.16*	NS	NS	NS	NS	NS
H1		0.27***	0.31***	NS	0.34***	0.17**	-0.38***	0.17**	0.36***	0.37***	0.3***	0.24***	NS
W1			NS	0.25***	NS	0.33***	NS	NS	NS	NS	0.31***	NS	NS
H2				NS	0.89***	NS	NS	NS	NS	NS	NS	0.42***	NS
W2					NS	0.63***	NS	0.14*	0.13*	0.2**	0.22**	NS	NS
H3						0.21**	NS	0.16*	0.16*	0.14*	NS	0.46***	NS
W3							NS	0.2**	0.2**	0.25***	0.16*	NS	NS
First flw date								NS	-0.71***	-0.65***	-0.27***	-0.33***	-0.33***
Last flw date									0.74***	0.66***	NS	NS	NS
Flw per										0.91***	0.27***	0.23***	0.28***
Total wks flw											0.34***	0.21**	0.3***
Avg inf num												0.26***	0.35**
Avg inf lgth													NS

*Cerc.* = *Cercospora* leaf spot; H = height; W = width; flw = flower; wks = weeks; inf = inflorescence; num = number; lgth = length; per = period. H1, W1, H2, W2, H3, W3 refer to measurements taken 3, 19, and 33 weeks after planting, respectively. NS, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.



and 5). This indicates that selection for plant height and width could be done at either 19 or 33 weeks after planting, which can be useful in expediting selection for this trait (Tables 4 and 5).

High negative correlations between first flowering date and flowering period were present in both environments ( $r = -0.84$  in field and  $r = -0.71$  in containers). First flower date and total weeks flowering were highly negatively correlated in the field and moderately correlated in containers. Earlier flowering plants had longer flowering periods and greater total weeks flowering. In addition, last flowering date and flowering period were highly correlated in the field and moderately correlated in containers, and correlation between last flowering date and total weeks flowering was moderate in both environments. High correlation between flowering period and total weeks flowering was present in both environments ( $r = 0.94$  in field and  $r = 0.91$  in containers) (Tables 4 and 5). Most plants from these populations flowered continuously during the growing season, suggesting that plants with a long flowering period were more likely to also have continuous flowering as opposed to intermittent flowering.

Although degree of correlation varied between the container and field environments, significance and direction of correlation were similar in both environments. Most differing correlations were higher in the field than in containers, but because values were still low, it is likely that selection for combined sets of traits either in containers or in the field could be successful. The lack of correlation of *Cercospora* leaf spot with other traits is especially helpful, because resistance can be selected along with any other set of desirable traits. The high or moderate correlations among desired qualities such as early flowering, long flowering period, and total weeks flowering will improve efficiency of selection in a *Vitex* breeding program.

In summary, traits that had differential expression in each environment such as first flower date, total weeks flower, and average inflorescence number would be best selected initially in containers, because consumer purchases are made early in the season on container-grown plants. Selection for plant height would be best conducted initially in the field, because height is important in

a landscape setting. Information generated in this study will be helpful in developing selection strategies in a *Vitex* breeding program and could potentially be applied to other ornamental breeding programs.

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