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ISOZYME, MORPHOLOGICAL, AND COLD HARDINESS VARIATION IN A *Cornus florida* L.PROVENANCE PLANTATION

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Alexander Fernandez

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ISOZYME, MORPHOLOGICAL, AND COLD HARDINESS VARIATION IN A *Cornus florida* L. PROVENANCE PLANTATION

By

Alexander Fernandez

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ABSTRACT

ISOZYME, MORPHOLOGICAL, AND COLD HARDINESS VARIATION IN A *Cornus florida* L. PROVENANCE PLANTATION

By

Alexander Fernandez

Isozyme and morphological variation were analyzed in 24 *Cornus florida* populations in a provenance plantation. Allelic variation was substantially lower than other woody plant species with similar life history traits. Also, no relationship was observed between allelic profiles and geographic origin. Conversely, principal component analysis of leaf and flower bud characters revealed considerable variation among provenances. Flower bud size and number of florets increased with population latitude. The relationship between flower bud size and latitude suggests an adaptive response to photoperiod throughout the species geographic range.

Cold hardiness evaluation of 10 provenances revealed significant variation in acclimation rates and mid-winter hardiness between provenances and between floral tissues. Northern provenances were more cold tolerant on all sampling dates with the greatest variation appearing during the acclimation period and decreasing by mid-winter. The peduncle proved to be the hardiest tissue and the receptacle and florets the least hardy.

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Guidance Committee:

The journal-article format was adopted for this thesis in accordance with departmental and university requirements. Each section was prepared as a self-standing manuscript. Section I was prepared for publication in the *Journal of the American Society of Horticultural Science*, and section II was prepared for the *Journal of Environmental Horticulture*.

SECTION I. ISOZYME AND MORPHOLOGICAL VARIATION IN A *Cornus florida* L. PROVENANCE PLANTATION REPRESENTING 24 GEOGRAPHICALLY DIVERSE POPULATIONS

Alexander Fernandez[1], Robert E. Schutzki[2], and James F. Hancock[3]

Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

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[1]Graduate Research Assistant

[2]Associate Professor

[3]Professor

Genetics and Breeding

Isozyme and Morphological Variation in a *Cornus florida* L. Provenance Plantation Representing 24 Geographically Diverse Populations

Additional index words. electrophoresis, flowering dogwood, principal component analysis, population genetics

Abstract. Starch gel electrophoresis and principal component analysis were used to determine the levels of genetic variation and the relationship between morphology and geographic origin for 24 *Cornus florida* (flowering dogwood) populations in a Michigan provenance plantation. The populations are representative of the species' geographic distribution, ranging from Texas to Georgia and north to Connecticut and Michigan. Allelic variation at 11 loci encoded by five enzymes was low in comparison to other plant species. On average, populations displayed 1.16 alleles per locus, 9.89% of loci polymorphic, with observed and expected heterozygosity values of 0.062 and 0.048, respectively. Genetic identity values ranged from 0.961 to 1.00 and displayed no relationship with geographic origin. The mean F_{ST} (0.169) and estimates of migration per generation ($Nm = 1.23$) revealed relatively high levels of gene flow in comparison with plant species with similar life history traits. The apparent high levels of gene flow may be attributable to efficient animal seed dispersal, increasing the potential for long

distance gene transfer between populations. The majority of variation (91%) in leaf and flower bud morphology was explained by four principal components. Leaf characters revealed no relationship with geographic origin. However, flower bud size and number of florets decreased with changes in latitude from northern, central and southern populations, respectively. The relationship between flower bud size and latitude suggests an adaptive response to photoperiod throughout the species' geographic range.

Introduction

Flowering dogwood (*Cornus florida* L.) is a small understory tree species native from southeastern Maine to northern Florida, and west through eastern Texas and Kansas (Elias, 1987). Wyman (1990) considers *C. florida* the best native ornamental tree in the northern United States because of its year-round ornamental interest. The species is widely planted throughout its native range with over 100 cultivar names listed in the nursery trade (Santamour and McArdle, 1985).

Interest in measuring genetic variation of native *C. florida* populations has been in response to numerous reports of disease, insect, and cold hardiness problems affecting trees in landscapes and native populations. Lack of flower bud hardiness in *C. florida* with southern origins is cited as a major cause of floral death or deformation in northern landscapes (Dirr, 1990). Few investigations into the levels of phenotypic and genetic variation in *C. florida* have been reported. Santamour and McArdle (1989) indicated a lack of genetic variation in anthracnose resistance for *C. florida* with no correlation between resistance and geographic origin. Heatley (1986) provided data describing variations in twig cold hardiness and fall phenology based on geographic origin. Northern dogwoods were more cold tolerant and displayed a more consistent, longer-lasting fall color display in comparison to southern dogwoods. At the DNA level, Culpepper et al. (1991) investigated the genus *Cornus* utilizing restriction fragment length

polymorphisms (RFLPs) and found high levels of genetic diversity between *Cornus* species, but low levels of genetic diversity between *C. florida* cultivars.

Levels of genetic variation in plant populations has been linked to the life history and ecological features of a species (Brown, 1979; Hamrick et al., 1979; Loveless and Hamrick, 1984). Species with wide distributions, high outcrossing rates, wind pollination, wind dispersal of seed, high fecundities, long generation times, and residents of late successional habitats have the potential for high levels of genetic variation (Hamrick et al., 1979). Numerous investigations of conifer species combining many of the variation-inducing life history traits have revealed high levels of genetic variability; however, relatively few studies have been conducted on woody angiosperms (Gottlieb, 1981; Hamrick, 1982; Mitton, 1983; Trembley and Simon, 1989). Studies on *Camellia japonica* (Wendel and Parks, 1985), *Robinia pseudoacacia* (Surles et al., 1989), *Alnus crispa* (Bousquet et al., 1987), and *Quercus* spp. (Hokanson et al., 1993) describe angiosperms with high levels of genetic variation residing within populations.

C. florida is a perennial angiosperm that possesses many of the variation-inducing traits with the notable exceptions of pollination and seed dispersal mechanisms. *C. florida* is insect pollinated (Gunatilleke and Gunatilleke, 1984) and seed dispersal is accomplished through animal ingestion and gravity (Elias, 1987). Both of these life- history traits would be expected to increase the genetic variation among populations while reducing variation within populations (Loveless and Hamrick, 1984).

This paper presents results of an isozyme and morphological evaluation of native *C. florida* trees in a provenance plantation. The objectives of the study were to determine the levels of genetic variation in native populations and to assess the relationship between geographic origin, allelic profiles, and morphometric traits. Determining genetic variation within and among native populations will lend insight into the potential for breeding and selecting cold-tolerant, disease-resistant flowering dogwoods.

Materials and Methods

Three *C. florida* provenance plantations in close proximity at the W.K. Kellogg Experimental Forest in Augusta, Mich. were used in this study. The plantations were established in 1975 by Drs. Kielbaso and Wright of the Dept. of Forestry, Michigan State University. Cooperators collected open-pollinated seeds in native populations representative of the flowering dogwood's geographic range (Fig. 1). Ten of the 23 populations used in this study are represented by seeds collected from a single maternal tree and the remaining 13 are represented by seeds collected from multiple trees per population (Table 1). Design, establishment, and maintenance of the plantations is described by Heatley (1986).

For sampling purposes, populations represented in the provenance plantations were collected by state of origin. Within each state, an attempt was made to sample five individual trees from two distinct populations. The minimum distance separating populations was approximately 45 miles. Due to tree mortality, not all

states were represented by two populations and five trees per population (Table 1). A total of 111 trees were examined, representing 23 populations across 14 states (Fig. 1).

Branches with dormant vegetative terminal buds were collected for electrophoresis on 8 Apr. 1993. The twigs were placed in labeled sealed plastic bags and immediately stored in an ice-filled cooler for transport back to East Lansing, Mich. Samples were then placed in a cooler at 5C for a maximum of two days until buds were removed and enzyme extractions completed.

Approximately 8 mg (8 to 10 terminal buds) of dormant buds were removed from the twigs and ground in a chilled mortar and pestle. Axillary buds were used when terminal bud material was limited. Extraction for all enzymes was achieved using a phosphate extraction buffer (Soltis et al., 1983) and insoluble polyvinylpolypyrrolidone (PVPP) (Sigma #P-6755) hydrated overnight in the extraction buffer. The extraction buffer and a spatula-tip (approximately 8 mg) of saturated PVPP were added to the mortar just prior to grinding. Buds were ground until the crude extract reached the consistency of a thick slurry. The bud extract was absorbed through a 35 μm nylon screen onto 3 x 4 x 10 mm wicks of Whatman no. 3 chromatography paper. Wicks were stored at -80C in Corning 96-well disposable ELISA plates wrapped with cellophane and sealed inside plastic bags.

Electrophoresis

Investigations of five enzyme systems and two gel/electrode buffer systems yielded 11 well-resolved putative loci. Enzyme systems, malate dehydrogenase (MDH, EC 1.11.1.7) and isocitrate dehydrogenase (IDH, EC 1.1.1.42), were resolved using the morpholine-citrate pH 6.1 system (Clayton and Tretiak, 1972). Ten-millimeter-thick gels of 11% starch were run at 50 Ma for 1 h and increased to 55-65 Ma for 5 h. Esterase (EST, EC 3.1.1.2), leucine aminopeptidase (LAP, EC 3.4.11.1), and alcohol dehydrogenase (ADH, EC 1.1.1.1) were resolved using the lithium borate, tris citrate pH 8.3 system (Scandalios, 1969). Ten-millimeter-thick gels of 11% starch were run at 75 Ma until 275 V were reached. The system was then maintained at 275 V for a total running time of 6 h.

Enzyme staining protocols were obtained from Vallejos (1983) for malate dehydrogenase (MDH); Soltis et al. (1983) for isocitrate dehydrogenase (IDH) and leucine aminopeptidase (LAP); and Wendel and Weeden (1989) for esterase (EST) and alcohol dehydrogenase (ADH). After staining was complete, slices were rinsed first with water, then with 1% acetic acid solution. Slices were fixed in 50% ethanol solution, placed in zip-lock bags, and stored at 4C until they could be analyzed and photographed.

Scoring of those enzymes with multiple loci were numbered with the fastest anodally migrating locus designated as one and each successively slower locus given a sequentially higher number. Alleles within a locus were numbered in the same manner. Locus and allele predictions of the observed banding patterns are

based on the predictability of enzyme structure and the expected number of loci from previous isozyme investigations (Kephart, 1990). Loci and allele predictions were not supported by a genetic analysis and are therefore reported as putative.

Statistical analysis

Levels of variation were determined based on measures of percent polymorphic loci, allele frequencies, mean alleles per locus, and expected heterozygosity. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Deviation from Hardy-Weinberg equilibrium and population differentiation as measured by F-statistics (F_{IS}, F_{IT}, F_{ST}) (Nei, 1977; Wright, 1978) and Nei's (1978) unbiased distance (D) calculated using the BIOSYS-1 program, release 1.7 (Swofford and Selander, 1989). F_{IS} measures the deviation in heterozygote frequencies of individuals within subpopulations (Nei, 1977). An average of all loci throughout all populations gives a mean F_{IS} for the species. F_{IT} measures the total heterozygote deviation from the reduction of heterozygosity of individuals in comparison to a hypothetical population in Hardy-Weinberg equilibrium. F_{ST} is a measure of differentiation between populations based on subpopulation deviations from equilibrium. To measure amount of gene flow occurring between populations, the number of migrants exchanged per generation (Nm) was calculated (Slatkin, 1981). N is equal to the population size, and m is the fraction of the population that is replaced by immigrants in each generation.

Principal component analysis of leaf and flower bud characters

Flower buds and leaves were collected from trees located in the same three provenance plantations described earlier. Leaf samples were collected from five trees for each of 24 populations. Due to tree mortality in the provenance plantation, not all populations were represented by five trees. Leaf sampling strategy followed the recommendations described by Blue and Jensen (1988) for species and population comparisons. Ten fully expanded leaves were collected on 25 July 1993 from twigs located in the southeast quadrant of the upper one-third portion of each tree. A random number table was used to select five leaves from the 10 leaves collected per tree. The leaves were labeled, pressed, and transported to East Lansing, Mich. On 9 Nov. 1993, 20 flower buds were collected from the same area of the tree previously described. The flower buds were sealed in plastic bags and transported in an ice-filled cooler to East Lansing, Mich. A random number table was used to select 10 flower buds from the 20 collected per tree.

Data for nine leaf characters and three flower bud characters were recorded for each tree (Table 2). Leaf surface area was calculated using a Delta-T Devices Area Measurement System. Leaf width and length measurements were recorded in millimeters by overlaying a transparent grid on pressed leaves. Flower bud width and length measurements were recorded in millimeters using Fowler & NSK Max-Cal electronic digital calipers. Statistical analysis was conducted on the means for each of the 12 variables within a population. SAS (SAS Institute Inc.,

1988) was used to perform principal component analysis on the 12-variable correlation matrix.

Results

Electrophoretic survey

A total of five enzyme systems resolved clearly and were interpreted for this study. The enzyme systems encoded 11 putative loci with a total of 16 putative alleles. Of the 11 loci examined, *Lap-1*, *Est-1*, and *Est-2* were polymorphic (Fig. 2 and 3). The remaining eight loci were monomorphic with IDH encoding two homozygous loci, and both MDH and ADH encoding three homozygous loci. The average number of alleles per locus (A) was 1.16, with a range of 1.0 to 1.3 (Table 1). The mean percentage of polymorphic loci (P) for all populations was 9.89%. The percent polymorphic loci for individual populations ranged from 0% to 18.2%.

Two populations, AL-1 and CT-1, displayed unique alleles. *Est-2*^{2} appeared in a single heterozygous individual of the AL-1 population and *Lap-1*^{1} was evident in two heterozygous individuals of the CT-1 population (Fig. 2 and 3). The mean expected heterozygosity (H_{exp}) for all populations was 0.048 with a range of 0 to 0.113 (Table 1). This value was slightly lower than the mean observed heterozygosity (H_{ob}) of .062. F_{IS} and F_{IT} also indicated an excess of heterozygotes for two of the three polymorphic loci with mean values of -0.455 and -0.210, respectively (Table 3). *Lap-1* was the only locus indicating a

heterozygote deficiency with a single population displaying a significant deviation from Hardy-Weinberg expectations. *Est-1* and *Est-2* revealed an excess in heterozygosity. *Est-1* revealed four populations with significant deviations from Hardy-Weinberg expectations. Levels of differentiation between populations and gene flow were measured using F_{ST}, Nei's unbiased distance, and Nm. Values revealed low levels of differentiation between populations with a relatively high level of gene flow. Identity values (I) for all populations ranged from 0.961 to 1.000. The greatest divergence in pairwise comparisons (I =0.961) was found in comparisons of CT-1 with MD-1, VA-1, and WV-1. The mean F_{ST} value for all loci was 0.169 (Table 3). F_{ST} values for individual loci ranged from 0.096 for *EST-2*, to 0.345 for *LAP-1*. Finally, the mean Nm estimate for the entire population was 1.23.

Ten populations studied are represented by open-pollinated seed sources collected from a single maternal tree. The maternal bias due to this collection method would be expected to lower the electrophoretically detectable variation within these populations. To determine the variation between the two collection methods, the populations were separated into single-tree collections and multiple-tree collections and analyzed separately using the BIOSYS-1 program. The differences in alleles per locus and polymorphic loci between the two collection methods were 0.03 and 1.4%, respectively. Direct count heterozygosities for single-tree collections revealed a slight increase in heterozygosity with a difference of 0.013. The F_{IS} for the single tree collections was −0.602 and for multiple tree

collections −0.339. The difference of 0.263 indicates a higher level of heterozygosity within the populations represented by single-tree collections.

Principal component analysis

Eighty-three percent of the total variation in the 12 leaf and flower bud characters was explained by three principal components (PC) (Table 4). Forty-eight percent of the total variance was explained by PC1. Leaf surface area, leaf length, primary veins, and the various leaf width measurements all had relatively high positive loadings. The three flower bud characteristics and petiole length were not significantly correlated to leaf measurements and contributed little to PC1. In PC2, accounting for 22% of the total variation, flower bud length and flower bud width had high positive loadings and were highly correlated with the number of florets. No leaf measurements were significantly correlated with the flower bud characteristics in PC2. Thirteen percent of the total variance was accounted for by PC3. Petiole length, leaf length, and leaf width at base had the highest positive loadings in PC3, contrasted with negative loadings from the remaining leaf width measures. The remaining nine PCs accounted for 17% of the total variance with each accounting for less than 8%.

The relationships between populations, geographic factors and the variance explained by the PC's were graphically visualized by plotting the three most variable PC's against each other. In Figures 4 and 5, PC1 is represented on the X-axis in comparison with PC2, and PC3 on the Y-axis. The remaining biplot

combinations displayed similar patterns evident in these biplots. Distinct patterns or clustering of populations were not obvious with any of the PC combinations. Figure 4 illustrates the correlation between leaf size and flower bud size, where leaf size is represented on the X-axis (PC1), and flower bud size on the Y-axis (PC2). Approximately half of the populations clustered near the center of the graph, with moderate leaf and flower bud sizes, and the remaining populations exhibited unique characteristics. Population MI-2 (14), TX-1 (21), and MO-2 (16) displayed the most atypical characteristics. Population MI-2 possessed the largest leaves and flower buds, TX-1 had moderate leaf sizes and the smallest flower buds, and MO-2 possessed the smallest leaves and moderately large flower buds (Table 5). The lack of correlation between leaf size and flower bud size is visualized in Figure 4.

Principal component 1 by PC3 exhibited a scattered pattern similar to the pattern found in Figure 4, with no clustering of populations. Although no distinct patterns were discernable, the biplot can be used to distinguish atypical populations. For example, in Figure 5, IL-1, GA-1, and GA-2 all displayed moderate leaf sizes as determined by PC1 but they had unique leaf shapes determined by PC3. These populations on average had longer leaves with wider leaf bases (positive loadings) and narrow widths at 25% of the leaf length (negative loading). Therefore, the overall leaf shape can be characterized as a long leaf which tapers off quickly from a wide leaf base.

To determine the relationship between the variance found for the 12

characteristics and geographic origin, numeric designations were assigned to each population according to latitude alone, and latitude by longitude (Table 6). Patterns in biplots with latitude by longitude designations were similar but not as obvious as latitude alone and are not included in this report.

Distinct clustering of latitude groups was most evident when PC2 was included on an axis. Northern populations had the largest flower buds and southern populations had the smallest flower buds with the central populations clustered between the two. In Figure 6, where PC2 is plotted on the Y-axis against PC1 on the X-axis, populations with the most northern latitudes exhibited large positive values for PC2, populations with central latitudes exhibited moderate values, and the most southern populations displayed large negative values. Overall leaf size, represented by PC1, appeared to have no relationship with latitude.

Clustering of latitude groups in biplots with PC3 or PC4 on an axis was apparent only when plotted against PC2. Therefore, distinct latitudinal relationships were distinguished by PC2, which accounted for 22% of the total variance.

Discussion

Total isozyme diversity

The level of isozyme diversity revealed through electrophoresis indicates that *C. florida* has a low degree of genetic variation, 1.16 (A) and 9.89% (P), in comparison to other perennial angiosperm species. Investigations of isozyme

diversity in *Alnus crispa* (Bousquet et al., 1987), *Camellia japonica* (Wendel and Parks, 1985), *Robinia pseudoacacia* (Surles et al., 1989), and *Quercus* spp. (Hokanson et al., 1993) revealed ranges of 2.16 to 2.80 alleles per locus with the percent polymorphic loci ranging from 45% to 71%. In reviewing the plant isozyme literature, Hamrick and Godt (1989) found all plant species studied had an average of 50% of their loci polymorphic and 1.96 alleles per locus.

C. florida displays many of the life history traits that would be expected to induce high levels of genetic diversity for plant species; however, it is insect pollinated and seed dispersal is accomplished through animal ingestion and gravity. Both of these mechanisms limit gene flow, resulting in smaller effective population sizes. The plant isozyme literature provides ample evidence documenting a reduction in isozyme diversity due to insect pollination and animal/gravity seed dispersal (Hamrick et al., 1979).

Observed heterozygosity across all populations slightly exceeded the expected values for populations in Hardy-Weinberg equilibrium but all levels of heterozygosity were very low (Table 1). However, the accuracy of the chi-square test for determining conformance to Hardy-Weinberg equilibrium was reduced due to the relatively limited population sizes. F-statistics also revealed a small excess in heterozygosity, with the majority of the deviation residing among individuals within subpopulations (F_{IS}) (Table 3). There was considerable variation in the F_{IS} values between loci, with *Est-1* contributing greatly to the overall heterozygote excess in contrast with *Lap-1* having a small heterozygote

deficiency. Investigations of other plant populations have revealed that the highest levels of variability and divergence are often found in loci coding esterases (Gottlieb, 1981).

Population differentiation

The genetic distance, F_{ST}, and gene flow values indicate that long distance gene transfer is occurring at levels greater than expected for a plant species combining insect pollination with animal dispersal of seeds. Genetic distance and F_{ST} values revealed low levels of differentiation among *C. florida* populations. F_{ST} values were lower and estimates of migrants per generation (Nm) were higher than several outcrossed, insect pollinated species with animal dispersal of seed (Hamrick, 1987).

Plant populations accomplish gene transfer through pollen movement and seed dispersal away from the parent plant. Insect-pollinated species are generally limited in long distance gene transfer, as most insect flight distances from plant to plant are relatively short (Levin and Kerster, 1974). The two most common pollinators of *C. florida* are honey bees (*Apies mellifera*) and bumble bees (*Bombus* sp.). Both of the species are reported to have narrow foraging areas and tend to make successive trips to the same plant (Levin and Kerster, 1974). Therefore, the potential for long distance pollen transfer between populations is limited.

The apparent long distance gene flow that is occurring between populations

must therefore be attributable to animal dispersal of seeds. It is possible that long distance gene flow can occur via birds; however, the effect on overall genetic structure of a population may be difficult to assess. The effect of animal dispersal on genetic structure is dependent on the location of dispersal (unpopulated site or established population), the method of dispersal (single or clustered), and the proportion of seeds actually dispersed over long distances (Hamrick and Loveless, 1986).

Stiles (1980) classified *C. florida* fruit in the high-quality fruit category for eastern North American forest species. Fruit of this type are consumed by birds in large quantities because of their high nutritional value. A report on the frugivory of *Cornus canadensis* (similar fruit structure to *C. florida*) revealed that 53% of the fruit was removed by animals, with the majority of the fruit being consumed by birds (Burger, 1987). The potential for long distance seed travel may also be enhanced by migratory patterns. Depending on latitude, ripening of *C. florida* fruit occurs between September and November. This period coincides with the peak migratory period of eastern deciduous forest birds (Stiles, 1980). The increased quantity of fruit consumed during the migratory period has the potential to enhance gene flow between populations over a greater distance. This scenario seems to contradict the review by Hamrick and Loveless (1984) which found surprising levels of differentiation among plant populations with animal-ingested dispersal mechanisms. However, given the two possible mechanisms for gene flow, animal-ingested seed dispersal would likely yield the greatest long

distance gene transfer between *C. florida* populations.

Further evidence to support the apparent high levels of gene flow is revealed by the relationship between genetic distance values and geographic separation. In pairwise comparisons of genetic distance, the maximum divergence was seen among populations clustered along the eastern portion of the geographic range (CT-1 vs. MD-1, VA-1, WV-1). These data suggest that geographic separation is not an important factor in the differentiation of populations. Due, in part, to the presence of unique alleles in two individuals, the CT-1 population revealed the lowest identity values (I = 0.961), which is still greater than the average identity calculated over all outcrossing species by Gottlieb (1981). The lack of correlation between geographic separation and population differentiation is unexpected. Throughout its native range, with the possible exception of the central region, *C. florida* exists in a widely dispersed, patchy distribution. This type of distribution would theoretically lead to divergent populations along the perimeter of the native range where populations are subjected to selection pressures. The isozyme data suggest that strong divergent selection is not occurring in native flowering dogwood populations at the electrophoretic loci we examined.

Principal component analysis

PC analysis revealed a distinct association between flower bud size and population latitude (Fig. 6). Leaf characteristics were highly variable with no apparent relationship with latitude. The variation in flower bud size can be

attributed to adaptations for the differences in photoperiodic conditions throughout the geographic range. Similar ecotypic differentiation is known to occur in many woody plant species with large north-to-south ranges (Kozlowski et al., 1991). The strong association between latitude and morphological variation suggests that *C. florida* has been located in its present location for many generations. Many deciduous forest species associated with *C. florida* have pollen and/or fruit records dating to approximately 10,000 years before present (Delcourt and Delcourt, 1987). Over the period of migration, plants suited to shorter daylength survived the migration and formed the genetic foundation for adapted populations with morphological and physiological variations.

The ecotypic variation revealed by PC analysis coincides with observations of phenologic and cold hardiness variations reported in *C. florida* (Dirr, 1990; Heatley, 1986). Plant processes that are reportedly affected by daylength include: duration of extension growth, internode extension, bud break, leaf abscission, and induction of dormancy (Wareing, 1956). When woody plant species with southern origins are moved northward, they will often respond with an extended growth period in late summer, delaying floral initiation and dormancy (Kozlowski et al., 1991). Therefore, many of the growth and cold-hardiness problems affecting trees in northern landscapes may be associated with the observed ecotypic variation.

Contradictory levels of genetic variation were revealed by isozyme and morphological analysis. Isozyme analysis showed low levels of genetic variation and higher than expected levels of gene flow. The high levels of gene flow may

be partially attributed to an increased efficiency in seed dispersal due to fruit composition and phenology. The genetic diversity appeared to reside within populations, and the genetic distances indicated no relationship between allelic profiles and geographic origin. Conversely, PC analysis revealed a considerable amount of genetic variation with a significant relationship between flower bud morphology and geographic origin. The inconsistency in genetic variation revealed by isozyme and morphological analysis suggests that the variability at the isozyme loci examined in this study may underestimate the amount of variability in the entire genome. Several studies indicate that isozyme loci are not under the same selection pressures as quantitative traits and that quantitative traits often reveal geographic patterns (Muona, 1989). The results of this study concur with these previous investigations and suggest that the considerable variation in morphological traits is a more representative measure of genetic variation and ecotypic adaptation in *C. florida* populations.

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Table 1. Population variability estimates for 23 *Cornus florida* populations evaluated for isozyme diversity at 11 loci encoded by five enzymes.[z]

Pop.	# of trees	Collection method[x]	A	P	H_{Ob}	H_{Exp}
AL-1	5	M	1.3	18.2	.055 (.039)[y]	.073 (.056)
CT-1	5	S	1.3	18.2	.145 (.098)	.113 (.077)
GA-1	4	M	1.1	9.1	.023 (.023)	.023 (.023)
GA-2	3	M	1.2	9.1	.091 (.091)	.067 (.067)
IL-1	5	M	1.1	9.1	.055 (.055)	.042 (.042)
IL-2	4	S	1.2	9.1	.091 (.091)	.065 (.065)
IN-1	5	M	1.0	0.0	.000 (.000)	.000 (.000)
IN-2	5	M	1.2	9.1	.055 (.055)	.046 (.046)
KY-1	5	S	1.2	9.1	.055 (.055)	.046 (.046)
KY-2	5	S	1.0	0.0	.000 (.000)	.000 (.000)
MD-1	5	S	1.1	9.1	.091 (.091)	.051 (.051)
MD-2	5	S	1.1	9.1	.036 (.036)	.032 (.032)
MI-1	5	S	1.1	9.1	.018 (.018)	.018 (.018)
MI-2	5	M	1.1	9.1	.036 (.036)	.032 (.032)
MO-1	5	M	1.2	9.1	.036 (.036)	.034 (.034)
MO-2	5	M	1.2	9.1	.091 (.091)	.063 (.063)
OH-1	5	M	1.2	18.2	.109 (.091)	.069 (.052)
OH-2	5	M	1.3	18.2	.091 (.091)	.091 (.064)
PA-1	5	M	1.1	9.1	.018 (.018)	.018 (.018)
TN-1	5	S	1.2	9.1	.073 (.073)	.055 (.055)
VA-1	5	S	1.1	9.1	.091 (.091)	.051 (.051)
VA-2	5	M	1.2	9.1	.073 (.073)	.055 (.055)
WV-1	5	S	1.1	9.1	.091 (.091)	.051 (.051)
Means	4.83		1.1	9.89	.062	.048

[z] Variability estimates are, (A) the mean number of alleles per locus,(P) the % polymorphic loci (95% criterion), (H_{Ob}) observed heterozygosity, and (H_{Exp}) expected heterozygosity.

[y] Numbers in parenthesis indicate standard error.

[x] Seed collection methods are, (S) for single tree collections, and (M) for multiple tree collections.

Table 2. Descriptions and abbreviations of nine leaf characters and three flower bud characters measured and evaluated using principal component analysis for 24 *Cornus florida* populations in a provenance plantation.

Abbreviation	Description
PL	petiole length (cm)
PV	number of primary veins
LL	leaf blade length (cm)
LWW	leaf width at widest point (cm)
LW25	leaf width at 25% of the total length (cm)
LW50	leaf width at 50% of the total length (cm)
LW75	leaf width at 75% of the total length (cm)
SA	total surface area (cm^2)
LWB	distance from the widest point to leaf base (cm)
BL	flower bud length (mm)
BW	flower bud width (mm)
FLO	number of florets per bud

Table 3. Summary of F-statistics for each polymorphic locus from 23 *Cornus florida* populations.

Locus	F_{IS}	F_{IT}	F_{ST}
Lap-1	.123	.426	.345
Est-1	-.532	-.314	.142
Est-2	-.111	-.004	.096
Mean	-.455	-.210	.169

Table 4. Eigenvectors, eigenvalues and % of total variance for the four principal components accounting for 83.3% of the total variation from 24 *Cornus florida* populations in a provenance plantation.

Character	PC1	PC2	PC3
PL	.117	-.186	.427
LL	.315	.140	-.000
PV	.312	-.118	.435
LWW	.395	-.027	-.214
LW25	.339	-.026	-.380
LW50	.399	-.022	-.193
LW75	.352	-.058	-.111
SA	.406	-.084	-.022
LWB	.234	-.096	-.585
BW	.073	.581	.055
BL	.065	.590	.108
FLO	.081	.474	.135
Eigenvalue	5.800	2.606	1.596
% Variance	48.3%	21.7%	13.3%

Table 5. Mean data for the 12 morphological characters[z] used to determine patterns of variation in 24 *Cornus florida* populations.

Pop.	PL (cm)	PV	LL (cm)	LWW (cm)	LW25 (cm)	LW50 (cm)	LW75 (cm)	SA (cm^2)	LWB (cm)	BW (mm)	BL (mm)	FLO
AL-1	1.10	11.45	11.40	6.87	5.86	6.70	4.40	63.66	6.14	5.12	4.46	17.83
CT-1	1.15	11.00	10.73	6.76	5.14	6.68	4.98	47.00	5.46	6.79	5.54	23.40
GA-1	1.38	10.60	11.34	6.04	4.44	5.93	4.25	42.93	5.57	4.31	3.94	19.33
GA-2	1.12	11.10	11.78	6.28	4.53	6.23	4.40	46.10	6.14	5.55	4.84	20.70
IL-1	1.30	10.76	11.01	5.58	4.42	5.52	3.71	39.00	5.34	5.80	5.21	20.00
IL-2	1.46	10.35	10.61	6.19	4.82	6.11	4.60	44.60	5.28	5.11	4.61	20.80
IN-1	1.32	10.72	10.42	6.25	5.18	6.10	4.02	41.80	4.81	5.51	4.54	19.58
IN-2	1.21	10.92	10.36	6.16	5.22	6.08	4.28	42.08	4.82	6.04	5.30	19.86
KY-1	1.20	11.44	10.27	6.64	5.32	6.54	4.75	46.04	5.12	5.94	4.82	19.92
KY-2	1.02	10.36	9.43	6.18	4.84	6.08	4.34	38.84	4.63	5.64	4.87	21.62
MD-1	1.03	10.96	9.36	6.00	4.92	5.92	4.20	37.92	4.62	6.45	5.36	19.84
MD-2	1.14	10.64	9.42	5.72	4.69	5.61	3.88	35.20	4.63	5.56	4.79	18.90
MI-1	1.14	10.84	10.17	5.72	4.62	6.62	3.92	36.76	4.91	6.95	6.14	23.69
MI-2	1.11	11.76	11.19	7.23	6.20	7.07	4.64	53.16	5.12	7.51	6.80	23.72
MO-1	1.07	10.92	9.88	5.65	4.46	5.58	4.04	36.72	4.90	5.36	4.86	18.36
MO-2	1.09	10.24	8.68	5.00	3.82	4.92	3.44	27.64	4.29	5.89	5.18	22.16
OH-1	1.18	10.32	9.79	6.54	5.62	6.39	4.27	42.80	4.50	7.03	5.61	21.30
OH-2	1.16	10.52	10.42	5.74	4.53	5.64	3.87	38.32	5.06	6.77	5.77	23.44
PA-1	1.22	10.76	10.30	6.76	4.32	5.65	3.82	38.08	5.17	7.18	6.59	22.84
TN-1	1.34	11.60	11.27	6.94	6.69	6.80	4.80	50.05	5.45	6.35	5.49	22.85
TX-1	1.17	10.20	9.84	6.29	5.36	6.13	4.27	41.20	4.67	2.52	3.14	19.67
VA-1	1.02	11.05	10.52	6.25	5.04	6.04	3.90	43.35	4.92	5.55	4.61	20.90
VA-2	0.99	10.52	9.85	5.34	4.07	5.26	3.79	33.80	4.96	5.67	4.90	18.64
WV-1	1.11	10.84	9.77	6.06	4.75	5.96	4.41	39.92	4.86	6.40	5.33	22.30

[z] Morpological character abbreviations are, (PL) petiole length, (PV) number of primary veins, (LL) leaf length, (LWW) leaf width at widest point, (LW25) leaf width at 25% of length, (LW50) leaf width at 50% of length, (LW75) leaf width at 75% of length, (LWB) distance from widest point to leaf base, (SA) leaf surface area, (BW) flower bud width, (BL) flower bud length, (FLO) number of florets per bud.

Table 6. State of collection, number of individuals, population designations, latitude designations, latitude and longitude of 24 *Cornus florida* populations used for principal component analysis.

State	Pop. ID	# of ind.	Lat. ID	Latitude	Longitude
AL-1	1	4	3	33° 00′	86° 30′
CT-1	2	5	1	41° 60′	73° 00′
GA-1	3	3	3	33° 30′	83° 42′
GA-2	4	2	3	33° 07′	84° 30′
IL-1	5	5	2	37° 80′	89° 30′
IL-2	6	4	2	37° 28′	88° 42′
IN-1	7	5	2	38° 17′	86° 33′
IN-2	8	5	2	38° 40′	85° 10′
KY-1	9	5	2	37° 40′	86° 60′
KY-2	10	5	2	37° 27′	83° 10′
MD-1	11	5	2	39° 60′	76° 60′
MD-2	12	5	2	39° 00′	75° 80′
MI-1	13	5	1	42° 50′	84° 40′
MI-2	14	5	1	42° 45′	84° 23′
MO-1	15	5	2	37° 30′	91° 51′
MO-2	16	5	2	38° 80′	91° 80′
OH-1	17	5	1	40° 47′	81° 54′
OH-2	18	5	1	40° 40′	80° 56′
PA-1	19	5	1	41° 35′	79° 16′
TN-1	20	4	2	36° 12′	84° 50′
TX-1	21	3	3	31° 00′	93° 80′
VA-1	22	4	2	37° 50′	78° 60′
VA-2	23	5	2	37° 50′	80° 40′
WV-1	24	5	2	39° 30′	78° 25′

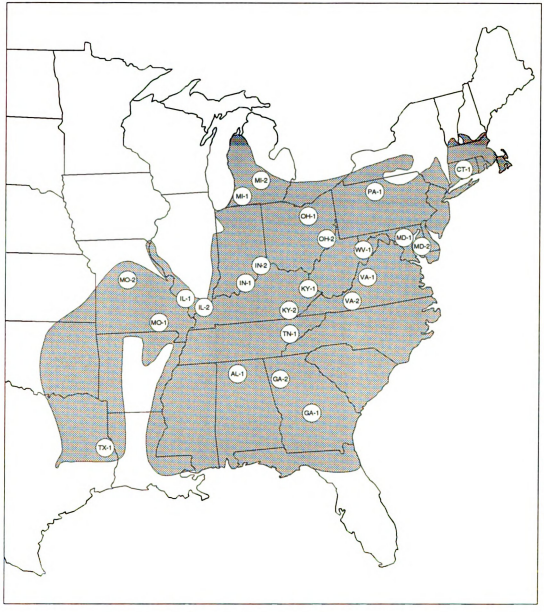

Figure 1. Natural range of *Cornus florida* and the location of specific seed sources represented in a provenance plantation at the W.K. Kellogg Experimental Forest, Augusta, Mich. (Adapted from Elias, 1987)

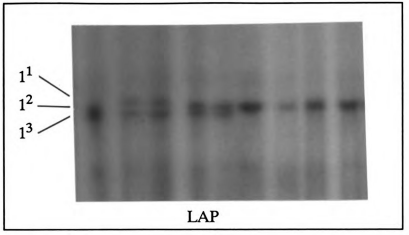

Figure 2. Electrophoretic banding pattern of leucine aminopeptidase (LAP) displaying a single polymorphic locus. LAP was scored as a single locus monomeric enzyme with 3 alleles. Allele 1 appeared only twice in two heterozygous individuals from the CT-1 *Cornus florida* population.

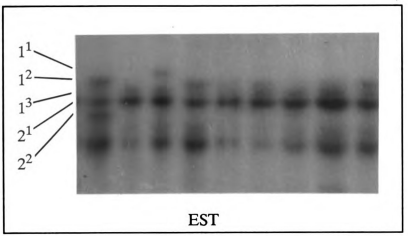

Figure 3. Electrophoretic banding pattern for esterase (EST) displaying two polymorphic loci. EST was scored as a monomeric enzyme encoded by two loci. Locus 1 contained 3 alleles. For locus 2, all *Cornus florida* trees were monomorphic for allele 1 except for a single individual from AL-1 (far left) scored as heterozygous for allele 1 and 2.

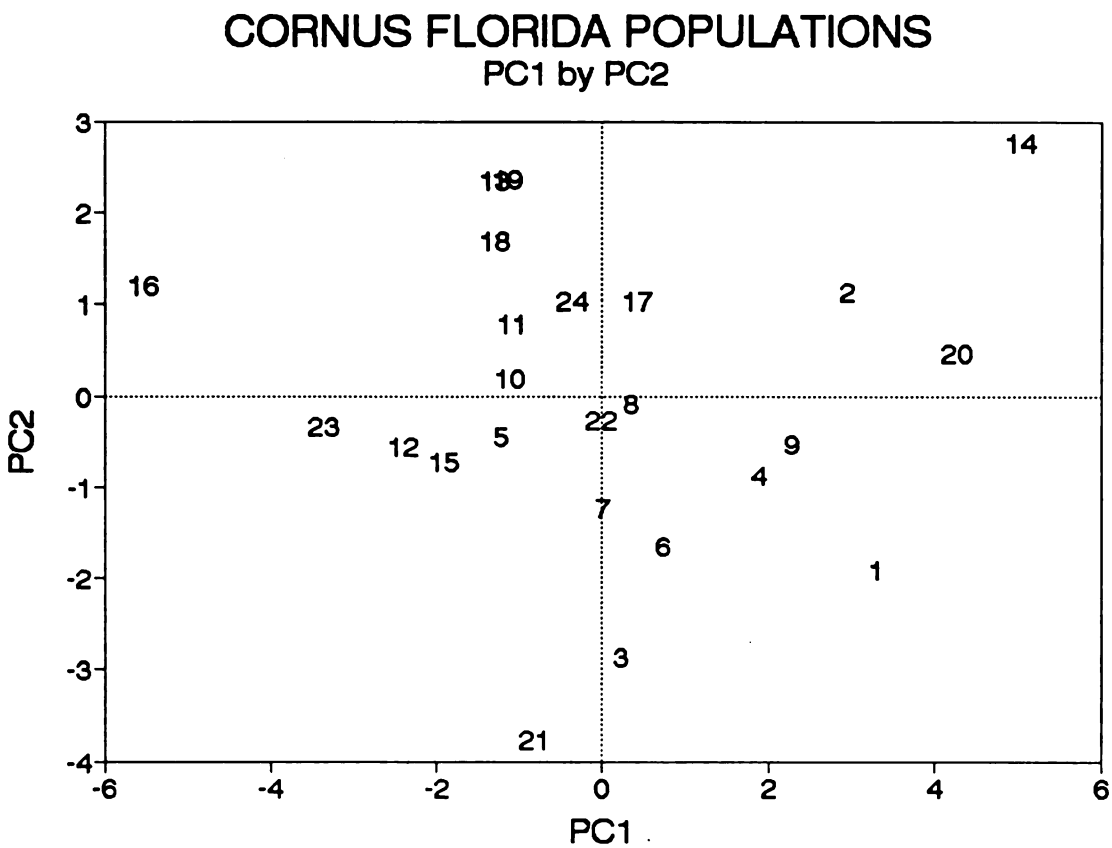

Figure 4. Relationship between eigenvector loadings derived from leaf and flower bud characters with *Cornus florida* populations listed in Table 5. PC1 (X-axis) accounted for 48% of the total variation and PC2 (Y-axis) accounted for 22%.

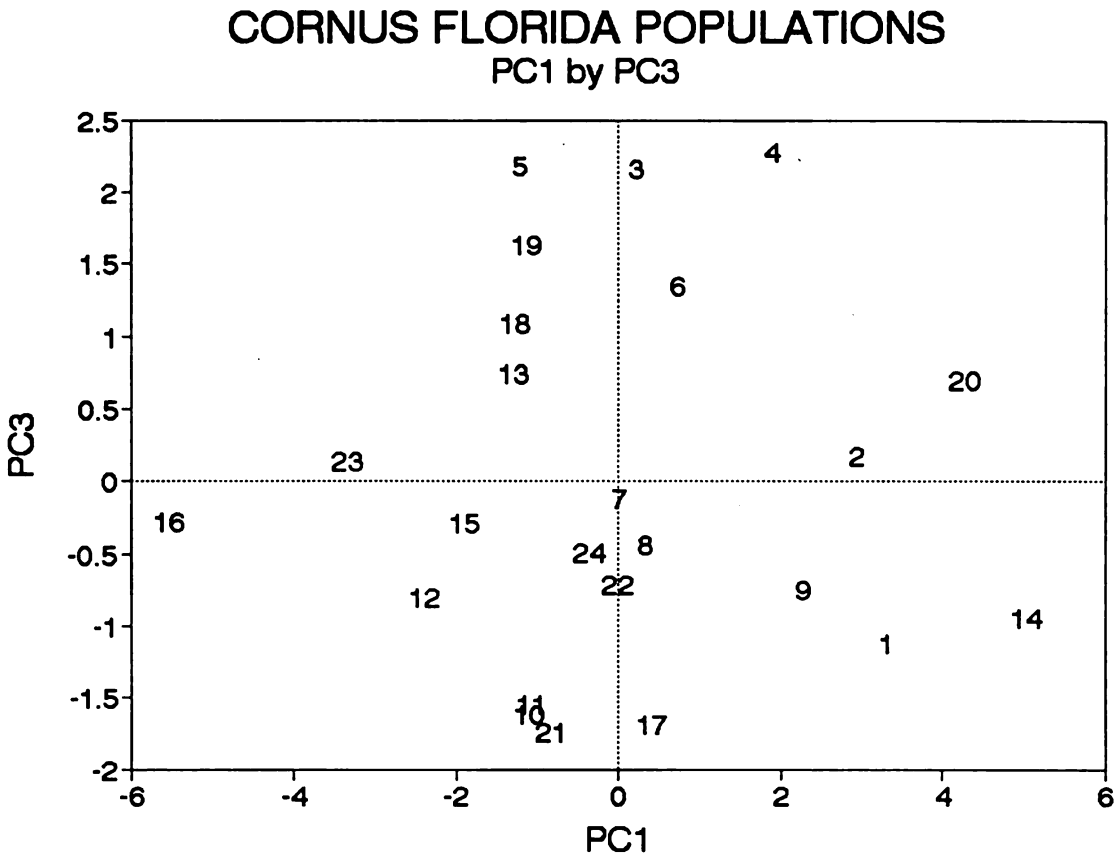

Figure 5. Relationship between eigenvector loadings derived from leaf and flower bud characters with *Cornus florida* populations listed in Table 5. PC1 (X-axis) accounted for 48% of the total variation and PC3 (Y-axis) accounted for 13%.

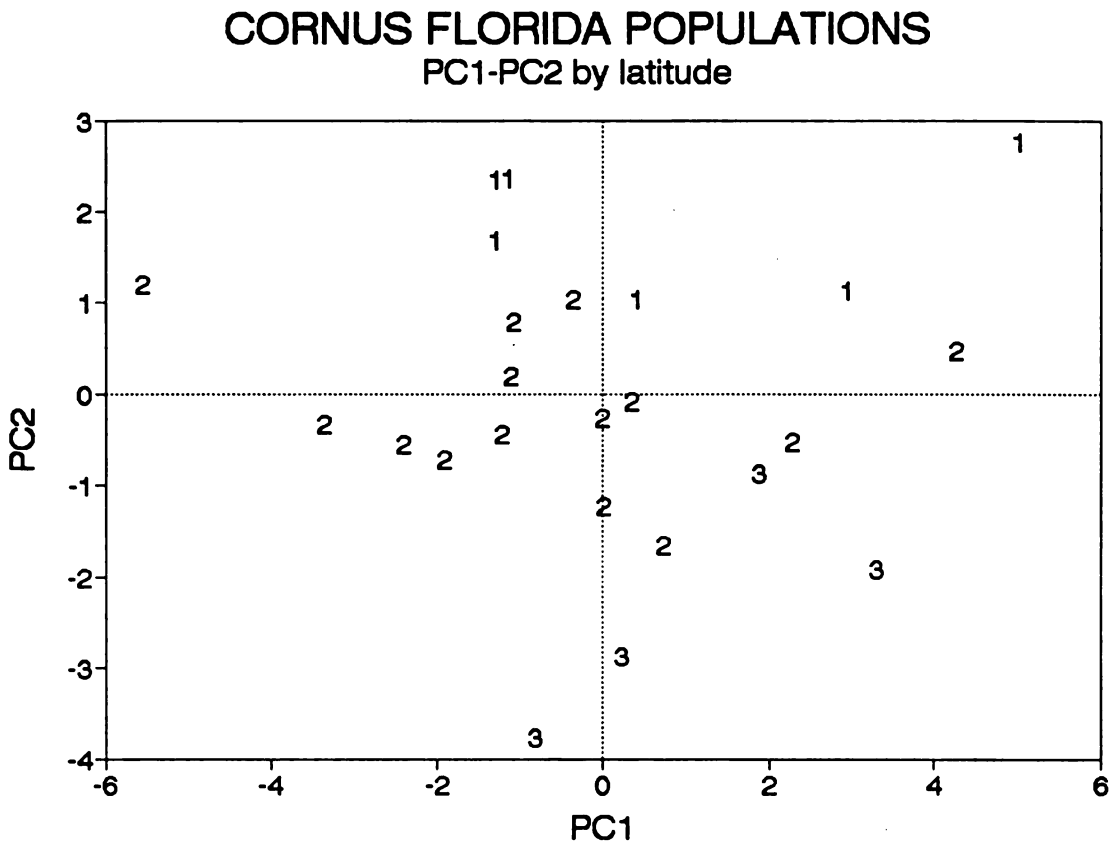

Figure 6. Relationship between flower bud characters represented by PC2 (Y-axis), and latitude of 24 *Cornus florida* populations in a provenance plantation. Latitude designations: 1 = >40°, 2 = 36-40°, 3 = <40°.

SECTION II. THE RELATIONSHIP BETWEEN GEOGRAPHIC ORIGIN AND COLD HARDINESS OF *Cornus florida* L. FLORAL TISSUES

Alexander Fernandez[1], Robert E. Schutzki[2], Gordon S. Howell[3]

Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

J. James Kielbaso[4]

Department of Forestry, Michigan State University, East Lansing, MI 48824-1325

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[1]Graduate Research Assistant

[2]Assistant Professor

[3]Professor

[4]Professor

The Relationship Between Geographic Origin and Cold Hardiness of *Cornus florida* L. Floral Tissues

Additional Index Words. flower bud hardiness, flowering dogwood, ecotypic variation, plant distribution

Abstract. Acclimation and mid-winter flower bud hardiness was evaluated for 10 flowering dogwood provenances using controlled freezing experiments. The peduncle proved to be the hardiest tissue in mid-winter (-28.9°C, -20.0°F) and the receptacle and florets the least hardy at -24.6°C (-12.3°F) and -24.3°C (-11.7°F), respectively. Outer bracts displayed significant variation among provenances; however, all trees had considerable damage caused by minimum temperatures in January potentially limiting the spring floral display. Northern provenances (MI, OH, PA) were on average more cold tolerant on all sampling dates. The greatest differences between northern and southern (AL, TN) provenances (approximately 6°C, 10.8°F) appeared during the acclimation period. Central provenances (IL, IN, KY, MD, WV) showed intermediate cold hardiness levels. The majority of the variation among provenances was reduced by mid-January with floret T_{50}'s of -25.4°C (-13.7°F), -24.4°C (-11.9°F), and -23.0°C (-9.4°F) for northern, central, and southern provenances, respectively. Significant damage was observed as a result of cold temperatures in January at the provenance plantation site with northern provenances displaying 29% to 41%

damage to florets, and southern sources 91% to 99%. *C. florida* flower buds are considerably less hardy than stem tissues demonstrating the importance of flower bud hardiness in determining the northern limits of native populations, as well as the ornamental effectiveness in northern landscapes.

Significance to the Nursery Industry

Cold temperature injury to flower buds of *Cornus florida* in northern landscapes significantly limits the ornamental effectiveness of the spring floral display. The degree to which flower buds are damaged is often related to the geographic origin of the original seed source. Consequently, trees with southern origins tend to have reduced cold hardiness capabilities and display increased flower bud damage in northern landscapes. This study investigated the relationship between cold hardiness and geographic origin of 10 *Cornus florida* seed sources. Also, the variation among floral tissues was determined within each bud. Our results indicate that flower bud damage may occur in northern landscapes regardless of the tree's geographic origin, but the extent of damage can be significantly reduced by selecting cultivars originating from seed sources with considerable mid-winter hardiness capabilities.

Introduction

Cornus florida L. (flowering dogwood) is a North American tree species that provides year-round ornamental interest. Considered one of the best native ornamental trees in the United States, it has been extensively cultivated throughout its geographic range (24). In fact, the 1978 census of agriculture registered annual *C. florida* sales of $10.3 million in the United States, more than cherry and plum trees combined (21). Another indication of the species popularity is the numerous selections of *C. florida* available in the nursery trade. Santamour and McArdle (17) list over 100 selections offered by the nursery industry with many new cultivars introduced since 1985 (5). The species has a wide geographic adaptability and is considered hardy in zones 5-9 (22). Dirr (5) lists *C. florida* as a zone 5 plant but cautions that seedling material from southern sources are less hardy than northern sources. Symptoms that have been associated with inadequate cold hardiness levels in landscape trees include; minimal flower production, distorted bracts, floret death, and stem dieback (5, 8). These problems have led researchers to investigate the cold hardiness capabilities of *C. florida* flower buds and stem tissues.

Cold hardiness investigations of *C. florida* flower bud and stem tissues have revealed considerable variation. Hardiness evaluations of flower buds using differential thermal analysis, revealed numerous low temperature exotherms between -13°C (9°F) and -23°C (-9°F) resulting in floret death (16). The number of recorded exotherms corresponded to the number of florets

demonstrating a wide range of floret hardiness within a single bud. Similar controlled freezing experiments on stem tissue hardiness showed a range in the level of cold temperature tolerance of -25 to -34°C (-13 to -29°F) with significant variation due to environmental adaptation in native populations (8, 15). Mid-winter stem hardiness of trees from northern seed sources were approximately 5°C (9°F) greater than those from southern seed sources (8).

The potential success of an ornamental plant in a new environment is often based on recommended plant hardiness zones. However, hardiness zone recommendations broadly categorize plants and do not account for the variation in cold hardiness observed across ecotypes and plant tissues in numerous ornamental plants (2, 10, 11, 12, 13).

Similar ecotypic variation has been observed in native populations of woody plants and the ecotypic differences have been correlated with latitude. Stem cold hardiness was closely related to latitude in *Quercus rubra*, and *Fraxinus americana* (1, 7). Smithberg and Weiser (19) observed earlier acclimation in northern clones of red-osier dogwood (*Cornus sericea*) compared to southern or warm climate clones and the observed ecotypic variation was attributed to photoperiodic adaptation throughout the species geographic range. In each of these studies, trees with northern origins sustained less cold temperature injury than those with southern origins. This relationship between latitude and cold hardiness has led to the assumption that cold temperature stress is an important selective force in determining the natural ranges of native woody plants (1, 7, 14).

The majority of experiments correlating latitude with cold hardiness in woody plants have focused on cold hardiness capabilities of vegetative tissues, principally xylem. Cold injury to xylem ray parenchyma cells results in black heart injury weakening and possibly killing the tree (4). However, flower buds have been found to be considerably less cold tolerant than vegetative tissues (15, 16). In *Quercus rubra*, Flint (7) found that, in all cases, stem cold hardiness levels were beyond the limit required for survival in the species range. Floral tissues of *Prunus spp.* were the least cold tolerant tissue and demonstrated a close relationship to the average minimum temperature at the species northern limit (14). In *C. florida*, the living bark, vegetative bud, and xylem survived experimental freezing temperatures sufficient to withstand expected minimum temperatures in the species northern range; however, florets were found to be considerably less hardy (15, 16). Sakai (16) suggests that flower bud hardiness may be the principal limiting factor in determining northern limits of distribution in *C. florida* populations.

The objectives of this study were to determine the cold hardiness capabilities of *C. florida* floral tissues, and investigate the relationship between flower bud hardiness and geographic origin. Information on maximum mid-winter hardiness, rates of acclimation, and tissue variation will allow horticulturalists to determine the ornamental potential for species and cultivars over a diverse range of locations.

Materials and Methods

Trees used in this study are located in an 18 year old provenance plantation at the W.K. Kellogg Forest in Augusta, Michigan. The experiment consisted of 10 provenances (AL, TN, IL, KY, IN, WV, MD, OH, PA, MI) with three trees per provenance representative of the species geographic distribution (Fig. 1, Table 1). Provenances were grouped into three regions based on latitude intervals of 3° 50′ (Table 1). Hardiness was tested on September 30, 1993, October 21, 1993, November 22, 1993, and January 12, 1994 to determine the variation during the acclimation and mid-winter periods. Deacclimation could not be evaluated due to extensive flower bud damage caused by extreme minimum temperatures in January; however, flower buds were sampled on February 17 and March 21, 1994 to determine the extent of damage caused by field temperatures. Twigs with terminal flower buds were collected to provide three buds per temperature treatment per tree. Samples were sealed in plastic bags, placed in a cooler and transported to East Lansing, Michigan where they were stored in a walk-in cooler at 3°C (37.4°F) overnight.

In preparation for artificial freezing, the samples were cut into 5-7.5 cm (2-3 in) long stems with terminal flower buds. For every temperature treatment, 30 samples (one per tree) were secured on a 112.5 cm (45 in) strip of masking tape, placed in contact with a strip of damp cotton gauze, and wrapped in aluminum foil bundles. Three bundles (replications) were prepared per temperature. Temperature within the bundle was monitored by a 26-gauge copper-constantan

thermocouple inserted into a twig in the center of the bundle and connected to a potentiometer. The bundles were placed in a Revco Ultralow freezer and the temperature was controlled by an Omega temperature controller. Three bundles were placed in a cooler at 3°C (37.4°F) and these served as the control. After stabilizing overnight at -3°C (26.6°F), the temperature was dropped at an interval of 3°C (5.4°F) per hour. When the predetermined temperature was reached, the bundle was removed and placed in a walk-in cooler at 3°C (37.4°F) to thaw. A temperature range was chosen for each sampling date so that the coldest temperature would kill all samples and the warmest temperature caused no tissue damage. After thawing overnight, the samples were removed from the bundles and placed into an aerated, high humidity container for 7 to 10 days.

The viability of florets, peduncles, receptacles, inner bracts, and outer bracts was based on presence or absence of oxidative browning (20). Flower buds were bisected and floral tissues were analyzed for damage using a dissecting microscope. T_{50} values (temperature at which 50% of the buds are killed) were calculated using the Spearman-Karber method (3). Chi-square analysis of viability data was accomplished using the SAS system for personal computers (18). Statistical significance was based on the total number of live versus dead tissues over the entire temperature range. The total number of samples per state varied between collection dates from 45 flower buds (3 trees x 3 replications x 5 temperatures) to 54 flower buds (3 trees x 3 replications x 6 temperatures). In addition, florets (15 to 30) within each flower bud were evaluated and totaled for

each provenance over the entire temperature range increasing the precision of the chi-square test.

Results and Discussion

The peduncle proved to be the hardiest floral tissue for all collection dates except for October when florets significantly increased in cold hardiness (Table 2). The receptacle was the least hardy, although many individual florets within a bud were killed at warmer temperatures than the receptacle. The T_{50} for florets was based on 50% floret death per bud; therefore, the T_{50} for an individual floret within a bud may vary considerably from the temperature calculated. The floret results parallel the findings reported by Sakai (16) and indicate a wide variation in floret hardiness within a single flower bud. Outer and inner bracts were significantly more cold tolerant than receptacles; however, it is possible that injury to the receptacle may disrupt the development of undamaged bracts leading to distorted or missing bracts in the spring (Table 2).

A significant amount of variation exists in the acclimation rates of flower buds from different provenances and between floral tissues (Fig. 2, Table 2). However, the total increase in hardiness from September to January was consistent at approximately 17°C (30.6°F) for all provenances (Table 3). Northern provenance hardiness was significantly greater for all tissues during the period between September 30, 1993 and January 12, 1994 (Table 3, 4). The differences in T_{50}'s between provenances were relatively small for the September collection, increased

between October and November, and narrowed again by January. The peduncle displayed the widest range in T_{50}'s with significant differences between all the northern and southern provenances (Table 3).

The greatest variation in cold hardiness occurred during the month of October. Northern provenances acclimated at a rate greater than central and southern sources reaching an average floret T_{50} of -21.7°C (-7.1°F) (Table 3). Central provenances revealed the widest range of T_{50}'s and acclimated at an intermediate rate. All floral tissues reacted similarly over this period, however, the florets displayed the greatest variation in acclimation rates with significant differences among many provenances (Table 4). Acclimation rates were relatively slower during the period between October and November for all provenances. Acclimation of the receptacle and florets of northern provenances slowed considerably with receptacles increasing approximately 1°C (1.8°F). However, considerable variation still existed between provenances for all floral tissues (Table 3, 4).

The January collection gives an indication of the mid-winter hardiness capabilities between provenances. Northern and central provenances acclimated at rates slower than southern provenances during the period from November to January (Fig. 2). The variation between northern and southern provenances was reduced in January with a difference of approximately 3°C (5.4°F).

On January 19, 1994, a minimum temperature of -29°C (-20.2°F) was reported at the W.K. Kellogg Forest. Based on the January T_{50} calculations, this

minimum temperature would be expected to cause considerable damage to all provenances (Table 3). The February 17, 1994 collection displayed extensive cold temperature damage in the controls and at all temperatures tested. The damage was especially severe for southern and central provenances. The two southern provenances, AL and TN, had 86% and 93% of the total control florets dead, respectively (data not shown). Central provenances displayed floret death ranging from 32% to 64% and northern provenances ranged from 23% to 34%. No tissues survived the lowest controlled freezing test temperature of -30°C (-22°F).

Similar results were seen in March with an increase in floret death for most provenances (data not shown). The increased floret damage over this period may represent the cumulative effect of minimum temperatures as temperatures in late February reached critical lows on two occasions (9). All tissues in the controlled freeze experiment were killed at -24°C (-11.2°F) showing a decrease in hardiness from February. Unfortunately, T_{50}'s could not be calculated for the deacclimation period due to the extensive damage caused by low field temperatures.

A final collection was completed on March 29, 1994 to determine the extent of flower bud damage (Table 5). A total of 20 buds per tree were collected and placed directly into a humidity chamber. Percentages of damaged tissues were similar to the control for the February and March collections. Floret death for southern provenances ranged from 91% to 99%, central provenances ranged from 30% to 81%, and northern provenances displayed the hardiest florets ranging from 29% to 41% (Table 5). The inner and outer bracts showed significant

differences in tissue damage among provenances. However, the variation in outer bracts was not as significant with the majority of all provenances showing damage in at least 90% of the buds examined (Table 5).

Effect of Flower Bud Hardiness on Geographic Distribution: Previous investigations into the levels of stem hardiness of *C. florida* found T_{50} values of -34.5 °C (-30.1 °F) for northern provenances in mid-winter (8). Temperatures recorded from 1970 to 1990 in East Lansing, Michigan, near the northern limit of the species, did not reach -34.5 C over the 21 year span. However, the probability of reaching the T_{50} of florets from northern provenances in the same time span is 48%, potentially limiting a population's reproductive capability. Central and southern provenances had T_{50} values considerably lower than the mean minimum temperatures expected in their regions with low probabilities of exposure to damaging temperatures (Table 1). It must be noted, however, that environmental conditioning plays an important role in determining cold hardiness capabilities (23). Therefore, conditions in Michigan during the winter of 1993-94, may have induced greater cold hardiness than what would be expected in the tree's original habitat. The T_{50} and weather data suggests that temperature is exerting a strong selective force on populations near the northern limit, and that floret hardiness is the principal limiting tissue in determining the northern boundaries of *C. florida* populations.

Varying rates of acclimation have also been shown to increase potential for

cold temperature injury before maximum hardiness is achieved (19). The acclimation rate among provenances varied considerably, but all provenances displayed hardiness levels sufficient to withstand expected minimum temperatures during the acclimation period (Figure 2). The ecotypic variation in acclimation rates of *C. florida* flower buds is similar to the two stage response observed in red-osier dogwood (19). Photoperiod apparently triggers the first stage of acclimation evidenced by considerable cold tolerance in absence of freezing temperatures in September. The second stage of acclimation is apparently triggered by freezing temperatures in October and November demonstrated by the three fold increase in T_{50} values over the two month period (Fig. 2).

The potential for floral damage during the acclimation period appears remote regardless of provenance. In 40 years, daily minimum temperatures at the W.K. Kellogg Forest have never reached the T_{50} values of floral tissues during the acclimation period. In November, temperatures were cold enough to cause damage to southern provenances in only two years, and only once to central provenances. Conversely, the probability for floral damage during mid-winter over the same time span was 55%, 50%, and 38% for southern, central, and northern provenances, respectively. Therefore, the critical period for native populations and ornamental plantings near the species northern limit appears to be mid-winter when plants are typically exposed to yearly minimum temperatures.

Effect of Flower Bud Hardiness on Fruit and Floral Display. Ecotypic variation in

maximum cold hardiness revealed in this study is of significant importance to the ornamental plant industry due to the popularity of *C. florida*. The majority of the selections listed by Santamour and McArdle (17) originated in nurseries located south of Kentucky and along the East Coast where native populations are abundant. This has led to observations of cold injury to flower buds, especially outer bracts, on landscape trees in the upper Midwest (5). Based on weather data from 1970 to 1990 in East Lansing, Michigan, damage to florets on trees in the upper Midwest can be expected to occur in at least 57% and 76% of the winters on cultivars with origins in the central and southern United States, respectively. It is interesting to note that even the hardiest flowering dogwoods with northern origins do not ensure successful flowering, with the probability of floret injury at 48%. However, a flower bud can sustain considerable floret damage and still have enough viable florets to remain ornamentally effective with the usual 3 to 8 fruit per cluster.

The floral structures associated with the floral display are the outer and inner bracts. Damage to the bracts leads to obvious distortions that drastically reduce the ornamental effectiveness of the display. Cold hardiness of both inner and outer bracts was significantly greater than florets and receptacles during the critical period in January and inner bracts proved to be the hardiest of the two bract tissues (Table 2). Comparisons of bract T_{50}'s and weather data indicate that bract damage can be significantly reduced if cultivars with northern origins are planted. The probabilities of outer bract injury between 1970 and 1990 were 0%,

33%, and 48% for northern, central, and southern provenances, respectively. However, it is not known what effect receptacle damage has on the development of bracts. Until the developmental relationship between these tissues is investigated through regrowth and floral evaluation studies, cold tolerance of receptacles should be used as the measure for determining the adaptability of new *C. florida* cultivars.

Considerable ecotypic variation in flower bud hardiness exists in *C. florida*. Flower bud tissues are considerably less hardy than stem tissues suggesting that the northern limits of the species range is limited by flower bud hardiness. In addition, variation in flower bud cold hardiness indicates a need for determining acclimation rates and maximum hardiness capabilities of existing *C. florida* cultivars and new introductions. This information could be used to classify individual cultivars according to minimum temperature tolerances, thus increasing the probability of success for ornamental landscape plants.

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Table 1 - Seed origins and the mean minimum temperatures expected (1970-90) for 10 *Cornus florida* seed sources. Seed origins were divided into three regions based on latitude[z].

Origin (county, state)	Latitude (°N)	Longitude (°W)	Mean Minimum Temp. °C (°F)	Region
Madison, Al.	33° 00′	86° 30′	-14 (6.8)	Southern
Anderson, Tn.	36° 12′	84° 50′	-17 (1.4)	Southern
Jackson, Il.	37° 80′	89° 30′	-22 (-7.6)	Central
Breathitt, Ky.	37° 37′	83° 10′	-21 (-5.8)	Central
Jefferson, In.	38° 40′	85° 10′	-19 (-2.2)	Central
Morgan, Wv.	39° 30′	78° 25′	-21 (-5.8)	Central
Harford, Md.	39° 60′	76° 60′	-18 (-0.4)	Central
Carroll, Oh.	40° 40′	80° 52′	-22 (-7.6)	Northern
Forest, Pa.	41° 35′	79° 16′	-28 (-15)	Northern
Ingham, Mi.	42° 45′	84° 23′	-24 (-11)	Northern

[z] Regions represent intervals of 3° 50′. Southern = 33° 00′ to 36° 50′, Central = 36° 51′ to 40° 00′, Northern = 40° 01′ to 43° 50′.

Table 2 - Mean floral tissue T_{50}'s of 10 *Cornus florida* provenances collected on four test dates.

Tissue	9/30/93 °C (°F)	10/21/93 °C (°F)	11/22/93 °C (°F)	1/12/94 °C (°F)	Tissue Mean
Floret[z]	-7.7 a[y]	-17.6 c	-19.9 bc	-24.3 a	-17.4 b
	(18.1)	(0.3)	(-3.8)	(-11.7)	(0.7)
Peduncle	-10.0 b	-17.4 c	-20.6 c	-28.9 d	-19.2 c
	(14.0)	(0.7)	(-5.1)	(-20.0)	(-2.6)
Receptacle	-8.6 a	-15.4 a	-16.7 a	-24.6 a	-16.3 a
	(16.5)	(4.3)	(1.9)	(-12.3)	(2.7)
Inner Bract	-9.0 a	-16.5 b	-19.5 b	-27.3 c	-18.2 b
	(15.8)	(2.3)	(-3.1)	(-17.1)	(-0.8)
Outer Bract	-8.6 a	-16.2 ab	-19.3 b	-26.4 b	-17.6 b
	(16.5)	(2.8)	(-2.7)	(-15.5)	(0.3)

[z] Floret T_{50}'s were based on 50% floret damage within a bud.
[y] Significance determined by chi-square analysis of dead vs. live samples. T_{50}'s followed by the same letter within a column are not significant.

Table 3 - Mean T_{50} values of northern, central, and southern *Cornus florida* provenances[z] for five floral tissues in a Michigan provenance plantation.

Date/Tissue	Northern T_{50} °C (°F)	Central T_{50} °C (°F)	Southern T_{50} °C (°F)
9/30/93			
Floret	-9.3 (15.3) b[y]	-7.7 (18.4) a	-6.0 (21.2) a
Peduncle	-13.5 (7.7) b	-9.1 (15.6) a	-7.3 (18.9) a
Receptacle	-10.2 (13.6) b	-8.1 (17.4) a	-7.5 (18.5) a
Inner bract	-10.9 (12.4) b	-8.5 (16.7) a	-7.5 (18.5) a
Outer bract	-10.8 (12.6) b	-7.8 (18.0) a	-7.3 (18.9) a
10/21/93			
Floret	-21.7 (-7.1) c	-17.1 (1.2) b	-14.0 (6.8) a
Peduncle	-21.3 (-6.3) c	-16.6 (2.1) b	-14.2 (6.4) a
Receptacle	-18.2 (-0.8) b	-14.6 (5.7) a	-13.3 (8.1) a
Inner bract	-20.2 (-4.4) b	-15.4 (4.3) a	-14.0 (6.8) a
Outer bract	-19.4 (-2.9) b	-15.1 (4.8) a	-14.0 (6.8) a
11/22/93			
Floret	-22.4 (-8.3) b	-20.8 (-5.4) b	-16.5 (2.3) a
Peduncle	-24.2 (-11.6) c	-20.9 (-5.6) b	-16.8 (1.8) a
Receptacle	-18.7 (-1.7) b	-16.6 (2.1) a	-14.8 (5.4) a
Inner bract	-23.3 (-9.9) c	-19.3 (-2.7) b	-16.0 (3.2) a
Outer bract	-23.3 (-9.9) c	-18.6 (-1.5) b	-16.0 (3.2) a
1/12/94			
Floret	-25.4 (-13.7) b	-24.4 (-11.9) ab	-23.0 (-9.4) a
Peduncle	-30.3 (-22.5) c	-29.0 (-20.2) b	-27.3 (-17.1) a
Receptacle	-25.3 (-13.5) a	-24.4 (-11.9) a	-24.0 (-11.2) a
Inner bract	-29.7 (-21.5) b	-26.8 (-16.2) a	-25.5 (-13.9) a
Outer bract	-28.3 (-18.9) b	-26.0 (-14.8) a	-24.8 (-12.6) a
MEAN	-20.3 (-4.5) c	-17.3 (0.9) b	-15.5 (4.1) a

[z] States represented in each region include: northern - MI, OH, PA, central - IL, KY, IN, WV, MD, and southern - AL, TN.

[y] Significance between regions (columns) determined by chi-square analysis.

Table 4 - Percentage of damaged florets over the entire temperature range on four collection dates in a *Cornus florida* provenance plantation.

STATE	9/30/93 (%)	10/21/93 (%)	11/22/93 (%)	1/12/94 (%)
AL	69 a[z]	58 a	69 a	54 c
TN	65 a	55 a	65 b	61 ab
IL	65 a	50 b	51 d	62 a
KY	59 b	48 b	58 c	56 bc
IN	65 a	32 c	34 ef	35 e
WV	50 c	23 d	38 e	36 e
MD	49 c	24 d	40 e	38 e
OH	36 e	5 f	32 f	38 e
PA	43 d	10 e	39 e	43 d
MI	56 b	7 ef	35 ef	37 e

[z] Significance within columns determined by chi-square analysis of dead vs live florets over the entire temperature range.
Temperature ranges for collection dates:
9/30/93 = -3 to -15 C 10/21/93 = -9 to -21 C
11/22/93 = -12 to -27 C 1/12/94 = -18 to -30 C

Table 5 - Percentage of damaged florets, inner bracts, and outer bracts on *Cornus florida* flower buds collected on March 29, 1994 following a field temperature of -29 C in January.

State	Total Florets Evaluated	Florets Damaged (%)	Inner Bracts Damaged (% out of 60)	Outer Bracts Damaged (% out of 60)
AL	997	91 b	87 b	91 ab
TN	1203	99 a	97 a	99 a
IL	1154	81 c	78 bc	92 ab
KY	1267	61 d	68 cd	88 bc
IN	1263	30 g	48 ef	95 ab
WV	1343	48 e	53 def	97 ab
MD	1322	39 f	60 de	92 a
OH	1512	41 f	22 g	75 c
PA	1298	29 g	38 f	97 ab
MI	1506	40 f	50 ef	95 ab

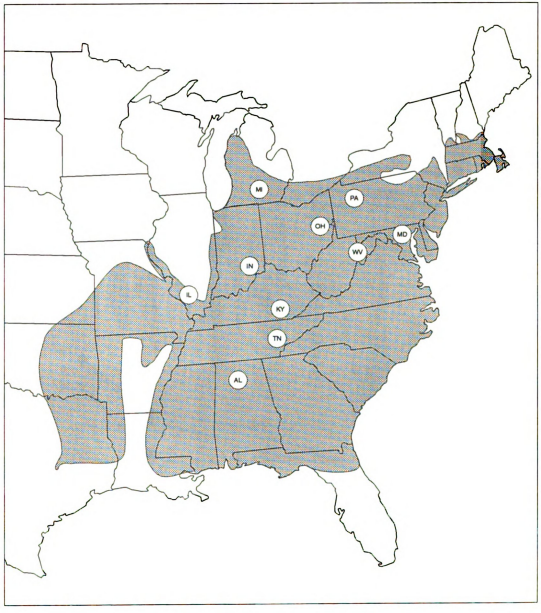

Figure 1 - Natural range of *Cornus florida* and locations of 10 seed sources evaluated for cold hardiness in a provenance plantation at the W.K. Kellogg Experimental Forest, Augusta, Michigan (Adapted from Elias, 1987).

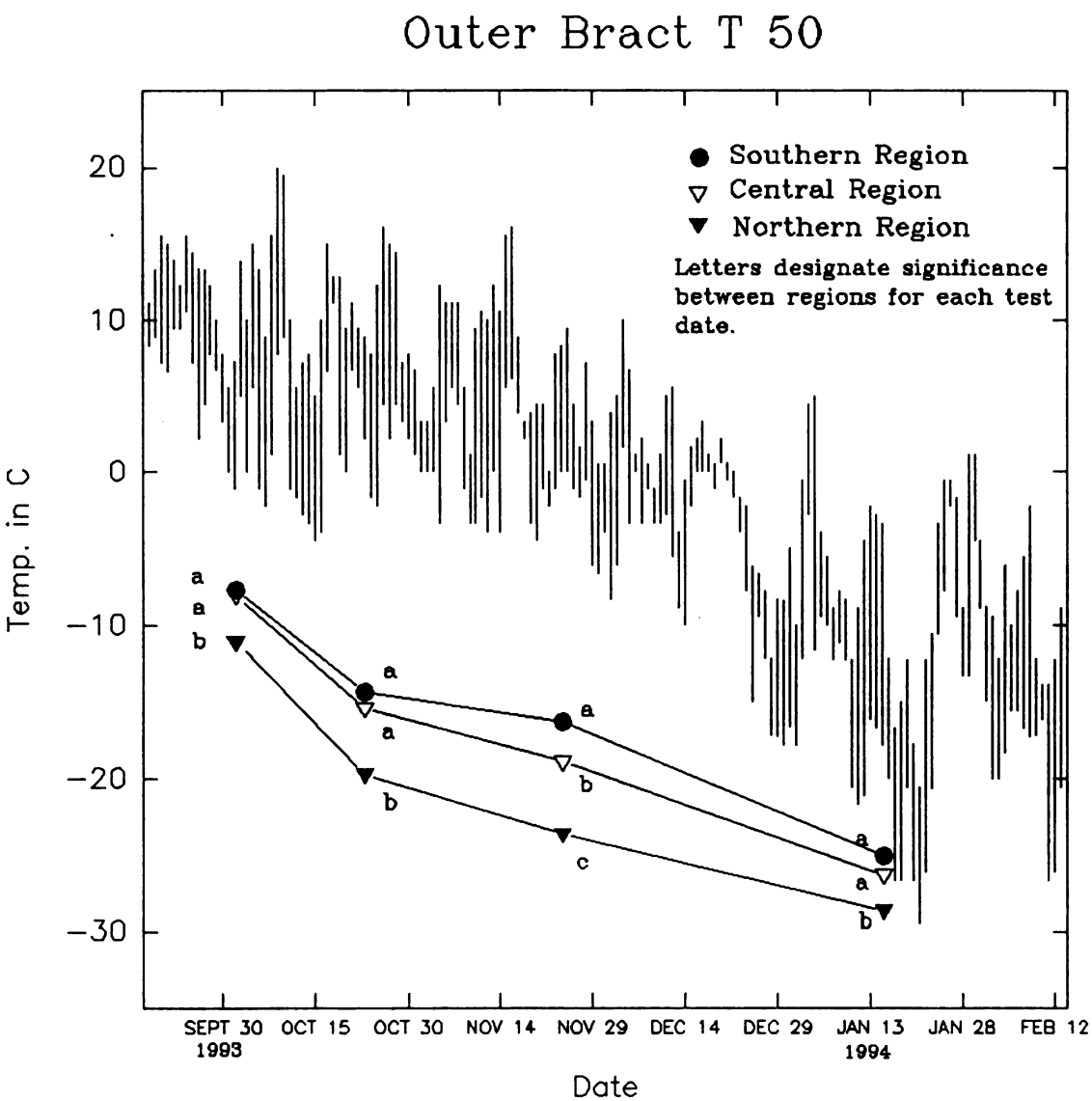

Figure 2 - Daily maximum/minimum temperatures recorded at the W.K. Kellogg Experimental Forest, and regional outer bract T_{50}'s of *Cornus florida* provenances located in Augusta, Michigan. Provenances within each region include: northern - MI, OH, PA, central - IL, KY, IN, MD, WV, southern - AL, TN.

SUMMARY AND CONCLUSIONS

Isozyme analysis of *Cornus florida* populations revealed low levels of variation in comparison to other woody perennial plant species. The genetic structure of plant populations is influenced by the species life history traits. Of the many life history traits, pollination and seed dispersal mechanisms are considered the most significant factors in determining population structure (Hamrick, 1986). The low levels of within population variation revealed in this study indicate that native *C. florida* populations are experiencing high levels of inbreeding resulting from short distance gene transfer. Conversely, low differentiation among populations indicate apparent high levels of long distance gene transfer.

Short distance gene transfer leading to inbreeding may be a result of insect pollination and gravity dispersal of seed in *C. florida*. Both of these traits result in relatively limited gene flow increasing the potential for inbreeding and homozygosity in plant populations. The apparent long distance gene transfer may be a result of seed dispersal by birds. The *C. florida* fruit matures in late October as many deciduous forest bird species begin their migratory patterns. During this period, birds are consuming large quantities of food and travelling relatively long distances (Levin, 1974). By this mechanism, long distance gene transfer can occur reducing the potential for divergence among native *C. florida* populations.

In contrast to the isozyme results, principal component analysis (PCA) of leaf and flower bud characters revealed considerable genetic variation among populations. Also, a distinct association between flower bud size and population

latitude was observed. The variation in flower bud size may be attributed to photoperiodic adaptations throughout the species geographic range. Similar ecotypic differentiation is known to occur in many woody plant species with large north-to-south ranges (Kozlowski et al., 1991). The ecotypic variation revealed by PCA coincides with observations of phenologic and cold hardiness variations reported in *C. florida* (Dirr, 1990; Heatley, 1986). Plant processes that are reportedly affected by daylength include: duration of extension growth, internode extension, bud break, leaf abscission, and induction of dormancy (Wareing, 1956). When woody plant species with southern origins are moved northward and exposed to longer daylengths in mid to late summer, they will often respond with an extended growth period, delaying floral initiation and dormancy (Kozlowski et al., 1991). The delay in floral initiation may in turn lead to the retardation in flower bud development observed in this study. Investigations into the phenology of extension growth and flower bud development will lend greater insight into the observed ecotypic variation and the influence environmental conditions, such as daylength, have on flower bud development.

Considerable ecotypic variation also exists in flower bud hardiness among *C. florida* populations with northern provenances displaying significant differences with southern provenances for every sampling period. The greatest variation was observed during the acclimation period. Florets and receptacles were the least hardy tissues, followed by outer bracts, inner bracts, and peduncles. The cold hardiness results coincide with the succession of damage observed under field

conditions as the florets and outer bracts appear damaged before the inner bracts. However, the effect of receptacle damage on bract development is not known. Therefore, until the developmental relationship is investigated, the receptacle results should be used as a measure of flower bud cold tolerance for new and existing *C. florida* cultivars.

Flower bud tissues were considerably less hardy than stem tissue hardiness reported by Heatley (1986), and Sakai (1982). Based on weather station data, central and southern provenances displayed hardiness levels sufficient to withstand temperatures in their native habitats. Northern populations, however, could be expected to have flower bud damage in nearly 50% of the of the winters from 1970 to 1990. This data suggest that flower bud hardiness has a significant influence on determining the northern distribution of *C. florida* populations.

The low level of isozyme variation revealed in this study was inconsistent with the significant variation observed in morphology and cold hardiness capabilities. Several studies have shown that isozyme loci are not under the same selection pressures as quantitative traits and that quantitative traits often reveal geographic patterns (Muona, 1989). This study concurs with the previous investigations as the morphological traits and cold hardiness capabilities revealed significant variation, and the observed variation displayed distinct geographic patterns. These results suggest that the quantitative traits are a more representative measure of genetic variation and ecotypic adaptation in native *C. florida* populations.

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