Wetland Plant Propagation: Comparative Growth and Reproduction of Micropropagated *Sagittaria latifolia* Ecotypes[1©]

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INTRODUCTION

Selecting and planting of native wetland plant species comprise the major tasks of most wetland habitat restoration/mitigation projects. There has been a shift from field harvesting of plants to increased reliance on nursery-propagated wetland plants (Pategas, 1992; Sutton, 1995). This shift to nursery production has generated concerns regarding the maintenance of genetic diversity and potential negative results following introduction of plant ecotypes genetically "mismatched" to specific wetland site conditions (Kane and Philman, 1997). Consequently, some regulatory agencies have established guidelines to restrict collection of propagules for nursery production only from populations within a limited radial distance from the restoration site. In principle, this policy appears ecologically sound. However, the relationship between geographical distance of source plants and the relevance to successful wetland habitat creation/restoration remains unclear.

Commercial micropropagation of wetland plants used for habitat restoration provides an alternative to field collection and traditional nursery propagation that could facilitate selection, rapid production, and storage of many genotypes that are physiologically adapted to specific habitat conditions (Kane, 1996; Seliskar, 1995). Reports of successful habitat restoration using micropropagated plants support this approach (Bird et al. 1994, Durako et al., 1993; Kane and Philman 1993). However, in most studies, survival and growth of single micropropagated clones were examined. Seasonal differences in ex vitro growth performance and reproduction of micropropagated wetland ecotypes of several species have been reported (Kane and Philman, 1997; Kane et al. 2000). Clearly, more information is needed concerning the degree of genotypic variation within wetland species and the advantages and limitations of exploiting this variation for habitat restoration. This information would be extremely valuable to commercial micropropagation laboratories and nurseries producing wetland plants.

Sagittaria latifolia Willd. (broad leaf arrowhead) is an important perennial herbaceous wetland plant, propagated by rhizomes, corms, or seed (Marburger, 1993; Sutton, 1995). In the current study, the ex vitro growth and corm formation of six micropropagated *S. latifolia* ecotypes, collected along a longitudinal gradient of the eastern seaboard, were compared when grown under nursery conditions for a growing season in north-central Florida.

MATERIALS AND METHODS

Rhizome shoot-tip explants excised from single plants collected from populations in Rhode Island (RI), North Carolina (NC), South Carolina (SC), and three Florida populations [(Lake Lochloosa (NFL), Lake Kissimmee (CFL), and Lake Okeechobee

(SFL)] were surfaced sterilized and established in vitro in a liquid basal medium (BM) consisting of half-strength Murashige and Skoog mineral salts (Murashige and Skoog, 1962), 0.56 mM myo-inositol and 1.2 µM thiamine supplemented with 87.6 mM sucrose. Indexed shoot cultures were used to clonally multiply each ecotype by rhizome production in BM supplemented with 0.25 mg liter^{-1} benzyladenine (BA) and solidified with 7 liter^{-1} TC Agar™ (PhytoTechnology Laboratories, Shawnee Mission, Kansas). Stage II shoot microcuttings of each ecotype were obtained from 28-day old cultures and acclimatized and rooted in 38-cell plug trays containing Fafard Mix #2 soilless medium (Fafard, Inc., Apopka, Florida) under intermittent mist (5 sec/10 min) for 14 days before being transferred to a glasshouse in holeless flats containing liquid Peters 20N-20P-20K (150 mg liter^{-1} N) for an additional 28 days. The nutrient solution was exchanged weekly.

After the 28-day period, plants were individually transplanted into 5-gal black plastic pots containing Fafard Mix #2 soilless medium. At the time of transplant, four Sierra Planting Tablets 16N-8P-12K +Minors (Scotts Company, Maryville, Ohio) were placed in each pot. An additional fertilizer tablet was added per pot after 2 months. Immediately after transplanting, on 20 June 2000, the containers were placed in an outdoor trough (119 cm × 734 cm) filled with water to a depth of 7.5 cm (Fig.1). The experiment was initiated (T_0) on 26 June by removing all but the youngest fully expanded leaf.

The experiment was a completely randomized block design consisting of three blocks each containing 6 plants of each genotype. Shoot production, leaf number, and plant height were determined at Week 12 (18 Sept.). Flowering was recorded at 2-week intervals for the first 12 weeks. At Week 22 (29 Nov.), corm production per plant and average corm dry weight were also determined. Data were statistically analyzed using the General Linear Model (GLM) procedure (SAS, 1985). Where appropriate, significant ($p < 0.05$) mean separation was determined using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Significant differences in shoot, leaf, and corm production were observed between the *S. latifolia* ecotypes. Shoot production occurred via rapid formation of rhizomes. The most northern ecotype (RI) exhibited significantly slower shoot and leaf production than the other ecotypes (Fig. 2A-C). All RI plants exhibited symptoms of high temperature stress including leaf yellowing and necrosis throughout the study. Maximum shoot production was observed in NC (Fig. 2A). No correlation was observed between latitudinal origin and leaf production (Fig. 2B). However, when grown under north central Florida conditions, the *S. latifolia* ecotypes from the more southern latitudes were significantly taller than the more northern ones (Fig. 2C).

Corm production per plant was high (40 to 51) in all ecotypes (Fig. 3A). No correlation was observed between latitudinal origin and corm dry weight. Corm shape and pigmentation varied between ecotypes. RI corms were significantly smaller both in size and dry weight (Fig. 3B). The onset of corm formation was not examined in this study. However, seasonal differences in the induction and duration of corm formation between *S. latifolia* ecotypes have been observed. Induction of corm formation occurred more rapidly in the northern ecotypes (Kane and Philman, 2000). Interestingly, the Florida ecotypes (NF, CFL, and SFL) all produced numerous corms by late fall in this study (Fig. 3A). This is in contrast to earlier

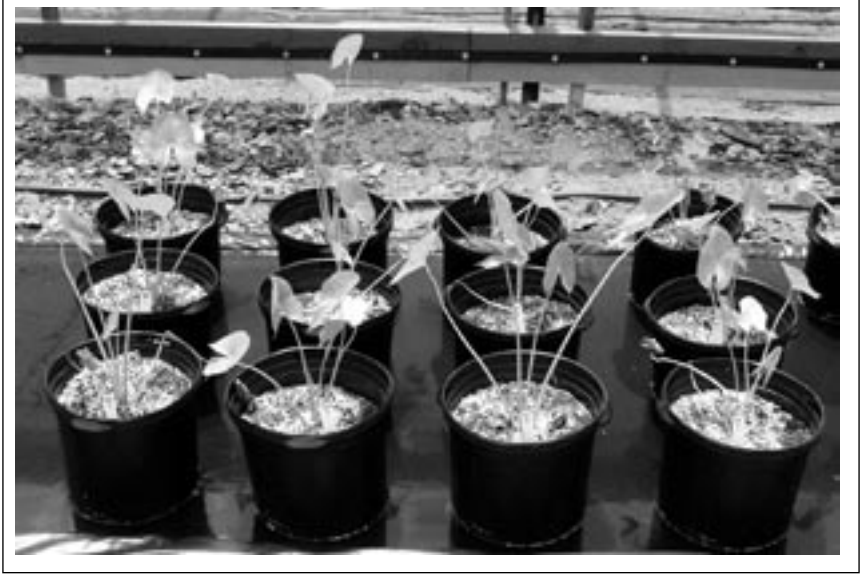

Figure 1: Tank culture system used to evaluate containerized *Sagittaria latifolia* ecotypes.

studies, in which the Florida ecotypes failed to produce corms when grown outdoors in smaller containers for 42 days, regardless of season (Kane, unpublished). The possibility exists that plants of the Florida ecotypes must reach a minimum size before corms are produced. The ecotypic differences in corm induction are probably the consequence of adaptation to latitudinal differences in natural photoperiod and temperature extremes.

Patterns of flowering and senescence also differed between the ecotypes. Flowering occurred earlier and was significantly more intensive in the northern RI, NC, and SC ecotypes (Fig. 4A & B). Between these three ecotypes, flowering duration was inversely correlated with latitude with flowering duration shortest in RI. In contrast, the Florida ecotypes (NFL, CFL, and SFL) displayed either no (SFL, NFL) or significantly less (CFL) flowering (Fig. 4B) when grown in containers. Plant senescence was observed first in RI and NC by Week 10 (5 Sept. 2000) followed by SC and the Florida ecotypes.

Clearly, *S. latifolia* ecotypes collected along the eastern U.S. coast display significant differences in growth and reproduction when grown under north central Florida conditions. No doubt, these differences can be attributed to adaptation to latitudinal differences in environmental factors prevailing where the plants were originally collected. The possibility arises that some of these adaptations may have long-term detrimental consequences should the ecotypes be planted far from their original source. Previously, we reported differences in growth and flowering under nursery conditions between micropropagated Florida ecotypes of the wetland species *Pontederia cordata* L. (Kane and Philman, 1997). These differences were observed in the same ecotypes following planting in wetlands (Kane, unpublished). However, long-term field studies are required before the ecological impacts of these differences can be realized.

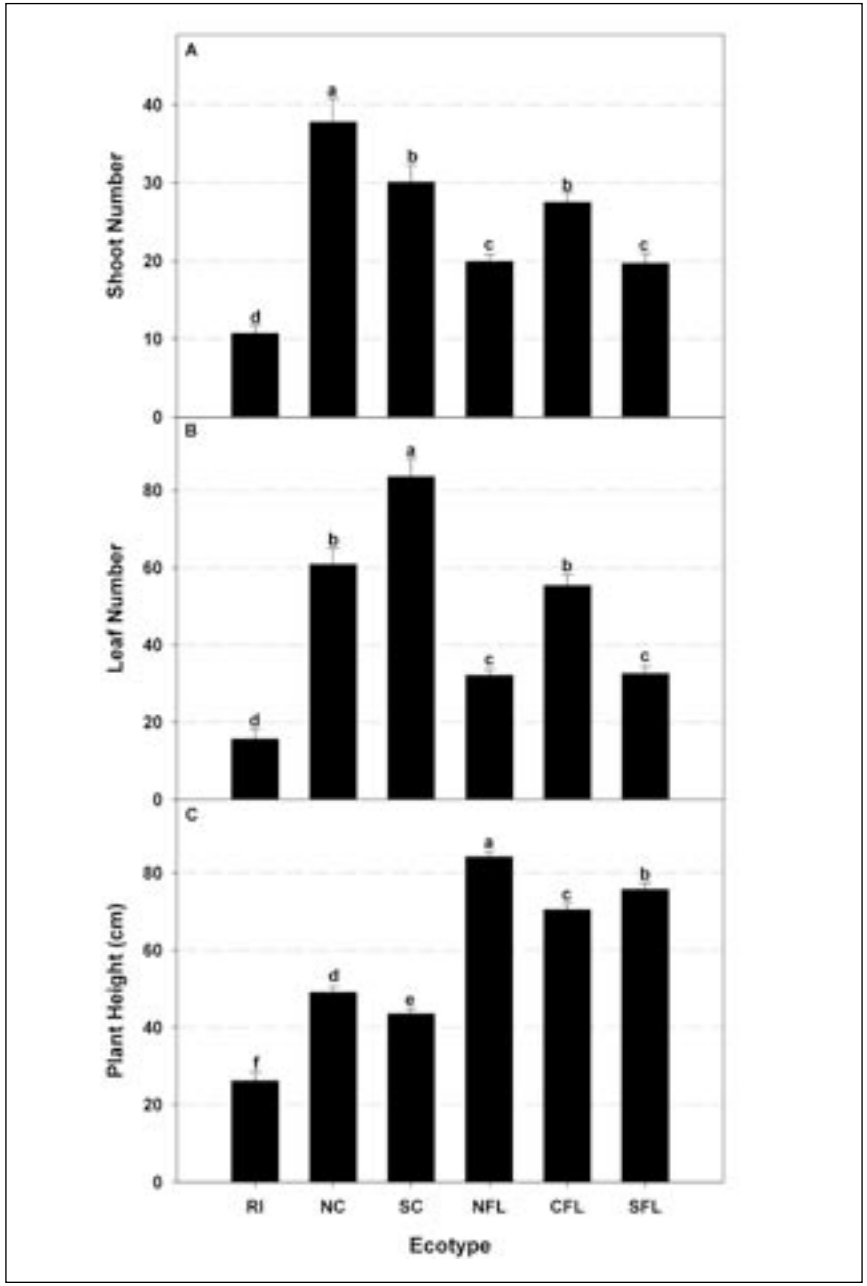

Figure 2: Comparative shoot (A) and leaf production (B) and plant height (C) between six *Sagittaria latifolia* ecotyopes after 12 weeks growth. The experiment was initiated 26 June with data collection on 18 Sept. 2000. Each histobar represents the mean response (±SE) of 18 plants. Histobars with the same letter are not significantly different at the 5% level.

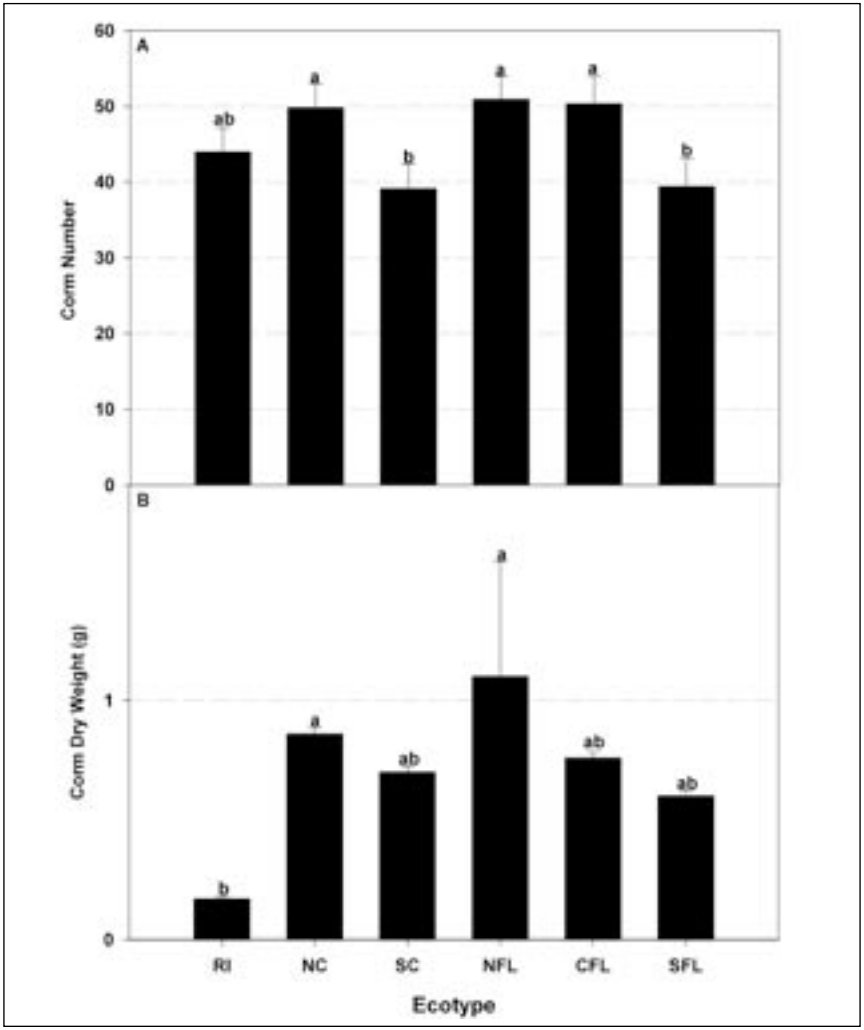

Figure 3: Comparative corm formation per plant (A) and mean corm dry weight (B) between six *Sagittaria latifolia* ecotypes after 22 weeks. The experiment was initiated 26 June. Corm harvesting occurred on 29 Nov. 2000. Each histobar represents the mean response (±SI) of 18 plants. Histobars with the same letter are not significantly different at the 5% level.

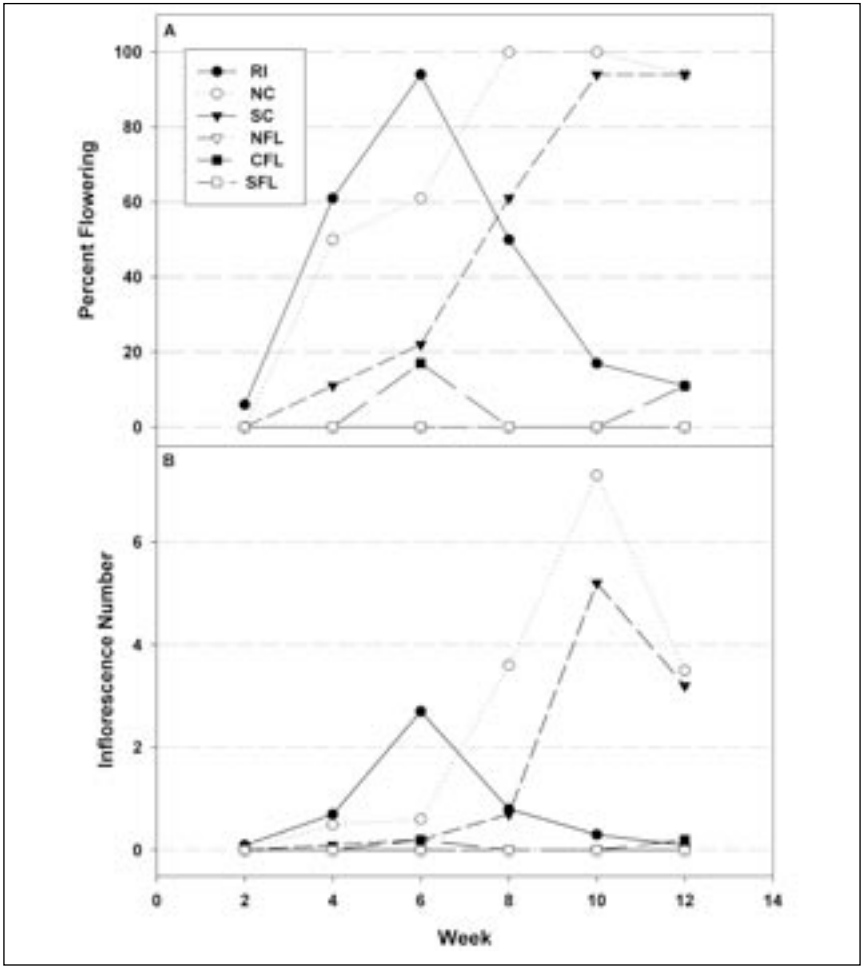

Figure 4: Comparative flowering responses of six *Sagittaria latifolia* ecotyopes. (A) percent flowering (B) number of inflorescences per plant. The experiment was initiated 26 June with data collection on 18 Sept. 2000. Each data point represents the mean response of 18 plants.

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Liner Production: Asset or Liability?©

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INTRODUCTION

Are we maximizing propagation departments to their fullest potential; or, are we merely satisfied fulfilling our production numbers at a reasonable cost? Can we generate more income from the propagation department?

Since we usually succeed in propagation more than we fail, I would like to share with you how you might consider developing the propagation department to increase your bottom line.

While an extremely high percentage of nurseries have a propagation department, only a handful of these nurseries produce liners for the open market. This somewhat untapped market of selling liners is the potential asset I would like discuss.

At Sarasota Growers, Inc., the concept of selling liners started a few years ago by chance. We had excess liners and took them to a trade show. What happened at the trade show opened our minds to the idea of developing a liner division. Our booth was constantly full of people looking at the liners and placing orders. As a consequence, we also were able to sell more finished material. We also realized that you do not have to be a big operation to sell liners all over the U.S.A.

Sarasota Growers, Inc, is a small nursery in Sarasota, Florida. We have approximately 7 ha (17 acre) in production, with 0.8 ha (2 acre) dedicated to propagation and liner production.

In 1999, sales were $94,000 per acre, all generated from sales of finished material. This year, we will have sales of $215,000 per acre. The importance of this increase in sales is that it was achieved with no additional labor nor increase in production acreage. Instead, increased sales was accomplished by reallocating more attention-to-detail and labor to the propagation department — among other things.

WHY PROPAGATION?

Increased Revenue per Unit of Land. We knew we could not dramatically increase our sales by increasing production volume of finished material because of the cost of developing additional acreage in our geographical area. We needed to generate more money per acre. An average flat of liners occupies 0.2 m^2 (2 ft^2) and sells for $22. An average spaced 1-gal container occupies 0.1 m^2 (1 ft^2), and in our market sells for $2.00.