In Vitro Propagation and Selection of Superior Wetland Plants for Habitat Restoration[1]

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Increased restrictions on field collection of wetland plants used for restoration and creation have promoted efforts to develop more efficient nursery production practices including the use of in vitro propagation (micropropagation). Selection and in vitro propagation of wetland plant genotypes from populations adapted to particular site conditions could enhance wetland restoration or creation success. In this preliminary study, comparison was made of the early ex vitro growth responses of in vitro propagated *Pontederia cordata* L. genotypes collected from five Florida populations. Significant differences in plant height, leaf production, and flowering between genotypes were observed. Correlation of these early growth differences with capacity for adaptation to specific site conditions will require evaluation of the survival and growth of these genotypes under field conditions.

INTRODUCTION

Once thought of as wastelands, wetlands are now considered important for maintaining water quality, recharging groundwater, providing unique wildlife habitats, and storing flood waters. Federal and state statutes require restoration of degraded wetlands, or replacement of destroyed wetlands (mitigation), through extensive planting and successful establishment of herbaceous and woody wetland species. Requirements for 100,000 plants for a single restoration project are not uncommon. Many wetland species are also used for ornamental purposes in water gardens and aquascaping of retention ponds. Consequently, these markets combined have resulted in rapid expansion of the wetland plant industry (Pategas, 1992).

Herbaceous and woody wetland plants used for habitat restoration are obtained from several sources: (1) bareroot transplants collected from natural populations; (2) seeds and vegetative propagules in mulch or peat from donor wetlands; and (3) nursery-grown seedlings and vegetatively propagated plants. Collection of bareroot transplants from donor wetlands has lead to over-collection in and subsequent damage to donor sites in some areas. Increased restrictions on field collection have promoted efforts to develop more efficient nursery production practices for wetland plants including the use of in vitro propagation (Brumback, 1990; Kane, 1996; Street, 1994; Sutton, 1990).

Nursery production for restoration/mitigation often raises two ecologically important concerns: (1) the lack of knowledge and maintenance of genetic diversity within vegetatively propagated species and (2) the potential negative results following introduction of plants genetically "mismatched" to wetland site conditions. Some

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regulatory agencies have attempted to set guidelines that restrict collections of either bareroot transplants or propagules for nursery production from only local provenance plants within a limited radial distance from a planting site. However, the relationship between geographical source distance and wetland plant adaptability is not known. The practice of selecting and propagating wetland plant ecological varieties (ecotypes) from populations adapted to particular site conditions may more effectively ensure wetland restoration or creation success. Application of micropropagation could facilitate genetic selection, production, and storage of wetland plant ecotypes exhibiting superior adaptation to specific site conditions (Kane, 1996). *Pontederia cordata* L. (pickerelweed), a perennial emergent herbaceous wetland plant, is one of the most frequently used plants in wetland restoration projects in the southeastern U.S. (Brown, 1988). In the present study, the early ex vitro growth responses of in vitro propagated *P. cordata* genotypes collected from five Florida populations were compared.

MATERIALS AND METHODS

Plant Source and Micropropagation. Single plants of *Pontederia cordata* were collected from five Florida populations: (1) shallow marsh, Steinhatchee, FL (**NWCF**); (2) deep-water site, Lake Kanapaha, Gainesville, FL (**NCF1**); (3) shallow retention pond, Gainesville, FL (**NCF2**); (4) white-flower variety, Paynes Prairie, Gainesville, FL (**NCF3**); and (5) shallow marsh, Tampa, FL (**WCF**). Collected plants were established in commercial soilless potting mix (Metro-Mix 500) contained in 1-gal plastic containers and maintained under greenhouse conditions prior to culture establishment. Specimens were verified as being genetically different using random amplified polymorphic DNA (RAPD) analysis (results not shown). Two *Pontederia* varieties, *P. cordata* var. *cordata* and *P. cordata* var. *lancifolia*, are recognized and separated by differences in flower characteristics and leaf morphology (Godfrey and Wooten, 1979). *Pontederia cordata* var. *cordata* and *P. cordata* var. *lancifolia* produce lobed ovate- and lanceolate-shaped leaves, respectively. With the exception of WCF, the genotypes screened were *P. cordata* var. *cordata*.

Each genotype was in vitro propagated by shoot culture and rooted in vitro using previously described methods (Kane et al., 1991). On 21 June 1993, rooted microcuttings were planted into 38-cell trays containing Metro-Mix 500 and acclimatized under mist (5 sec every 10 min) in partial shade for 7 days. Trays were then moved to a 50% shade house when initial measurements were made. After 7 days, 10 plants of each genotype were transplanted into individual 6-inch azalea plastic pots containing Metro-Mix 500. On 6 July, pots were placed in full sun in an outdoor vinyl-lined trough (119 cm × 734 cm) with a water depth of 7.5 cm containing 20-20-20 liquid Peters fertilizer (300 mg liter^{-1} N). A completely randomized block design was used. Plant height, leaf, shoot, and flower number were recorded weekly. However, with the exception of flowering data, only the 9th week data collection is shown. At the end of the Week 9 study (31 Aug. 1993) the area of two of the newest fully expanded leaves from each plant was determined with a Li-Cor Model LI-3000A area meter. Shoot and root dry weights were also determined. Data were statistically analyzed using the General Linear Model (GLM) procedure (SAS, 1985). Where appropriate, significant ($p \leq 0.05$) mean separation was achieved using Duncan's Multiple Range Test.

RESULTS

The *Pontederia* genotypes screened exhibited significant differences in growth and shoot regeneration during the 9-week growth study. By Week 9, the white-flowered genotype NCF3 exhibited significantly greater shoot and leaf production than the other genotypes evaluated (Fig. 1). Both NCF1 and NWCF were significantly taller than the other genotypes (Fig. 1). The WCF genotype exhibited the shortest plant height and lowest shoot and leaf production (Fig. 1). Genotype had a significant effect on leaf area (Fig. 2). Not surprisingly, the smallest leaf area was observed in WCF, a specimen of the narrow-leaf *P. cordata* var. *lancifolia* (Fig. 2).

Patterns of flowering also differed between genotypes. Flowering was first observed by Week 5 in NCF1 and NCF3 (Fig. 3). By the Week 7 all genotypes were flowering. However, by the Week 9 both NCF1 and WCF ceased flowering. Among the genotypes, flowering was significantly greater (3 flowers/plant) in NCF3. Enhanced flowering in NCF3 may be the result of greater shoot production (Fig. 1). Shoot and root dry weights for NWCF, NCF1, NCF2 and NCF3 were not significantly different. Both shoot and root dry weights were significantly lower in WCF. There were no significant differences in root : shoot dry weight ratios between genotypes.

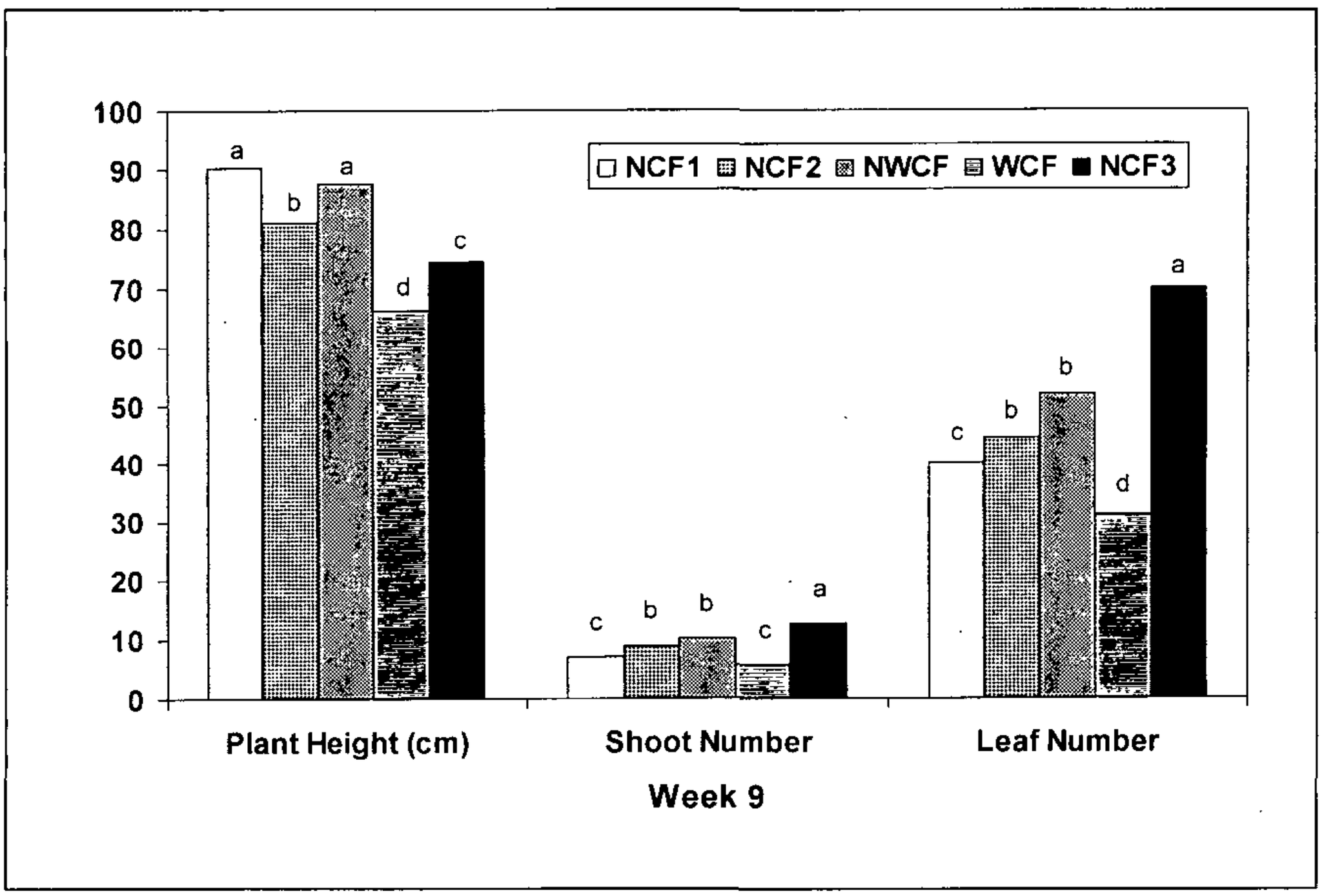

Figure 1. Comparison of plant height, and shoot and leaf production by five *Pontederia cordata* genotypes after 9 weeks growth. Each histobar represents the mean response of 10 plants. Histobars with the same letter are not significantly different at the 5% level.

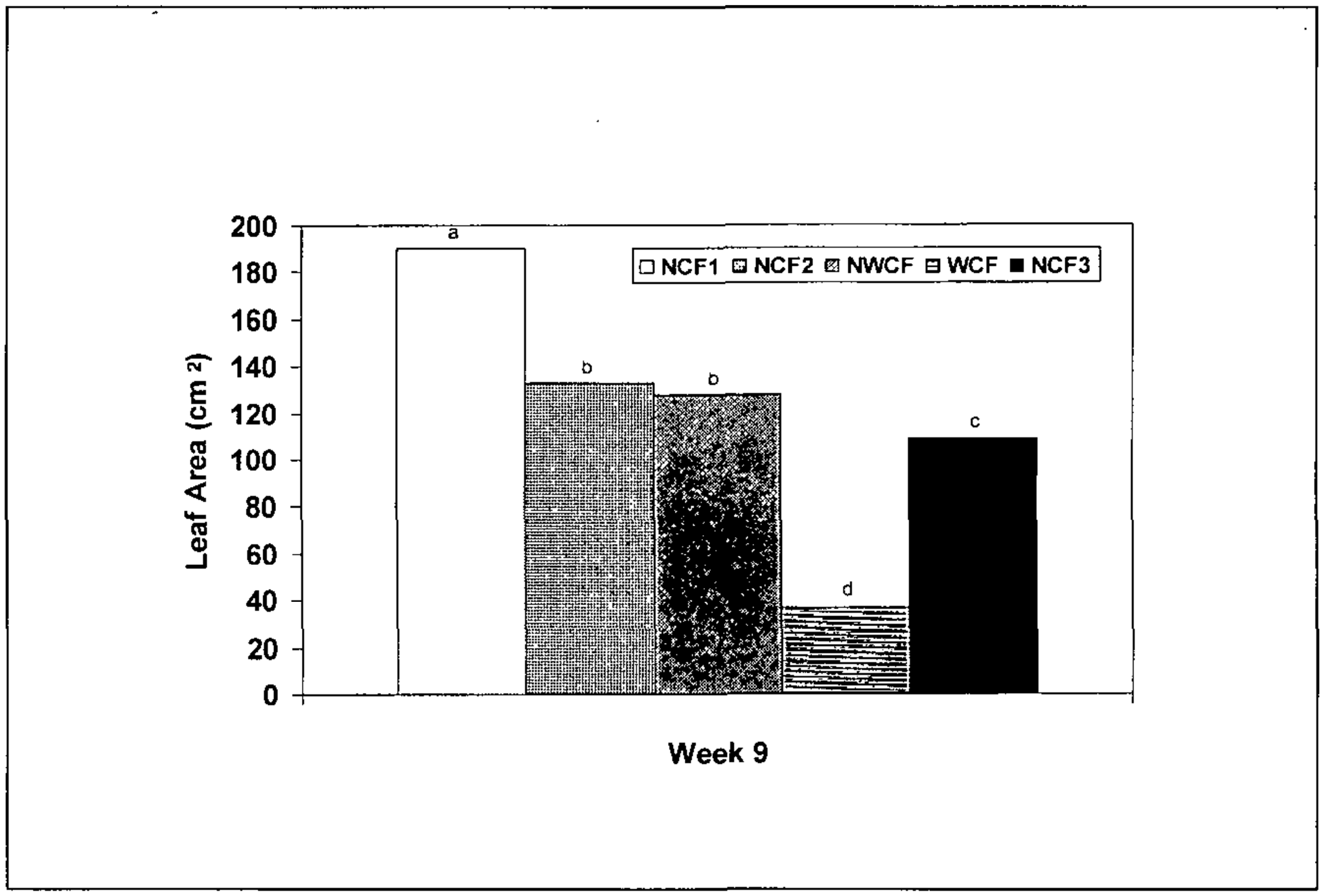

Figure 2. Comparison of leaf area of five *Pontederia cordata* genotypes after 9 weeks growth. Each histobar represents the mean area of the two newest fully expanded leaves from 10 plants. Histobars with the same letter are not significantly different at the 5% level.

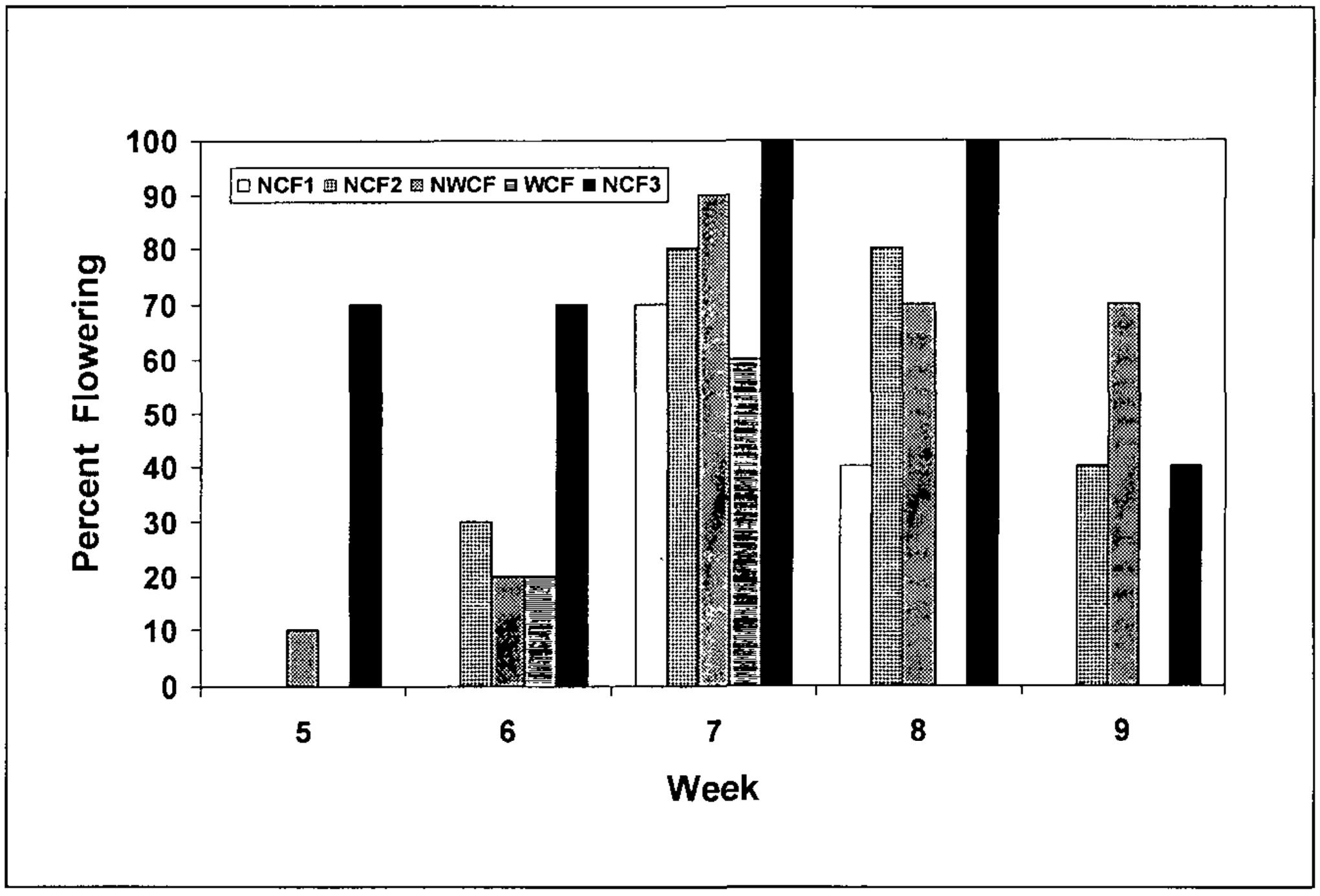

Figure 3. Comparison of flowering response of five *Pontederia cordata* genotypes during 9 weeks growth. No plants flowered prior to Week 5. Each histobar represents the percent flowering of 10 plants.

DISCUSSION

The RAPD analysis revealed that the *P. cordata* specimens collected from the five Florida wetland populations exhibited a high degree of genetic divergence. This is not surprising since *P. cordata* possesses a breeding system in which populations contain individual plants producing one of three floral types (tristyly). Tristyly promotes outcrossing and enhancement of genetic diversity (Price and Barrett, 1982). Apparently, this genetic variation can be observed as differences in the early ex vitro growth and development of in vitro propagated plants.

Presumably genetic variation is beneficial for most species since it allows populations to adapt to changing conditions. Environmental conditions at the donor sites possibly play a role in genetic selection in natural *Pontederia* populations. For example, NCF1 plants, in vitro propagated from a tall large leaf specimen growing in deep water, exhibited similar morphological traits when grown under controlled conditions. Conceivably, these early growth and morphological differences can be correlated with capacity for adaptation to specific site conditions.

Although plants in this study were grown under nonflooded conditions, this screening procedure could be modified to evaluate responses to different hydrologic conditions and soil types. Validation of this procedure would require subsequent field evaluation of the screened wetland genotypes. Survival and growth of four *Pontederia* genotypes screened in this study have been examined under field conditions and results will be reported elsewhere. Selecting and micropropagation of multiple wetland plant genotypes from populations physiologically adaptable to particular site conditions may prove both commercially valuable and useful in ensuring wetland restoration/mitigation project success.

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