THE POPULATION ECOLOGY OF A PERENNIAL CLONAL HERB *ACORUS CALAMUS* L. (ACORACEAE) IN SOUTHEAST OHIO, USA

A dissertation presented to

the faculty of

the College of Arts and Sciences of Ohio University

In partial fulfillment of

the requirements for the degree

Doctor of Philosophy

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March 2005

This dissertation entitled

THE POPULATION ECOLOGY OF A PERENNIAL CLONAL HERB *ACORUS CALAMUS* L. (ACORACEAE) IN SOUTHEAST OHIO, USA

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The Population Ecology of a Perennial Clonal Herb *Acorus calamus* L. (Acoraceae) in Southeast Ohio, USA (153pp.)

Director of Dissertation: Brian C. McCarthy

Acorus calamus L. (Sweetflag, family Acoraceae) is an economically important helophyte found in temperate and subtropical wetlands. I examined edaphic factors influencing distribution of *A. calamus* populations; potential of the rhizome in wetland restoration; genetic diversity of populations; and, environmental factors influencing seed germination. Redundancy analysis (RDA) indicated that rhizome length and biomass, total number of leaf scars and leaf scars per unit length in *A. calamus* are positively related to a soil calcium content gradient. Shoot density is influenced most by a silt and nitrogen gradient but negatively related to organic matter. Multivariate Analysis of Variance indicated that light ($\lambda = 0.762$, $P < 0.001$), nutrient ($\lambda = 0.449$, $P < 0.001$) and moisture ($\lambda = 0.508$, $P < 0.001$) had significant effects on rhizome growth. *A. calamus* grows best in open light, waterlogged conditions and is best planted at the very onset of a wetland mitigation project.

I estimated genetic diversity in populations of *Acorus calamus* L. in southeast Ohio using Intersimple sequence repeats. Indices of genetic diversity, evenness, expected heterozygosity and percentage of polymorphic loci were low. Analysis of Molecular variance (AMOVA) indicated that most of the variation (58%) is

among populations and a moderate amount (42 %) is within populations. Cluster analysis, Principal Coordinates Analysis and the coefficient of genetic variation among populations (G_{st} = 0.7188) and gene flow (N_m = 0.1956) indicated little gene flow among populations. My results suggest that *A. calamus* populations in southeast Ohio are primarily clonal in nature.

Seed germination occured only in light and varied significantly (F = 8.42, p < 0.0001) across temperatures with seeds germinating maximally in spring (15/25^{o}C) and summer (20/35 ^{o}C) in submerged conditions. Germination velocity (Timson's Index) was greater in moist acidic conditions in spring temperatures. There was no significant difference in seed germination afte storage for 24 months. Results indicate that *A. calamus* seeds are adapted to germinate in wide variety of wetlands in summer and spring but are limited by light availability. They can create persistent seed banks. Seeding of *A. calamus* in wetland restoration projects is best conducted at the very onset when there is more light at the soil surface.

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Acknowledgements

I am grateful to the following for making this research possible:

- My advisor Brian C. McCarthy, for his support and guidance, and, other members of my committee – Irwin A. Ungar, Harvey E. Ballard, Arthur Trese and Winfred Just for their timely advice.
- Jill E. Brown, Elizabeth Martin, Wendy Dobrowolski, Mary-Vaughn Johnson and Vijayanand Nadella for technical assistance.
- Harold E. Blazier, Dr. Sarah Wyatt and Dr. Ivan Smith for technical advice.
- Paul Strauss, Ora Anderson and United Plant Savers for use of their property.
- My colleagues and friends Matthew Albrecht, Kurt Hartman, Zachary Rinkes, Corrine McCamment, Erin Larson, Russell Campbell, Eirik Stijfhoorn, Connie Pollard, Aurea Cortes-Palomec, Cheryl McCreary and Ross McCauley for their encouragement and dark humor.
- My parents Vasanth and Baby Pai, soulmates - Aarthi Pai and Cathy Hamlin, and, my spouse Vijaykishore Bhatt - for their unconditional love, support and patience through this journey.
- Funding sources -- Sigma-Xi Grants in Aid of Research, Ohio University John Houk Memorial Research Grant, Ohio Biological Survey, Earl E. Core Award (Southern Appalachian Botanical Society), Herb Society of America and the Departments of Biological Sciences and Environmental and Plant Biology, Ohio University.

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INTRODUCTION

Clonal propagation is characteristic of plants growing in resource poor, stressful environments (van Groenendael et al. 1997, Pennings and Callaway 2000). Though ramets can exist independently, physiological integration within a genet facilitates translocation of photoassimilates, water and minerals (Jonsdottir and Watson 1997). Clonal propagation promotes persistence of successful genotypes, higher survival of nascent ramets, overall growth of the genet and rapid regeneration after local damage (Oborny and Bartha 1995, Alpert 1995, Pitelka and Ashmun 1985, Hutchings and Wijesinghe 1997) thus possibly increasing fitness (Pan and Price 2002).

Clonal plants exhibit plasticity in response to habitat quality and microenvironment (de Kroon and Schieving 1991, Cain 1994, Cain et al. 1996) though they vary among species (de Kroon and Hutchings 1995). These responses, referred collectively to as “foraging behavior”, are manifested as variation in spacer (stolon or rhizome internode, *sensu* de Kroons and Hutchings 1995) length, branching intensity, and branching angle (Oborny and Cain 1997). Foraging in clonal species is an adaptation to habitat heterogeneity. Plasticity in spacer length and branching allows placement of ramets in favorable microhabitats and avoidance of unfavorable patches (Alpert 1995, de Kroons and Hutchings 1995, Hutchings 1997, Stueffer et al. 1997). The foraging syndrome has been observed in several species e.g., *Glechoma hederacea* L. (Slade and

Hutchings 1987 a, b)*, Brachypodium pinnatum* L. (de Kroon and Hutchings 1995)*, Carex flava* L. (de Kroon and schieving 1990), *Trientalis europaea* L. (Dong etal. 1997), *Hydrocotyle bonariensis* (Evans and Cain 1995), *Elymus lanceolatus* (Huber-Sanwald et al. 1997, Keijen and Groenendael 1999) and *Fragaria chiloensis* (Tworkoski et al. 2001). The foraging syndrome is not displayed uniformly among all clonally propagating plants species but depends on morphological and physiological constraints (Oborny and Cain 1997).

Clonal propagation, especially by rhizomes is the primary means of reproduction in emergent macrophytes in wetlands. In these plants, rhizomes often serve both as a carbohydrate storage organ and spacers; i.e., a mode of dispersal through lateral growth (Cronk and Fennessy 2001). As in other clonal plants (Suzuki and Stuefer 1999), carbohydrate reserves in the rhizome of emergent macrophytes are important in helping tide over temporal disturbances and in rapid foliage growth at the onset of the growing season. Emergent macrophytes are subject to sporadic flooding and inundation from the adjacent wetland body. Given the periodic disturbance and concurrent physiological stress (e.g., anoxia) that emergent macrophytes are subjected to, these plants have evolved life history strategies that reflect stress tolerance. They are usually geophytic and sequester nutrients in belowground tissues (Cronk and Fennessey 2001). The distribution, population structure and growth of these species are dependent on the flooding regime of the wetland and local soil characteristics (van den Brink et al. 1995, Ferreira and Moreira 1999, Owen 1999, Campbell et

al. 2002). While several studies on rhizome architecture and plasticity exist for other terrestrial species, (Cheplick 1995, Stuefer et al. 1996, Alpert 1999, Dong 1993), studies on rhizomes of emergent macrophytes are generally lacking.

I examined plasticity in rhizome morphology in populations of *Acorus calamus* L. (Sweetflag, Acoraceae), an emergent macrophyte, in southeast Ohio. *Acorus calamus* has a ubiquitous distribution in wetland areas in temperate and subtropical regions of the world (Motley 1994). Phylogenetically, the genus *Acorus* is considered the primal extant monocot (Grayum 1987, Duvall et al. 1993). Both diploid fertile varieties and triploid sterile varieties of the species are found in North America (Buell 1937, Packer and Ringius 1984).

Sweetflag has sword-like foliage, with equitant leaf bases, arising from a pinkish white rhizome. Diploid populations of *A. calamus* reproduce predominantly by rhizome but also by seed. Polyploid varieties rely solely on clonal propagation by rhizome. The rhizome grows sympodially by means of terminal and lateral buds and growth is of the “phalanx” type (Lovett Doust 1981), consisting of tightly clustered ramets growing radially. During the dormant season, leaves die, leaving annular scars, while the terminal bud persists. *Acorus calamus* rhizomes have a high physiological tolerance for anoxia, surviving up to three months in completely submerged conditions by anaerobic fermentation of its carbohydrate reserves (Weber and Braendle 1996, Schluter and Crawford 2001, Joly and Braendle 1995).

The rhizome has an aromatic odor and contains several essential oils, sesquiterpenes and asarones. It is popular in several traditional forms of medicine (e.g., Ayurveda) mainly as a stomachic (Motley 1994). It was used by Native Americans (Moerman 1998) and may have been planted intentionally around settlements and hunting trails (Gilmore 1930). The rhizome is being explored for medicinal and pesticidal properties (Nawamaki and Kuroyanagi 1996, Rahman and Schmidt 1999).

Despite its evolutionary and ethnobotanical significance, the ecology of Sweetflag is largely unexplored. In this study, I investigated edaphic factors characterizing sites where *A. calamus* populations are distributed in southeast Ohio. I then examined variation in rhizome morphology of these populations with respect to site edaphic factors. I hypothesized that *A. calamus* would exhibit a greater ramet density in nutrient rich areas. I expected that rhizome length (i.e. spacer length) would be increased in areas of intermediate nutrient quality.

METHODS

Study Sites

Eleven populations of *A. calamus* were surveyed throughout the unglaciated Allegheny plateau region of southeast Ohio, U.S.A. The bedrock stratum in this area consists of sedimentary rocks of the Ordovician, Silurian, Devonian, Mississippian, Pennsylvanian, and Permian series (Cusick and Silberhorn 1977).

Watersheds in the area lie within the northern Ohio River drainage system. The climate is temperate continental with distinct seasons. Mean annual temperature varies from a mean monthly high of 24^{o}C in July to a mean monthly low of –2^{o}C in January with an annual mean of 11.6^{o}C. The annual precipitation is a total of 98 cm, varying from a monthly mean of 5.6 cm in February to 11.7 cm in June (National Climatic Data Center 2001).

Wetlands in Ohio have decreased from 2,023,428 ha to 195,463 ha (ca. 90% decline) in the last 200 years owing to farmland conversion and development activities (Gordon 1969, Cusick and Silberhorn 1977, Ohio Department of Natural Resources 2002). Several human-made reservoirs and water bodies exist in the area and the older water bodies are dominated by *Typha*, *Carex*, *Scirpus* and *Eleocharis* species in the emergent zones. While the larger wetlands in Ohio are found in the northern regions of the state, most of the wetland bodies in southeast Ohio (i.e., the unglaciated Allegheny plateau) consist of ephemeral streams, vernal pools, and human-made impoundments (Boreman 2001). For this study I selected eleven population patches of *A. calamus* in southeast Ohio: one in Belmont County, seven in Athens County, and three in Meigs County (Table 1).

Field Methods

Populations lay linearly along the edge of a water body. At each site, the population patch was transected at its longest axis. Soil samples (each about

1000 cm^3 in volume) were collected at different locations along the transect. Soil was sampled at the center of the patch (referred to as “inner” samples), at the periphery of the population (referred to as “peripheral” samples), and at a distance of 20 m from the periphery of the population (referred to as “outer” samples). Soil was also sampled at a distance of 20 m from the periphery of the population along a second transect that was uphill, away from the water body and perpendicular to the initial transect (referred to as “uphill” samples). A total of eight soil samples were collected from each population patch. Edaphic variables estimated included soil texture (% sand, clay and silt), pH, moisture, percent of organic matter, and concentration of inorganic ions: nitrogen, phosphorous, potassium, aluminium, calcium, and magnesium. Laboratory analyses follow procedures summarized by McCarthy (1997). Nitrogen was measured using cadmium reduction reactions. Soil moisture was measured using the gravimetric method. Soil pH was quantified using the pH/ion analyzer 350 (Corning laboratories). Organic matter and ash content were measured after ashing soil in a muffle furnace. Soil texture was measured using the hydrometer method. Mechlich’s extracts were made to measure P, Ca, Mg, K, Al. Phosphorous determination was conducted using the colorimetric method. Ca, Mg, K, and Al were measured using Spectra AA 220 atomic absorption analyzer (Varian Techtron Pty, Ltd. Australia).

Abundance and density of above ground shoots (ramets) were measured in 10 0.5 m^2 plots along the central line of transects and then extrapolated to

determine the density of shoots per 1 m^2. One shoot was selected randomly from each plot and its rhizome was severed from the remainder of the genet. Thus, a total of ten rhizomes were gathered from each population. Length of rhizome and number of leaf scars on each rhizome was noted and the number of leaf scars per unit length of rhizome was derived. The rhizomes were dried at 80^o C for 72 hours and then the dry weight biomass was noted. Both edaphic and biotic samples were coded using abbreviations for population location (Table 1).

Statistical analyses

A multivariate analysis of variance (MANOVA) was used to test for significant differences in soil samples due to location (i.e., inner, outer, upper and peripheral). A randomized block design MANOVA was used with location of soil sample as a fixed effect and patch (N = 11) as a random effect. All edaphic variables were included in the MANOVA except for sand which was excluded to avoid collinearity of data. MANOVA was also used to discern differences in biotic variables (i.e., shoot density, rhizome biomass, rhizome length, total number of leaf scars and number of leaf scars per unit length) among sites. Edaphic and biotic variables contributing to statistically significant differences among locations and population patches were further examined with univariate analysis of variance (ANOVA). Fisher's test of least significant difference (LSD) was used to distinguish groups with similar means for both the edaphic and biotic variables.

Spearman's rank correlation was used to assess the relationship between the biotic variables and the variables of the inner, outer, and peripheral soil samples. Significant (at $P < 0.01$) interactions were then used for further interpretation. A redundancy analysis (RDA, Rao 1964) was used to discern patterns of variation in biotic variables with respect to abiotic variables. RDA is based on a linear response of biotic variables to environmental variables (McCune and Grace 2002). It is a direct extension of multiple regression to modeling multivariate response data. Ordination of the response variables is constrained by the descriptive variables and the ordination vectors are linear combinations of the descriptive variables (Legendre and Legendre 1998). Only seven of the twelve edaphic variables were used in the RDA to avoid distortion of the RDA due to covariance of variables. Edaphic variables used in the RDA included calcium, nitrogen, phosphorous, moisture, clay, silt, and organic matter.

All data were subjected to tests for normality, equal variance, and equality of covariance using the D'Agostino Omnibus test, Modified Levene equal variance test, and Box's M test (Hintze 2000). Data that did not meet assumptions of normality were transformed. The MANOVA, ANOVA, correlation and tests for assumptions were all conducted using NCSS 6.0 (Hintze 2000). The RDA was computed using CANOCO 4 (ter Braak 1997, ter Braak and Smilauer 1998). Significance of the RDA model was evaluated using Monte Carlo simulation (1000 permutations, $P = 0.05$).

RESULTS

The MANOVA (Table 2) indicated no significant difference in edaphic variables among locations within population sites, however, there was a significant difference among sites. Examination of each edaphic variable using one way ANOVA showed a significant difference in moisture ($F = 4.71$, $P < 0.005$), clay ($F = 3.23$, $P < 0.05$) and silt ($F = 3.52$, $P < 0.05$) content due to location. Inner soil samples had significantly more moisture (figure 1A) and clay (figure 1B) content as compared to samples from peripheral, outer or upper locations. Inner and peripheral samples had less silt content as compared to outer and upper samples (figure 1C). There is considerable variation in all edaphic variables among population sites (Table 3). One way ANOVA indicated a significant difference in Ca ($F = 152.89$, $P < 0.05$), Al ($F = 20.50$, $P < 0.05$), Mg ($F = 40.42$, $P < 0.05$), P ($F = 19.47$, $P < 0.05$), N ($F = 5.86$, $P < 0.05$), moisture ($F = 4.06$, $P < 0.05$), Clay (4.38, $P < 0.05$), organic matter content ($F = 152.89$, $P < 0.05$), and pH ($F = 2.47$, $P < 0.05$) among sites.

The MANOVA indicated a significant difference ($\lambda = 0.451$, $P < 0.001$) in shoot density and all morphometric variables among populations. An examination of each of the variables using one way ANOVA showed significant differences in density of shoots ($F = 5.31$, $P < 0.05$), rhizome biomass ($F = 3.63$, $P < 0.05$), rhizome length ($F = 2.77$, $P < 0.05$), total number of leaf scars ($F = 4.01$, $P < 0.05$) and leaf scars per unit length ($F = 3.52$, $P < 0.05$) among populations.

Fisher's LSD indicated that GBR, LSNF and RID have significantly greater rhizome biomass than all other population patches (figure 2A). RID and LSNF also have longer rhizomes than other population patches while OALI, PSP and PSPP have the shortest rhizomes (figure 2B). DYS and GBS have the greatest total number of leaf scars and also number of leaf scars per unit length of rhizome (figures 2C and 2D). LSFF, OAS and PSP have greater shoot density as compared to other population patches. PSPP and OALI have the lesser shoot density while DYS has the least among all the populations.

Spearman's rank correlation of the edaphic variables and biotic variables (Table 4) indicated that rhizome length is positively correlated with high levels of calcium ($r = 0.75$, $P < 0.05$), magnesium ($r = 0.55$, $P < 0.05$) and negatively correlated with high levels of aluminum content ($r = - 0.55$, $P < 0.05$). Rhizome biomass is positively correlated with rhizome length ($r = 0.82$, $P < 0.05$) and negatively correlated with leaf scars per unit length ($r = 0.65$, $P < 0.05$). The total number of leaf scars is positively correlated to calcium content ($r = 0.57$, $P < 0.05$). There is also significant correlation between some of the edaphic variables; e.g., calcium is positively correlated to magnesium ($r = 0.65$, P 0.05) and phosphorous content ($r = 0.58$, $P <$ 0.01), but is negatively correlated to aluminum content ($r = - 0.74$, $P < 0.05$).

In the RDA (figure 3), eigenvalues for Axis 1 ($\lambda = 0.51$) and Axis 2 ($\lambda = 0.25$) indicate their importance in explaining the variation in biotic variables. This is further emphasized by the population environment correlation of Axis 1 ($r = 0.87$) and Axis 2 ($r = 0.98$). The cumulative percentage variance in biotic data explained by Axis 1 is

50.6 % and by Axis 2 is 75 % respectively. Cumulative percentage variance of the species-environment relationship explained by Axis 1 is 62.3 % and by Axis 2 is 92.6 %. The Monte Carlo simulation indicated that the first axis ($F = 1.94$, $P < 0.05$) and the overall model ($F = 0.743$, $P < 0.05$) were significant.

In the RDA, lengths of gradients associated with Ca, P, organic matter and silt indicate their relative importance in influencing biotic variables. Soil calcium and organic matter content have the strongest influence when sorting biotic variables with respect to edaphic variables in the ordination space. Phosphorous and silt content also contribute significantly to gradients in axes 1 and 2. Silt content is negatively correlated to Axis 1 ($r = - 0.4427$). Soil organic matter content is best correlated to Axis 2 ($r = 0.71$), along with calcium content ($r = - 0. 69$). Soil phosphorous is moderately correlated with both Axis 1 ($r = 0.34$) and Axis 2 ($r = 0.36$).

The ordination suggests that rhizome length, rhizome biomass, total number of leaf scars, and leaf scars per unit length are positively related to calcium and phosphorous content of the soil, but negatively related to the organic matter content. Shoot density is influenced by silt, nitrogen, moisture, and clay content and negatively correlated with organic matter content. The ordination separates the *A. calamus* populations into three sets. While one set of points (*viz.* RID and GBS) has elevated calcium and phosphorous content, another (*viz.* PSP, LSNF, LSFF, OAS and PSR) has increased silt and nitrogen content, with the third cluster (*viz.* PSPP, OALI and GBR) having greater soil organic matter content. DYS is pushed to the edge of the ordination space along the calcium – phosphorous gradient.

DISCUSSION

Soils inhabited by *Acorus calamus* are characteristic of wetland areas; exhibiting low pH and nitrogen, and elevated moisture, organic matter, and clay content. Soils within a patch where *A. calamus* is found are generally homogenous but I observed a great deal of heterogeneity among the population patches. *Acorus calamus* is able to establish and proliferate under a wide range of soil conditions, a factor that accounts for its ubiquitous distribution. There is greater moisture and clay content within population patches than surrounding areas. These edaphic factors may limit local proliferation of *A. calamus*.

I observed a large degree of plasticity in rhizome-related features both within and among populations. The ordination results suggest that shoot density is positively influenced by moisture, clay, silt and nitrogen content. Growth, ramet production and increased shoot density in *A. calamus* reflect colonization of suitable habitats. Silt and clay, important sources of plant nutrients, characterize *A. calamus* population sites. Clay has a higher cation binding and moisture holding capacity as compared to silt. There is a significant correlation between clay and N content of the soil (Table 5); clay patches are nutrient rich and hence provide *A. calamus* suitable microenvironments to colonize. Shoot density is negatively correlated with organic matter content. Increased organic matter content indicates anoxic wetland soils (Histosols) with low nitrogen levels, a result of slow decomposition of accumulated organic matter due to anaerobic conditions (Cronk

and Fennessy 2001). Populations located in areas with high organic matter *viz.* GBR, OALI and PSPP (figure 3), have low rhizome biomass and significantly shorter rhizome length and shoot density than other populations (figure 2).

The ordination suggests that rhizome biomass, rhizome length, total number of leaf scars and leaf scars per unit length are positively related to calcium and phosphorous content of the soil but negatively related to the organic matter content. The data (Table 5) indicate that rhizome length and the number of leaf scars are correlated with calcium content. Magnesium, which is also correlated with rhizome length (Table 5), was not included in the ordination since it is highly correlated with calcium.

Calcium, phosphorous and magnesium are macronutrients important for plant metabolism and growth. Models of foraging in clonal plants based on resource availability indicate that allocation to spacers (i.e., rhizome length) will be low at both low and high patch quality but will be greater at intermediate qualities and large-scale patchiness (de Kroon and Schieving 1991, Oborny 1994, Wijesinghe and Hutchings 1997). This is a result of the opposite responses of branching intensity and spacer length to factors influencing patch quality. Plasticity in rhizome length among populations of *A. calamus* suggests that the plant possibly displays the foraging syndrome by varying this biotic variable. Both GBS and RID population patches have longer rhizomes, greater rhizome weight and less shoot density (figure 3). These populations are located

in areas that have greater amounts of phosphorous and calcium but low quantities of nitrogen, possibly an indication of intermediate habitat quality.

Despite a heterogeneous habitat that may contain high amounts of calcium and phosphorous conducive to rhizome growth, some other environmental variable may limit certain populations (e.g., DYS). Closer examination reveals DYS to be the only population growing under a forest canopy. With *A. calamus* being a heliophyte, availability of sunlight is likely to be the limiting factor to the growth of this population. The population may have been established earlier when the canopy was open, with canopy growth and shading ultimately becoming detrimental to growth of the population.

The RDA indicates that total number of leaf scars and leaf scars per unit length respond to gradients of increasing calcium and phosphorous. The total number of scars is correlated with Ca. In clonal plants, allocation to leaves is increased under conditions of high nutrient availability (Hutchings 1997). Thus, large numbers of leaf scars may indicate nutrient rich sites. Leaf scars also indicate age of ramets, especially if a fixed number of leaves are produced each growing season. In such a scenario, short rhizomes with large number of leaf scars may represent stunted growth and hence nutrient poor conditions. Short rhizomes with less number of leaf scars and high shoot density may also indicate nutrient rich patches where the genet has high ramet production. Not much is known about the phenology of *A. calamus* and variation in annual production of leaves.

It is not clear if the populations of *A. calamus* L. that I surveyed in southeast Ohio are entirely diploid or triploid. Polyploidy is a common occurrence in several species of angiosperms (Masterson 1994), especially aquatic macrophytes and has long term evolutionary consequences often providing the opportunity for sympatric speciation (Les and Philbrick 1993). Polyploid varieties of species tend to have larger individuals. If ploidy varies between populations this can influence both rhizome biomass and length. However, substantial phenotypic plasticity exists even within a population patch suggesting that genets are influenced by local environmental factors. The relationship between patterns of rhizome morphology, shoot density and edaphic variables in *A. calamus* populations in southeast Ohio suggest that *A. calamus* is a stress tolerator (sensu Grime 1977). *A. calamus* is adapted to growing in nutrient deficient, highly anoxic soil. The rhizome acts as both a storage organ and displays characteristics of the foraging syndrome. Rhizome biomass and length vary with nutrient gradients. Ramet production is increased in nutrient rich areas enabling genets to forage effectively.

Distribution patterns of *A. calamus* in wetlands suggest that succession can play an important role in the proliferation and maintenance of *A. calamus* populations. With increasing levels of silt, *A. calamus* populations will have the potential to expand. However, if wetlands are drained, either due to anthropogenic activities or woodland encroachment, consequent low moisture levels can result in population decline. On the other hand, soil flooding and water

impoundment for extremely long periods of time, along with build up of anaerobic sediment will also likely contribute to population declines of the species.

LITERATURE CITED

Alpert, P. 1995. Does clonal growth increase plant performance in natural communities? Abstracta Botanica 19:11-16.

Alpert, P. 1999. Effects of clonal integration on plant plasticity in *Fragaria chiloensis*. Plant Ecology 141:99-106.

Boreman, J. 2001. Ohio Chapter protects and restores threatened wetlands. Ohio Landscapes. The Nature Conservancy. “Fall” pp 8-11.

Buell, M. F. 1937. *Acorus calamus* in America. Rhodora 37:367-369.

Cain, M. 1994. Consequences of foraging in clonal plant species. Ecology 75:933 - 944.

Cain, M. L., D. A. Dudle, J. P Evans. 1996. Spatial models of foraging in clonal plant species. Amer. J. Bot. 83:76-85.

Campbell, D. A., C. A. Cole, R. P. Brooks. 2002. A comparison of created and natural wetlands in Pennsylvania USA. Wetlands Ecology and Management 10:41-49.

Cheplick, G. P. 1995. Genotypic variation and plasticity of clonal growth in relation to nutrient availability in *Amphibromus scabrivalvis*. J. of Ecol. 83:459-468.

Cronk, J. L and M. S. Fenessy. 2001. Wetland Plants*:* Biology and Ecology. Lewis Publishers, Boca Raton, FL, USA.

Cusick, A. W. and G. M. Silberhorn. 1977. The Vascular plants of unglaciated Ohio. Bulletin of the Ohio Biological Survey, Volume 5, Number 5. Ohio State University, Columbus, OH, USA.

De Kroon, H. and F. Schieving. 1991. Resource allocation patterns as a function of clonal morphology*:*a general model applied to a foraging clonal plant. J. Ecol. 79:519-530.

De Kroon, H. and M. J. Hutchings. 1995. Morphological plasticity in clonal plants*:*the foraging concept reconsidered. J. Ecol. 83:143-152.

Dong, M. 1993. Morphological plasticity of the clonal herb *Lamiastrum galeobdolon* (L.) Ehrend. & Polatschek in response to partial shading. New Phytologist 124:291-300.

Duvall, M. R., G. H. Learn JR., L. E. Eguiarte and M. T. Clegg. 1993. Phylogenetic analysis of rbcL sequences identifies *Acorus calamus* as the primal extant monocotyledon. Proceedings of the National Academy of Sciences of the United States of America 90:4641-4644.

Evans, J. P. and M. L. Cain. 1995. A spatially explicit test of foraging behavior in a clonal plant. Ecology. 76:1147 - 1155.

Ferreira, M. T. and I. S. Moreira. 1999. River plants from an Iberian Basin and environmental factors influencing their distribution. Hydrobiologia 415:101-107.

Gilmore, M. 1930. Dispersal by Indians as a factor in the extension of discontinuous distribution of certain native species. Papers of the Michigan Academy of Science, Arts and Letters 13:89-99.

Gordon, R. B. 1969. The natural vegetation of Ohio in pioneer days. Bulletin of the Ohio Biological Survey Volume III Number 2. Ohio State University, Columbus, OH, USA.

Grayum, M. 1987. A summary of evidence and arguments supporting the removal of *Acorus* from the Araceae. Taxon 36:723 – 729.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Amer. Nat. 111:1169-1194.

Hintze, J. L. 2000. NCSS 6.0 Users Manual. Number Cruncher Statistical Systems. Kaysville, UT, USA.

Hutchings, M. J. 1997. Resource allocation patterns in clonal herbs and their consequences for growth. p. 161 – 190. *In* Bazzaz, F.A. and J. Grace (ed.) Plant Resource Allocation. Academic Press, San Diego, CA, USA.

Hutchings, M. J. and D. K. Wijesinghe. 1997. Patchy habitats, division of labor and growth dividends in clonal plants. Trends in Ecology and Evolution 12:390-394.

Jonsdottir, I. S. and M. A. Watson. 1997. Extensive physiological integration: an adaptive trait in resource-poor environments? p. 109 – 136. *In* de Kroon,

H. and J. van Groenendael (ed.) The ecology and evolution of clonal plants. Backhuys publishers, Lieden, The Netherlands.

Joly, C. A. and R. Braendle. 1995. Fermentation and adenylate metabolism of *Hedychium coronarium* J. G. Koenig (Zingiberaceae) and *Acorus calamus* L. (Araceae) under hypoxia and anoxia. Functional Ecology 9:505-510.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. Second English Edition. Elsevier Science, Amsterdam, The Netherlands.

Les, D.H., C.T. Philbrick. 1993. Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. Aquatic Botany 44:181-228.

Lovett Doust, L. 1981. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*) I. Dynamics of ramets in contrasting habitats. J. Ecol. 69:743-755.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiospserms. Science 264: 421-423.

McCarthy, B. C. 1997. Lab protocols for testing of eastern deciduous forest soils. URL:www.plantbio.ohiou.edu/epb/soils/soils.htm

McCune, B. and J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software, Gleneden Beach, OR, USA.

Moerman, D. E. 1998. Native American ethnobotany. Timber Press, Portland, OR, U.S.A.

Motley, T. J. 1994. The ethnobotany of sweet flag, *Acorus calamus* (Araceae). Economic Botany 48:397-412.

National Climatic Data Center. 2001. National virtual data system. URL:http://nndc.noaa.gov/

Nawamaki, K. and M. Kuroyanagi. 1996. Sesquiterpenoids from *Acorus calamus* as germination inhibitors. Phytochemistry 43:1175-1182.

Oborny, B. 1994. Growth rules in clonal plants and environmental predictability - a simulation study. J. Ecol. 82:341-351.

Oborny, B. and S. Bartha. 1995. Clonality in plant communities an overview. Abstracta Botanica 19:115-127.

Ohio Department Of Natural Resources. 2002. A history of Ohio wetlands. URL:http://www.dnr.state.oh.us/wetlands/history.htm.

Owen, C. R. 1999. Hydrology and history: landuse changes and ecological responses in an urban wetland. Wetlands Ecology and Management 6:209-219.

Packer, J. G. and G. S. Ringius. 1984. The distribution and status of *Acorus* (Araceae) in Canada. Can. J. Bot. 62:2248-2252.

Pan, J. J. and J. S. Price. 2002. Fitness nad Evolution in clonal plants: the impact of clonal growth. Evolutionary Ecology 15:583-600.

Pennings, S. C. and R. M. Callaway. 2000. The advantages of clonal integration under different ecological conditions: a community wide test. Ecology 81:709-716.

Pitelka, I. F. and J.W. Ashmun. 1985. Physiology and integrationof ramets in clonal plants. p. 399 – 343. *In* Jackson, J. B. C., L. W. Buss and R. E. Cook (ed.) Population Biology and evolution of clonal organisms. Yale University Press, New Haven.

Rahman, M. M. and G. H. Schmidt. 1999. Effect of *Acorus calamus* (L.) (Araceae) essential oil vapours from various origins on *Callosobruchus phaseoli* (Gyllenhal) (Coleoptera: Bruchidae). J. Stored Products Research. 35:285 – 295.

Rao, C. R. 1964. The use and interpretation of Principal components analysis in applied research. Sankhya A 26:329-358.

Schluter, U. and R. M. M. Crawford. 2001. Long term anoxia tolerance in leaves of *Acorus calamus* L. and *Iris pseudacorus* L. J. of Experimental Bot. 52:2213-2225.

Stueffer, J. F., H. De Kroon and H. J. During. 1996. Exploitation of environmental heterogeneity by spatial division of labor in a clonal plant. Functional Ecol. 10:328-334.

Suzuki, J. and J. F. Stuefer. 1999. On the ecology and evolutionary significance of storage in clonal plants. Plant Species Biology 14:11-17.

Ter Braak , C. J. F. 1997. CANOCO-A FORTRAN program for canonical community ordination by partial detrended canonical correspondence analysis, principal components analysis and redundancy analysis (version 2.1). DLO – Agricultural Mathematics Group, Wageningen, Netherlands.

Ter Braak, C. J. F. and P. Smilauer. 1998. CANOCO reference manual and user's guide to CANOCO for Windows: software for canonical community ordination (v.4). Center for Biometry, Waginengen, Netherlands.

Van Groenendael, J. M., L.M. Klimes, J. Klimesova and R. J. J. Hendriks. 1997. Comparative ecology of clonal plants. p 191-209. *In*: Silvertown, J., M. France and J. L. Harper (eds.). Plant life histories: ecology, phylogeny and evolution. Cambridge University Press, Cambridge, United Kingdom.

Van Den Brink, F. W. B., G. Van Der Velde, W. W. Bosman and H. Coops. 1995. Effects of substrate parameters on growth responses of eight helophyte species in relation to flooding. J. Aquat. Bot. 50:79-97.

Weber, M. and R. Braendle. 1996. Some aspects of the extreme anoxia tolerance of the Sweet Flag, *Acorus calamus* L. Folia Geobotanica Phytotaxa 31:37-46.

Wijesinghe, D. K. and M. J. Hutchings. 1997. The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechoma hederacea*. J. Ecol. 85:17-28.

Table 1.1: Location of eleven *Acorus calamus* L. populations surveyed in southeast Ohio for rhizome morphology and edaphic variables.

Site ID	Location	Coordinates
LSNP	Lake Snowden, Athens County	N 39^0 14.536' W 82^0 11.371'
LSNF	Lake Snowden, Athens County	N 39^0 14.516' W 82^0 11.362'
OALI	Lavelle Road, Athens, Athens County	N 39^0 19.460' W 32^0 8.224'
OAS	Lavelle Road, Athens, Athens County	N 39^0 19.468' W 32^0 8.265'
GBR	Route 56, Athens County	N 39^0 20.791' W 82^0 14.670'
GBS	Route 56, Athens County	N 39^0 20.778' W 82^0 14.645'
RID	Ridges, Ohio university, Athens County	N 39^0 18.920' W 82^0 06.959'
DYS	Dysart woods, Belmont County	N 39^0 59.550' W 80^0 50.'634
PSP	United Plant Savers, Meigs County	N 39^0 04.829' W 82^0 10.429'
PSPP	United Plant Savers, Meigs County	N 39^0 04.855' W 82^0 10.482
PSR	United Plant Savers, Meigs County	N 39^0 04.808' W 82^0 10.469

Table 1.2: Multivariate analysis of variance (MANOVA) of edaphic variables measured at 11 *Acorus calamus* L. populations in southeast Ohio.

Source	Wilk's λ	df 1	df 2	*F*	*P*
Edaphic variables					
Within population patches	0.29200	27	65	1.26	0.220
Among population patches	0.00006	90	254	9.01	0.000
Within among X population patches	0.00019	270	333	3.09	0.000

Table 1.3: Means (± standard errors) of soil variables measured at inner, periphery, outer and upper locations adjacent to eleven *Acorus calamus* L. populations in southeast Ohio.

	Inner	Peripheral	Outer	Upper
Al (μgl^{-1})	601.52 (± 30.48)	601.36 (± 31.59)	592.93 (± 49.24)	600.46 (± 39.35)
Ca (μgl^{-1})	937.12 (± 193.92)	1847.48 (± 187.85)	1979.53 (± 290.08)	1862.82 (± 278.04)
Mg (μgl^{-1})	253.72 (± 15.74)	241.27 (± 12.69)	200.81 (± 14.75)	238.14 (± 23.80)
N (μgl^{-1})	3.18 (± 0.34)	3.13 (± 0.28)	3.23 (± 0.24)	3.15 (± 0.24)
P (μgl^{-1})	26.44 (± 5.303)	29.48 (± 9.12)	37.73 (± 14.51)	26.98 (± 4.29)
PH	5.72 (± 0.15)	5.75 (± 0.13)	5.47 (± 0.19)	5.63 (± 0.16)
Moisture (%)	58.02 (± 5.68)	43.78 (± 3.40)	46.10 (± 5.35)	34.60 (± 4.55)
Clay (%)	14.44 (± 0.54)	12.55 (± 0.59)	12.31 (± 0.38)	12.90 (± 0.61)
Sand (%)	36.86 (± 1.42)	34.31 (± 1.16)	31.40 (± 1.21)	32.08 (± 1.34)
Silt (%)	49.2 (± 1.93)	52.7 (± 1.34)	54.91 (± 1.19)	54.87 (± 1.71)
Organic (%)	1.09 (± 0.20)	1.20 (± 0.17)	1.97 (± 0.49)	2.03 (±0.40)

Table 1.4: Spearman's rank correlation (*r*) of abiotic and biotic variables surveyed from eleven populations of *A. calamus* L. in southeast Ohio. Letters underlined and in bold indicate $r > 0.50$, $P < 0.05$.

	Rhizome Biomass	Rhizome length	Total no. of leaf scars	Leaf scars/ length	Density	Ca	Al	Mg	P	N	pH	Moisture	Sand	Clay	Silt	Organic matter
Rhizome biomass (g)	1	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Rhizome length (cm)	0.82	1	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Total no. of leaf scars	0.21	0.45	1	_	_	_	_	_	_	_	_	_	_	_	_	_
Leaf scars/ length	- 0.65	- 0.38	0.45	1	_	_	_	_	_	_	_	_	_	_	_	_
Density (ramets/m^2)	0.18	0.25	- 0.04	- 0.39	1	_	_	_	_	_	_	_	_	_	_	_
Ca (μgl^{-1})	0.39	0.75	0.57	0.03	0.40	1	_	_	_	_	_	_	_	_	_	_
Al (μgl^{-1})	- 0.41	- 0.55	- 0.22	0.12	- 0.47	- 0.74	1	_	_	_	_	_	_	_	_	_
Mg (μgl^{-1})	0.49	0.55	0.42	- 0.37	0.50	0.65	-0.39	1	_	_	_	_	_	_	_	_
P (μgl^{-1})	- 0.16	0.27	0.29	0.24	0.29	0.58	- 0.16	0.42	1	_	_	_	_	_	_	_
N (μgl^{-1})	- 0.28	- 0.42	- 0.36	- 0.34	0.19	- 0.37	0.59	0.24	0.03	1	_	_	_	_	_	_
pH	- 0.20	0.07	0.13	0.11	- 0.16	0.32	- 0.25	0.02	- 0.06	- 0.04	1	_	_	_	_	_
Moisture (%)	- 0.33	0.06	0.04	0.06	0.21	0.38	- 0.23	0.10	0.22	0.13	0.85	1	_	_	_	_
Sand (%)	0.22	0.17	0.34	- 0.22	- 0.17	- 0.20	0.57	0.30	0.01	0.41	- 0.18	- 0.16	1	_	_	_
Clay (%)	0.18	0.05	- 0.20	- 0.52	0.37	- 0.10	0.40	0.49	0.32	0.74	- 0.51	- 0.21	0.53	1	_	_
Silt (%)	- 0.23	- 0.25	- 0.52	0.04	0.23	0.12	- 0.45	- 0.19	0.08	- 0.13	0.08	0.10	- 0.92	- 0.25	1	_
Organic matter (%)	0.27	0.18	- 0.48	- 0.67	- 0.10	- 0.12	0.17	0.28	0.06	0.42	0.05	0.07	0.31	0.51	- 0.09	1

Figure 1.1 Soil moisture and clay content with respect to relative position of the patch of *Acorus calamus*. Inner = within the patch, outer = 20 m outside the patch (along contour), peri = at the periphery of the patch and upper = 20 m upslope of the patch. Means with same letter are not significantly different from each other ($P > 0.05$).

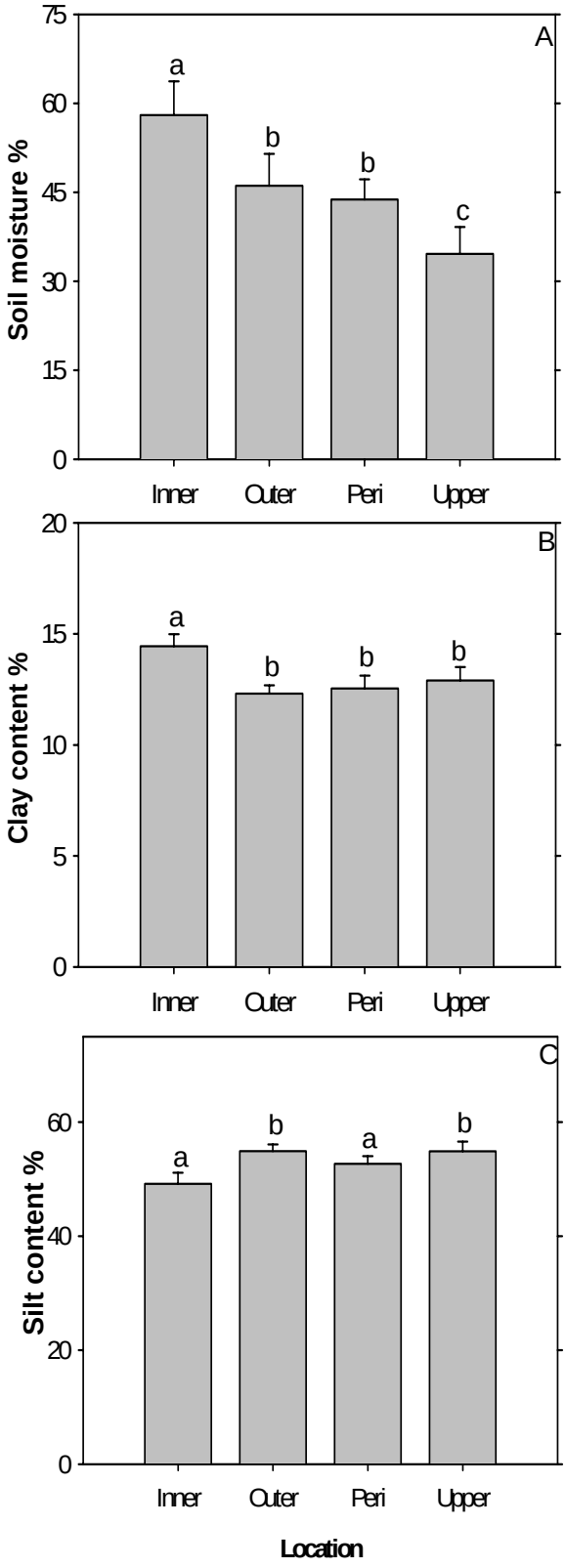

A
Soil moisture %
75
60
45
30
15
0
a
b
b
c
Inner
Outer
Peri
Upper
B
Clay content %
20
15
10
5
0
a
b
b
b
Inner
Outer
Peri
Upper
C
Silt content %
60
40
20
0
a
b
a
b
Inner
Outer
Peri
Upper
Location

Figure 1.2 Variation in rhizome biomass, length, total number of leaf scars, leaf scars per unit length and shoot density per m^2 of *Acorus calamus* shoots sampled in eleven populations in southeast Ohio. Means with same letter are not significantly different from each other ($P > 0.05$).

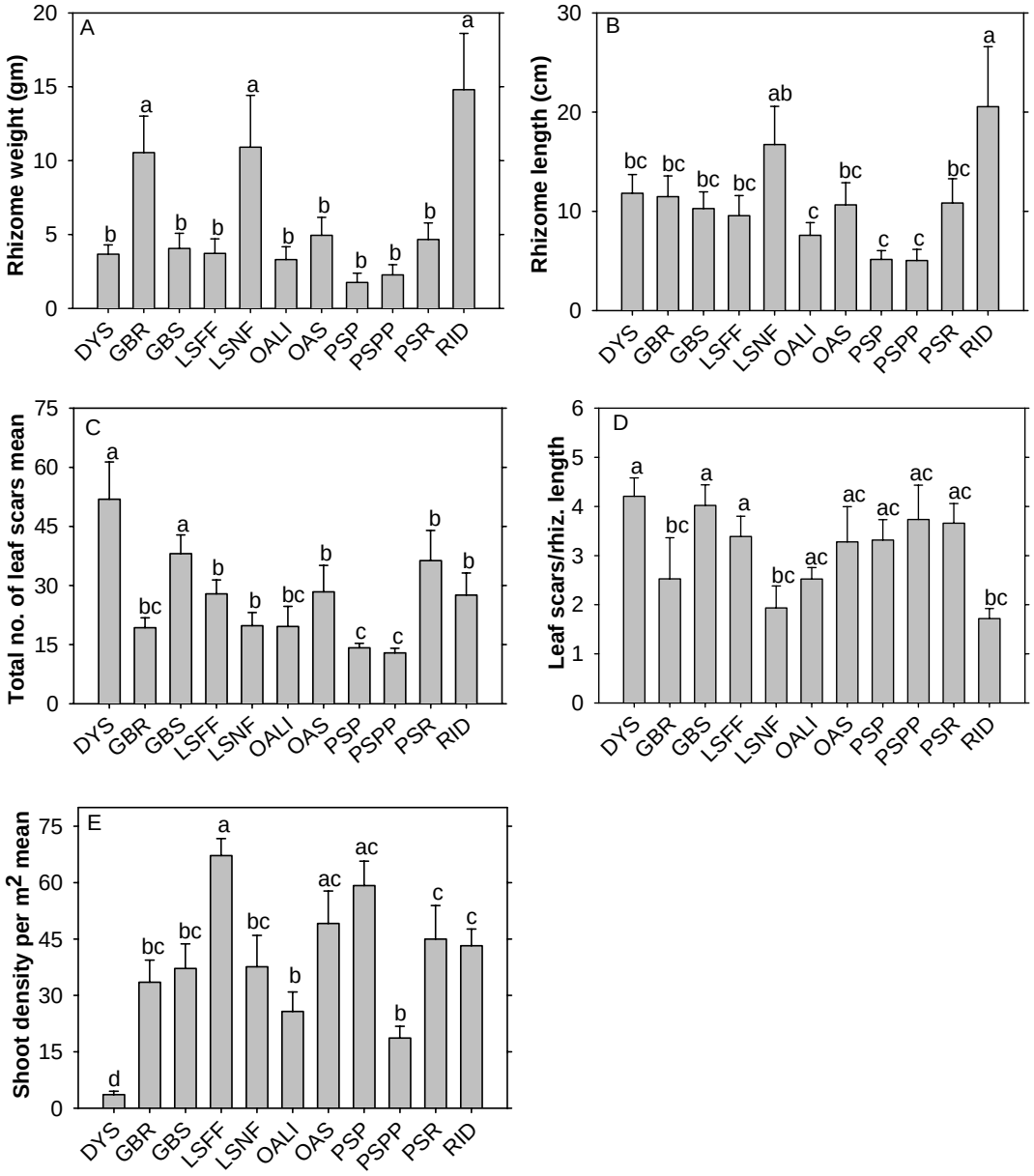
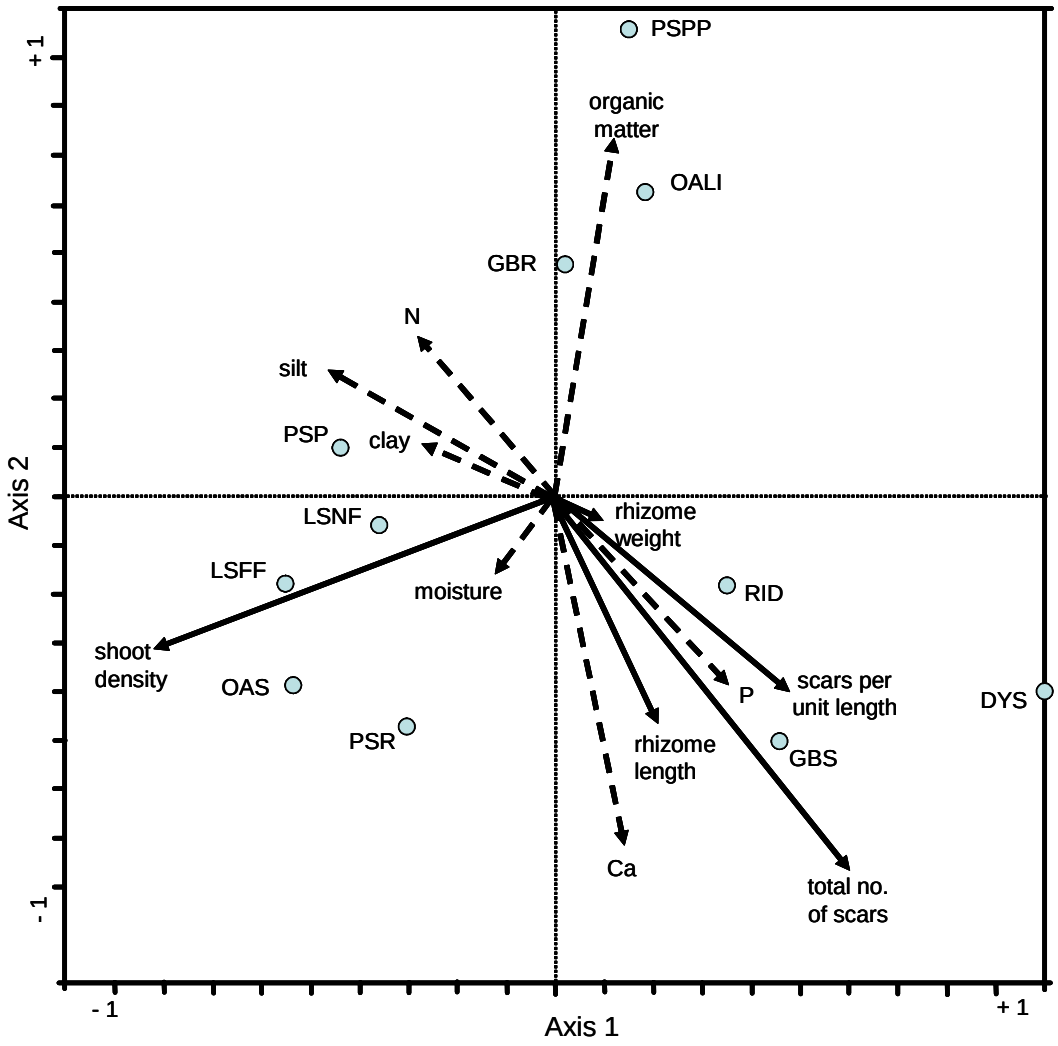

A
Rhizome weight (gm)
0
5
10
15
20
b
a
b
b
a
b
b
b
b
a
DYS
GBR
GBS
LSFF
LSNF
OALI
OAS
PSP
PSPP
PSR
RID
B
Rhizome length (cm)
0
10
20
30
bc
bc
bc
bc
ab
c
bc
c
c
bc
a
C
Total no. of leaf scars mean
0
15
30
45
60
75
a
bc
a
b
b
bc
b
c
c
b
b
D
Leaf scars/rhiz. length
0
1
2
3
4
5
6
a
bc
a
a
bc
ac
ac
ac
ac
bc
E
Shoot density per m2 mean
0
15
30
45
60
75
d
bc
bc
a
bc
b
ac
ac
b
c
c

Figure 1.3 Ordination triplot of Redundancy Analysis (RDA) of morphological features of *Acorus calamus* rhizome with respect to soil variables in populations of the plant in southeast Ohio. Soil variables are depicted as thick arrows, morphological variables as dash arrows and populations as circles. Abbreviations for population sites are the same as in Table 1.2.


PSPP
organic matter
OALI
GBR
N
silt
PSP
clay
LSNF
LSFF
moisture
rhizome weight
RID
shoot density
OAS
PSR
P
scars per unit length
DYS
rhizome length
GBS
Ca
total no. of scars
+ 1
- 1
Axis 2
- 1
+ 1
Axis 1

Chapter 2: Potential of *Acorus calamus* L. rhizomes in wetland restoration and mitigation.

INTRODUCTION

Following a large-scale loss of freshwater wetlands in the last two centuries, several states in the USA have made wetland restoration a priority for natural resource managers (World Wildlife Fund 1992). Development activities damaging a wetland are mandated by the Clean Water Act (National Research Council 1995) to compensate by restoring or creating new wetlands. Successful wetland restoration depends on the establishment of appropriate vegetation. Screening and evaluating plant species that are able to establish populations and facilitate natural succession in wetland ecosystems is a critical step in wetland restoration. While the ability of a plant species to thrive in a wetland area depends on its life history characteristics and adaptations to local site conditions (Budelsky and Galatowitsch 2000, Keddy 2000, Kowalski and Wilcox 2003), the actual selection of species for wetland restoration is also influenced by socio-economic factors and management goals (Mitsch and Gosselink 2000).

Initial species composition plays an important role in long-term vegetation succession of a wetland (Kellogg and Bridham 2002). At the onset of wetland construction, soil in the area is subjected to severely nutrient poor and reduced oxygen conditions (Mitsch and Gosselink 2000), with available nitrogen often dropping to values near zero (Cronk and Fenessy 2001). These conditions remain for a period of time (Gilliam et al. 1999) and can retard the establishment of plant communities. Nitrogen fertilization is not advisable in these

circumstances since it can encourage encroachment by weeds such as *Phalaris arundanacea* which can in turn suppress natural sedge and fen communities (Green and Galatowitsch 2000, Perry et al. 2004). Plant material used for initial stages of wetland creation or mitigation should be indigenous species able to rapidly establish and stabilize soil substratum in spite of anoxic, reduced and nutrient poor conditions. It should also be adapted to a broad range of water depths and not susceptible to overgrazing by wild animals (Kentula et al. 1992).

Natural resource managers also have to contend with the fact that world over, wetlands are an important source of income and food security to communities of people yielding economically important resources e.g., fish, rice paddy (*Oryza* spp.), sago palm (*Metroxylon sagu*), bull thatch (*Sabal*), willow (*Salix*), reeds (*Phragmites* spp.), etc. (Maltby 1986). Hence it is important that socio economic values be realized when restoring a wetland (Ramchandra 2001). Perception of a direct monetary benefit can serve as an incentive for local inhabitants to conserve a wetland (Morrison 2002). In polluted areas, wetland plant crops that utilize nitrate loads draining into watersheds through agricultural run off can increase the value of wetlands to local farm owners (Hey 2002). Economic returns can offset the significant lag costs associated with wetland restoration. For example, restoration lag costs in Ohio range from $3,640 per acre to $49,811 per acre, nearly 50% of total restoration costs (Gutrich and Hitzhusen 2004). The harvest and marketing of economically important plants can help to recover some of these costs. Thus a multidimensional approach

incorporating both ecological and socio economic aspects is beneficial during species selection.

Emergent macrophytes are most suited to the initial stages of wetland construction and restoration (Mitsch and Gosselink 2000). Besides being able to stabilize soil, they can play an important role in bioremediation and biorestoration of contaminated water (Venosa et al. 2002). The importance of emergent macrophytes in wetland restoration has prompted research on their propagation (Rogers 2003, Zhou et al. 2003). Rhizomes and tubers are successful planting stock for emergent macrophytes (Mitsch and Gosselink 2000) and are often more available than seed banks within post restoration soil (Combroux et al. 2002). In nature, rhizomes serve as disaspores and a means of dispersal for many species of emergent macrophytes (Maun 1984).

Acorus calamus L. (Sweetflag), an emergent aquatic macrophyte, is listed as a species suitable for wetland restoration (Mitsch and Gosselink 2000). The species has a ubiquitous global distribution in temperate and subtropical Asia, North America, Europe and South Africa. It is locally abundant in North America (Braun 1921, Buell 1937, Packer and Ringius 1984) often occurring during the successional stages of wetlands (Van der Walk and Bliss 1971). In southeastern Ohio, populations of the plant occur in variety of wetland types and severely nutrient poor areas (Pai and McCarthy, *in press*). In summers, *A. calamus* has long sword-like foliage with equitant leaf bases arising from a sympodial rhizome. Lateral buds produce ramets resulting in clonal growth. In winters, only the

rhizome and, terminal and lateral buds persist. The species is adapted to anoxia tolerance (Weber and Braendle 1996, Schluter and Crawford 2001, Joly and Braendle 1995) enabling its populations to withstand long periods of flood and snow.

A. calamus rhizome is important several traditional systems of medicine in Asia, Africa, Europe and North America (Motley 1994, Van Wyke et al. 1997, Moerman 1998, Kumar et al. 2000). Its widespread distribution is attributed to intentional introduction by humans along trade and colonization routes (Motley 1994). Early settlers planted the rhizome around settlements while Native Americans distributed the plant along hunting trails (Gilmore 1930, Motley 1994, Les and Mehroff 1999). The rhizome contains several essential oils that are being investigated for pharmacological and pesticidal properties (Rahman and Schmidt 1999, Acuna et al. 2002, Mehrotra et al. 2003). It is widely used as raw material in the global pharmaceutical and perfume industry. On the Internet, the rhizome powder is available for US $20 per kg and its essential oil (calamus oil) is priced between US $250 to $700 per liter. Populations of the plant have been reported as threatened in the Indian subcontinent and Europe due to over harvest and habitat loss (Indian Institute of Forest Management 1998, Lange 1998). Market demand for the rhizome has prompted cultivation of the plant in India (Lokesh and Chandrakanth 2002), especially as an intercrop in abandoned paddy fields (Oudhia 2002).

A. calamus rhizome is sold by the North American horticultural industry (Winterrowd 1990). It has the potential for phytoremediation in constructed wetlands (U. S. Department of defense 1999, Pivetz 2001, Wilson et al. 2001). The Food and Drug Administration (FDA) banned commercial use of the plant in the United States in 1968 due to the presence of β-asarone, a carcinogen, detected in Asian varieties of the rhizome (Motley 1994). However recent reports indicate that North American populations may have lower quantities of β-asarone as compared to other global populations (European Agency for Evaluation of Medicinal Products 2003, Belanger et al. 2000). Despite its potential as an export commodity, landowners in the United States consider the plant to be a weed (Les and Mehroff 1999). Recent surveys indicate population declines of *A. calamus* in North America (Robbins 1998), possibly due to habitat loss from wetland drainage.

Its socio-economic importance, indigenous nature and suitability for wetland habitats indicate that *A. calamus* might be an apt candidate for wetland restoration. However its potential for wetland construction and restoration has not been studied. I evaluated the potential of *A. calamus* as a species for wetland restoration under different environmental conditions by subjecting the rhizome to experimental manipulations of light, water and nitrogen. These environmental variables were manipulated to mimic environmental conditions that occur at the early (i.e., less nitrogen and, more light and water) and late (i.e., more nitrogen, lesser light and water) stages of a wetland construction or

rehabilitation project. I hypothesized that *A. calamus* rhizomes would proliferate best under conditions of high moisture, low nitrogen and light -- indicating its suitability at the onset of a wetland restoration and construction project.

METHODS

Rhizomes of *Acorus calamus* were acquired from a population and sized to 5 cm leaving terminal buds intact. Leaves and roots on each rhizome were trimmed to 1 cm. The rhizomes were planted in aquatic plant containers (12 cm diameter) half-filled with potting soil (Sierra Perennial mix ®, Scotts-Sierra Horticultural Products Company, Marysville, OH) and placed in a greenhouse for two weeks to allow for acclimatization. During this period the rhizomes were subjected to full light and watered with 400 ml of 10 $\mu g \cdot ml^{-1}$ Nitrogen solution twice a week. After two weeks, the potted rhizomes were allocated to different light, nutrient and moisture treatments in shade houses located in the West State Street research gardens maintained by the Department of Environmental and Plant Biology at Ohio University.

Light treatments were full sunlight (1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and shade (900 $\mu mol \cdot m^{-2} \cdot s^{-1}$), each treatment being replicated thrice. The full light treatment sought to imitate open wetlands, i.e., wetlands in their initial stages without any canopy cover while the shade treatment imitated wetlands in later stages of succession when some vegetation cover had been established. Full sunlight

treatments were established by three shade house frames covered with clear polyethylene. For shade treatments three shade house frames were covered with a layer of fifty percent shade cloth (Sudden Shade knitted shade cloth, Waldo and Associates, Inc., Perrysburg, OH) in addition to the polythene. Thirty two potted rhizomes were placed in each shade house and randomly designated to nutrient and moisture combinations. Placement of the pots was randomized twice every week.

Nutrient treatments were high Nitrogen (10 $\mu g \cdot ml^{-1}$ N) and low Nitrogen (4 $\mu g \cdot ml^{-1}$ N) administered twice a week using Peters Professional Water Soluble Fertilizer® (Scotts-Sierra Horticultural Products Company, Marysville, OH), a greenhouse fertilizer for perennials. The low nitrogen treatment was similar to nitrogen levels in anoxic soils. Moisture treatments were 400 ml (high) or 200 ml (low) of the nutrient solution. The high moisture treatment led to constantly waterlogged soil. Thus, within each shade house there were four treatment combinations: high nutrient-high moisture (HNHM), high nutrient-low moisture (HNLM), low nutrient-high moisture (LNHM), and, low nutrient-low moisture (LNLM). A total of 192 rhizomes were subjected to the experiment with eight rhizomes in each treatment combination within a shade house (figure 1).

The experiment was carried out for 14 weeks concurrent with the growing season of *A. calamus* in southeast Ohio. After 14 weeks, various morphometric measurements on the rhizome were recorded. The number of buds, number of mature buds (those that produced leaves), and rhizome length were recorded.

Leaf area was measured using a Li Cor leaf area meter (LI-3100, Li-cor Inc, USA). Leaves, rhizomes and roots were dried in a forced air oven at 80^{o} C for 72 hours before their dry biomass was measured.

Specific leaf area (SLA) was computed as

$$\frac{total\ leaf\ area}{total\ leaf\ biomass}$$

Shoot-root ratio was calculated as the relationship between aboveground and below ground tissue

$$\frac{leaf\ biomass}{root + rhizome\ biomass}$$

A multivariate analysis of variance (MANOVA) was performed on pre-treatment rhizome variables (rhizome length and weight) in each treatment combination. Post-treatment growth data was analyzed using a split plot design with nitrogen and moisture treatments nested as factorial treatments within light. Data were analyzed using the PROC GLM MANOVA procedure (Littell et al. 2002) in SAS Version 8 (SAS Institute 2003). Variables included in the MANOVA were leaf area, leaf biomass, rhizome length, rhizome biomass, root biomass and total biomass. Specific leaf area and shoot-root ratio were analyzed using one way ANOVAs with the PROC GLM procedure in SAS. The split plot model degenerated when a light into nutrient or light into moisture error term was specified due to problems with the H matrix having zero degrees of freedom. Hence only the moisture into nutrient error could be obtained in the analyses.

The Tukey Kramer multiple comparison procedure was used to distinguish moisture and nutrient combinations with similar means within each light treatment (Hintze 2000) respectively.

All data were subjected to tests for normality, equal variance, and equality of covariance using the D'Agostino Omnibus test, Modified Levene equal variance test, and Box's M test (Hintze 2000). Data that did not meet assumptions of normality were appropriately transformed before further analyses.

RESULTS

The pretreatment MANOVA did not indicate any significant ($\lambda = 0.9538$, $P > 0.50$) difference in weight and length of rhizomes assigned to various treatments. Increase in wet weight of the rhizomes varied from 3.5 g to 20 g (with a mean increase of 11 g). A large proportion of the dry biomass is invested in below ground structures. Leaves amounted to a mean of 40 % of the dry biomass. MANOVA on post treatment rhizome variables indicated that light, nutrient and moisture significantly influenced rhizome growth (table 1). In addition, the moisture and nutrient interaction was also significant for a few growth variables (table 2) resulting in variation of above and below ground variables of the rhizomes measured after the experiment (table 3).

Both in full light and shade, total plant biomass was significantly greater in high moisture conditions irrespective of nitrogen availability. In full light, when

nitrogen availability was greater, rhizomes invested significantly more in relative leaf biomass as compared to treatments with less nitrogen. Treatments with low nitrogen invested more in belowground biomass. In shade, the combination of more nitrogen and moisture resulted in relative leaf biomass that was significantly greater than in combinations of less nitrogen with less water but not significantly different from the remaining two treatments. In full light, as well as shade, rhizomes grown in less nitrogen but with more moisture, invested in significantly greater root biomass (figure 2).

Response of above ground variables

One way ANOVAs indicated that light significantly influenced all above ground variables except the total number of buds and mature buds. Nitrogen also significantly influenced all above ground variables. Moisture influenced all above ground variables except specific leaf area and shoot-root ratio (table 3). The Tukey Kramer multiple comparison procedure indicated that that the total number of leaves, leaf area and specific leaf area were greater in full light as compared to shade (figure 3).

All aboveground variables responded significantly to changes in nitrogen and moisture though the pattern of responses varied with the light treatment. In full light, total number of buds produced was significantly greater when increased nitrogen and moisture were present but in shade, the total number of buds was greater in waterlogged conditions irrespective of levels of nitrogen (figure 3-A).

In full light, the number of buds that matured into shoots was significantly greater only with increased levels of both nitrogen and moisture. However in shade, only the combination of both low moisture and less nitrogen produced significantly less mature buds than other combinations (figure 3-B).

In full light, significantly more leaves were produced only when the waterlogged treatment occurred with elevated levels of nitrogen. In shade, only the combination of both low moisture and less nitrogen produced significantly less numbers of leaves (figure 3-C). Total leaf biomass in both light and shade was greatest only when moisture occurred along with increased nitrogen (figure 3-D).In full light, the combination of greater moisture and greater nitrogen resulted in significantly larger leaf area but in the shade, both high nitrogen treatments had similar leaf area which was significantly greater than in the low moisture treatments (figure 3-E). In full light, specific leaf area did not differ significantly among nitrogen or moisture treatments but in shade, the low nitrogen low moisture treatment had significantly less specific leaf area than other treatments (figure 3-F).

Response of below ground variables

One way ANOVAs indicated that light significantly influenced rhizome length. Moisture influenced all belowground variables. Nitrogen significantly influenced all belowground variables except rhizome length (table 3). The Tukey Kramer multiple comparison procedure indicated that rhizome length was greater

in shade than full light. In both full light and shade, rhizome length was significantly greater in waterlogged conditions (figure 4-A).

In both full light and shade, rhizome biomass produced in treatments of greater nitrogen and moisture availability was significantly greater than in other treatments. However in full light, rhizomes growing in greater moisture and low nitrogen availability also produced significantly greater rhizome biomass than those grown in a combination of less nitrogen and moisture (figure 4-B). Root biomass had similar patterns in both full light and shade. Rhizomes grown under conditions of less nitrogen but high moisture availability had significantly greater root biomass than other treatments except rhizomes grown in both low nitrogen and moisture availability (figure 4-C).

DISCUSSION

Fluctuations in light, moisture and nitrogen are characteristic of constructed wetland habitats, both when nascent or after large periods of time. Initially, flooding regimes and consequent anoxia create waterlogged conditions and nitrogen limitation. On the other hand, autogenic processes over large periods of time can result in terrestrialization. Coupled with canopy encroachment, plants in terrestrialized environments can face light and moisture limitation. These challenges influence the populations of *A. calamus* inhabiting wetlands. I examined biomass allocation in *A. calamus* rhizomes with respect to

environmental variables. Biomass allocation patterns in response to environment impact the relative growth rate and competitive ability of populations of a species during succession (Tilman 1988, Bazzaz 1997). The results of our study suggest that all three environmental parameters that were investigated have a profound influence on biomass allocation patterns in *Acorus calamus*. Patterns of growth in *A. calamus* are consistent with the theory of optimal biomass allocation (Bloom et al. 1985): morphological plasticity allows plants to track resource availability and preferentially allocate biomass to organs responsible for acquisition of a limiting factor.

Though total biomass was not different among the two light treatments, patterns of allocation to above- and belowground biomass varied with moisture and nitrogen availability. *A. calamus* rhizomes sequester more total biomass in greater moisture conditions despite low availability of nitrogen. Moisture is the most important factor influencing overall growth. Most of the total biomass accumulated by *A. calamus* is in belowground organs, i.e., the rhizome and roots, especially when nitrogen availability is low.

I observed dramatic changes in several aboveground variables under different light treatments. In *A. calamus*, a clonal plant, the number of total buds indicates potential ramification and population expansion. Each mature bud is a shoot bearing leaves but there is a tradeoff in numbers of buds that can mature into shoots. While more leaves increase chances of acquiring light, more shoots imply maintenance of nascent ramets. Clonal plants regulate densities of ramets

(i.e., shoots) in suboptimal conditions (de Kroon and Hutchings 1995, Jonsdottir and Watson 1997, Hutchings 1997). When in shade, *A. calamus* produced more mature buds in waterlogged conditions and in high nitrogen conditions resulting in an increase in numbers of leaves and total leaf area for light interception and photosynthesis. When light was not a limiting factor, rhizomes produce significantly greater numbers of mature buds only when both moisture and nitrogen are available, increasing the density of ramets only in favorable conditions.

There is no difference in total leaf biomass between full light and shade treatments; specific leaf area is greater in the shade. Low light conditions induced larger and thinner leaves. This is a morphological response that has been consistently observed in other terrestrial species (Poorter and van der Werf 1989). Variation in SLA occurs with concurrent changes in mesophyll, lamina, water content and support tissue (Meziane and Shipley 1999) and is most pronounced in monocots (Van Andel and Biere 1989). SLA influences relative growth rate (Dijkstra 1998, Meziane and Shipley 2001). When nitrogen is not a limiting factor, *A. calamus* invests more in leaves, increasing photosynthetic capacity and relative growth rate, a phenomenon that has been observed in other terrestrial species (McDonald 1989, Aerts et al. 1992, van der Werf et al. 1993). In the shade, even though greater numbers of leaves are produced than in other treatments, substantial leaf biomass (implying thicker leaves) is accumulated only when both nitrogen and moisture are available.

Rhizomes are longer and thinner in shade and also when nitrogen limitation occurs in waterlogged conditions. In clonal plants, increase in rhizome or spacer length is observed as a response to intermediate resource availability. The adaptation aids a genet to forage for resources (Cain 1994, Hutchings 1997). When placed in a suitably moist or waterlogged environment, *A. calamus* rhizomes may attempt to forage for light or nitrogen by plasticity in spacer length.

Nitrogen limitation induces *A. calamus* to invest more in root biomass in an attempt to acquire the limiting factor, especially in moisture rich conditions. This results in a lower shoot-root ratio. Greater rhizome biomass is sequestered only if more moisture is available in full sunlight. In the shade, increased nitrogen leads to more investment in aboveground tissue. Belowground biomass sequestration is important in resource poor habitats as it allows plants to capitalize on nutrient pulses and store reserves for periods of scarcity (Suzuki and Stueffer 1999). In *A. calamus* carbohydrate reserves play an important role in its anoxia tolerance (Joly and Braendle 1995). Belowground biomass is critical to the long term maintenance of marshy emergent wetlands (Mendelssohn et al. 1981). With high nutrient loads, biomass is preferentially allocated to aboveground structures and belowground biomass diminishes (Morris and Bradley 1999). This can lead to erosion of wetland edges, especially when above ground vegetation dies back periodically (Turner et al. 2004).

Waterlogged conditions are the ideal habitat for a rhizome. In waterlogged conditions, the rhizome proliferates and adjusts biomass allocation

patterns in response to the other limiting factors allowing for rapid colonization of a potentially ideal habitat. Rhizomes are able to adjust morphometric variables to acquire light or nitrogen. The rhizome can perform in shaded areas by adjusting aboveground parameters, though investment in aboveground parameters maybe at the cost of the rhizome and roots.

Nitrogen is not the primary limiting factor for *A. calamus*. *A. calamus* has broad ecological amplitude with respect to nitrogen. While the plant sequesters its maximum biomass during high nitrogen availability, it fares well in nitrogen-limited habitats, as long as an ample amount of water is available in the microenvironment. The species is adapted to resource poor environments. Its ability to grow in nitrogen-limited environments may explain its broad distribution in a variety of wetlands.

A. calamus grows best in open light, waterlogged conditions and in spite of nitrogen limitation. Hence, it is most suitable for planting at the onset of a wetland restoration or construction project. Populations of *A. calamus* can be affected when anthropogenic activities drain wetlands or, natural succession changes hydrology, moisture availability, and light quantity.

Optimal growth of *A. calamus* is achieved if the plant is grown in conditions of high Nitrogen and Moisture. However, if grown in an open, inundated wetland area, receiving full sunlight, biomass sequestered by *A. calamus* rhizomes in less nitrogen will not be significantly different from that in greater nitrogen conditions.Its ability to thrive in nitrogen limited conditions makes

A. calamus an ideal crop that can be harvested periodically from nitrogen deficient wetlands such as those constructed newly by natural resource managers or in abandoned nutrient depleted paddy fields. *A. calamus* will not require any fertilizer inputs enhancing its value as an economic crop.

LITERATURE CITED

Acuna, U. M., D. E. Atha, J. Ma, M. H. Nee and E. J. Kennelly. 2002. Antioxidant capacities of ten edible North American plants. Phytotherapy Research 16:63-65.

Aerts, R., H. de Caluwe and H. Konings. 1992. Seasonal allocation of biomass and nitrogen in four carex species from mesotrophic and eutrophic fens as affected by nitrogen supply. The Journal of Ecology 80:653-664.

Bazzaz, F.A. 1997. Allocation of resources in plants: state of the science and critical questions. Pages 1-38 in F.A. Bazzaz and J. Grace, editors. Plant resource allocation. Academic Press, San Diego, CA, USA.

Belanger, A., F. Boudreau and N. Grondin. 2000. Essential oil from *Acorus calamus*. Research Summary Volume 27, 1999-2000, Horticultural Research and Development center, Agriculture and Agri-food Canada, Quebec Canada. http://res2.agr.ca/stjean/publication/resume9900/resume9900_e.pdf

Bloom, A. J., F. S. Chapin III and H. A. Mooney. 1985. Resource limitation in plants-an economic analogy. Annual Review of Ecology and Systematics 16:363-392.

Braun, L. E. 1921. Composition and Source of the Flora of the Cincinnati Region. Ecology 2:161-180.

Buell, M. F. 1937. *Acorus calamus* in America. Rhodora 37: 367-369.

Budelsky, R. A. and S. M. Galatowitsch. 2000. Effects of water regime and competition on the establishment of a native sedge in restored wetlands. Journal of Applied Ecology 37:971.

Cain, M. 1994. Consequences of foraging in clonal plant species. Ecology 75:933-944.

Combroux, I. C. S., G. Bornette and C. Amoros. 2002. Plant regenerative strategies after a major disturbance: the case of a riverine wetland restoration. Wetlands 22:234-246.

Cronk, J. L. and M. S. Fenessy. 2001. Wetland Plants*:* Biology and Ecology. Lewis Publishers, Boca Raton, FL, USA.

de Kroon, H. and M. J. Hutchings. 1995. Morphological Plasticity in clonal plants: the foraging concept reconsidered. Journal of Ecology 83:143-152.

Dijkstra, P. 1998. Cause and effect of differences in specific leaf area. Pages 124-140 in H. Lambers, H. Poorter and M.I. Van Vuuren editors. Inherent variation in plant growth. Backhuys publications, Leiden, The Netherlands.

European Agency for the Evaluation of Medicinal Products 2003 Evaluation of medicines for human use: Final Paper on the use of herbal medicinal products containing Asarone. EMEA, Canary Wharf, London, UK.

Gilmore, M. R. 1930. Dispersal by Indians as a factor in the extension of discontinuous distribution of certain species of native plants. Papers of the Michigan Academy of Science, Arts and Letters 13:89-94.

Gilliam, F. S., J. D. May, M. A Fisher and D. K. Evans. 1999. Short-term changes in soil nutrients during wetland creation. Wetlands Ecology and Management 6:203-208.

Green, E. K. and S. M. Galatowitsch. 2001. Differences in wetland plant community establishment with additions of nitrate-N and invasive species (*Phalaris arundinacea* and *Typha* × *glauca*). Canadian Journal of Botany 79:170-178.

Gutrich, J.J. and F. J. Hitzhusen. 2004. Assessing the substitutability of mitigation wetlands for natural sites: estimating restoration lag costs of wetland mitigation. Ecological Economics 48:409-424.

Hey, D. L. 2002. Nitrogen farming: harvesting a different crop. Restoration Ecology 10:1-10.

Hintze, J. L. 2000. NCSS 6.0 Users Manual. Number Cruncher Statistical Systems. Kaysville, UT, USA.

Hutchings, M.J. 1997. Resource allocation patterns in clonal herbs and their consequences for growth. Pages 161-190 in F. A. Bazzaz and J. Grace,

editors. Plant resource allocation. Academic Press, San Diego, California, U.S.A.

Indian Institute of Forest Management. 1998. Conservation Assessment and management of Non timber forest products in India. Indian Institute of Forest Management. Available from IIFM, Nehru Nagar, Post Box 357, Bhopal, India.

Joly, C. A. and R. Braendle. 1995. Fermentation and adenylate metabolism of *Hedychium coronarium* J.G.Koenig (Zingiberaceae) and *Acorus calamus* L. (Araceae) under hypoxia and anoxia. Functional Ecology 9:505-510.

Jonsdottir, I. S. and M. A. Watson. 1997. Extensive physiological integration: an adaptive trait in resource-poor environments? Pages 109-136 In H. de Kroon and J. van Groenendael editors. The ecology and evolution of clonal plants. Backhuys publishers, Lieden, The Netherlands.

Keddy, P. A. 2000. Wetland ecology: principles and sonservation. Cambridge University Press. Cambridge, UK.

Kellogg, C. H. and S. D. Bridgham. 2002. Colonization during early succession of restored freshwater marshes. Canadian Journal of Botany 80:176-185.

Kentula, M.E., Brooks, R.P., Gwin, S.E., Holland, C.C., Sherman, A.D. and J.C. Sifneos. 1992. An approach to improving desion making in wetland restoration and creation. Edited by A.J. Hairston. U. S. Environmental Protection Agency, Environmental Research laboratory, Corvallis, Oregon.

Kowalski, K. P. and D. A. Wilcox. 2003. Differences in sedge fen vegetation upstream and downstream from a managed impoundment. American Midland Naturalist 150:199-220.

Kumar, V. S., R. K. Srivastava, A. Krishna, V. K. S. Tomar, A. K. Singh and S. Kumar. 2000. Cultivation, chemistry, biology and utilization of bach (*Acorus calamus*): A review. Journal of Medicinal and Aromatic Plant Sciences 22:338-348.

Lange, D. 1998. Europe's Medicinal and Aromatic plants: their use, trade and conservation. Available from TRAFFIC International, 219c Huntington Road, Cambridge CB3 0DL, United Kingdom.

Les, D. H. and L. J. Mehrhoff. 1999. Introduction of nonindigenous aquatic vascular plants in southern New England: a historical perspective. Biological Invasions 1:281-300.

Littell, R. C., R. J. Freund, W. Stroup. 2002. SAS for Linear Models. John Wiley and Sons, Inc. New York, NY, USA.

Lokesh, G. B., Chandrakanth, M. G. 2002. A study for commercial cultivation of sweet flag (*Acorus calamus*) in Tumkur district of Karnataka. Journal of Medicinal and Aromatic Plant Sciences 24:984-989.

Maltby, E. 1986. Waterlogged wealth. Why Waste the world’s wet places. Pp. 200. Earthscan, Washington D.C., USA.

Maun, M. A. 1984. Colonizing ability of *Ammophila breviligulata* through vegetative regeneration. Journal of Ecology 72:565.

McDonald, A.J.S. 1989. Phenotypic variation in growth rate as affected by N-supply: its effects on NAR, LWR and SLA. Pages 35-44 in H. Lambers, H. Poorter and M.I. Van Vuren editors. Inherent variation in plant growth. Backhuys Publications, Leiden, The Netherlands.

Mehrotra, S., Mishra, K.P., Maurya, R., Srimal, R.C., Yadav, V.S., Pandey, R. and Singh, V.K. 2003. Anticellular and immunosuppressive properties of ethanolic extract of *Acorus calamus* rhizome. International Immunopharmacology 3:53.

Mendelssohn, I. A. K. L. McKee and W. H. Patrick Jr. 1981. Oxygen deficiency in *Spartina alterniflora* roots: metabolic adaptation to anoxia. Science 214:439-441.

Meziane, D. and Shipley, B. 1999. Interacting determinants of specific leaf area in 22 herbaceous species: Effects of irradiance and nutrient availability. Plant Cell and Environment. 22: 447-459.

Meziane, D. and Shipley, B. 2001. Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. Annals of Botany 88: 915-927.

Mitsch, W.J. and J. G. Gosselink. 2000. Wetlands. 3rd edition. John Wiley and Sons, NY, USA.

Moerman, D. E. 1998. Native American Ethnobotany. Timber Press, Portland, OR, U.S.A.

Morris, J .M. and P. Bradley. 1999. Effects of nutrient loading on the carbon balance of coastal wetland environments. Limnology and Oceanography 44:699-702.

Morrison, M. 2002. Understanding local community preferences for wetland quality. Ecological Management and Restoration 3:127-134.

Motley, T. J. 1994. The ethnobotany of Sweetflag *Acorus calamus* (Araceae). Economic Botany 48:397-412.

National Research Council (NRC) 1995. Wetlands: Characteristics and Boundaries. National Academy Press, Washington, D.C., USA.

Oudhia, P. 2002. Rice-acorus intercropping: a new system developed by innovative farmers of Chhattisgarh (India) international rice receaserach notes. 57:56. http://www.irri.org/publications/irrn/pdfs/vol27no1/irrn27%2D1notes.pdf

Packer, J. G. and G. S. Ringius. 1984. The distribution and status of *Acorus* (Araceae) in Canada. Canadian Journal of Botany 62:2248-2252.

Pai, A. and B.C. McCarthy. (in press). Variation in shoot density and rhizome biomass of *Acorus calamus* L. with respect to environment. Castanea.

Perry, L. G., S. M. Galatowitsch and C. J. Rosen. 2004. Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. Journal of Applied Ecology 41:151-162.

Pivetz, B. E. 2001 Phyto remediation of contaminated soil and groundwater at hazardous waste sites. United states Environmental Protection agency Groundwater Issue. EPA/540/S-01/500, Washington, D. C.

Poorter, H. and Van der Werf, A. 1998. Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. Pages 309-336 in H. Lambers, H. Poorter and M.I. Van Vuuren editors. Inherent variation in plant growth. Backhuys Publications, Leiden, The Netherlands.

Rahman, M. M. and G. H. Schmidt. 1999. Effect of *Acorus calamus* L. (Araceae) essential oil vapours from various origins on *Callosobruchus phaseoli* (Gyllenhal) (Coleoptra Bruchidae). Journal of Stored Product Research 35:285-295.

Ramachandra, T. V. 2001. Restoration and management strategies of wetlands in developing countries. Electronic Green Journal. 15. http://egj.lib.uidaho.edu/egj15/index.html

Robbins, C. 1999. Medicine from the U.S. Wildlands: An assessment of native plant species harvested in the United States for medicinal use, trade and evaluation of the conservation and management implications. *Report from TRAFFIC prepared for the Nature Conservancy*. Available from http://www.nps.gov/plants/medicinal/pubs/traffic.htm (cited April 2004).

Rogers, S. M. D. 2003. Tissue culture and wetland establishment of the freshwater monocots Carex, Juncus, Scirpus, and Typha. In Vitro Cellular and Developmental Biology - Plant 39:1-5.

SAS Institute Inc. 2003. SAS/STAT® software. Version 8. SAS Institute Inc. Cary, NC.

Schluter, U. and R. M. M. Crawford. 2001. Long term anoxia tolerance in leaves of *Acorus calamus* L. and *Iris pseudacorus* L. Jounal of Experimental Botany 52:2213-2225.

Suzuki, J. and J. F. Stuefer. 1999. On the ecological and evolutionary significance of storage in clonal plants. Plant Species Biology 14:11-17.

Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, New Jersey.

Turner, R. E., E. M. Swenson, C. S. Milan, J. M. Lee and T. A. Oswald. 2004. Below-ground biomass in healthy and impaired salt marshes. Ecological Research 19:29-35.

U. S. Department of Defense 1999. The use of constructed wetlands to phytoremediate explosives-contaminated groundwater at the Milan army ammunition plant, Milan, Tennessee. http://www.estcp.org/documents/techdocs/199520.pdf

Van Andel, J. and A. Biere. 1989. Ecological significance of variability in growth rate and plant productivity. Pages 257-268 in H. Lambers, H. Poorter and

M.I. Van Vuuren editors. Inherent variation in plant growth. Backhuys publications, Leiden, The Netherlands.

Van der Valk, A. G. and Bliss, L. C. 1971. Hydrarch succession and net primary production of oxbow lakes in Central Alberta. Canadian Journal of Botany 49:1177-1199.

Van der Werf, A., Visser, A.J., Schieving, F., Lambers, H. 1993. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast- and slow-growing species. Functional Ecology 7:63-74.

Van Wyke, B., B. van Oudtshoorn and N. Gericke. 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa.

Venosa, A. D., K. Lee, M. T. Suidan, S. GarciaBlanco, S. Cobanli, M. Moteleb, J. R. Haines, G. Tremblay and M. Hazelwood. 2002. Bioremediation and biorestoration of a crude oil-contaminated freshwater wetland on the St. Lawrence River. Bioremediation Journal 6:261-281.

Weber, M. and R. Braendle. 1996. Some aspects of the extreme anoxia tolerance of the Sweet Flag, *Acorus calamus* L. Folia Geobotanica Phytotaxa 31:37-46.

Wilson, P. C., S. J. Kline and T. Whitwell. 2000. Phytotoxicity, uptake and distribution of 14C-simazine in *Acorus gramineus* and *Potendria cordata*. Weed Science 48:701-703.

Winterrowd, W. 1990. Just add water. Horticulture 68:24.

World Wildlife Fund. 1992. Statewide wetland strategies: A guide to guide to protecting and managing the resource. Island Press, Washington, D.C., USA.

Zhou, C. F., P. Qin and M. Xie. 2003. Vegetating coastal areas of east China: species selection, seedling cloning and transplantation. Ecological Engineering 20:275-286.

Table 2.1: Multivariate analysis of variance (MANOVA) of growth variables measured for *Acorus calamus* grown under low and high light, nutrient and moisture treatments. The experiment was set up as a split plot design with nutrient and moisture nested within light treatments.

Source	Wilk's λ	df1	df2	*F*	*P*
Light	0.762	11	164	4.66	< 0.001
Nutrient (Light)	0.449	22	328	7.33	< 0.001
Moisture (Light)	0.508	22	328	6.01	< 0.001
Nutrient (Light) × Moisture (Light)	0.709	22	328	2.80	< 0.001

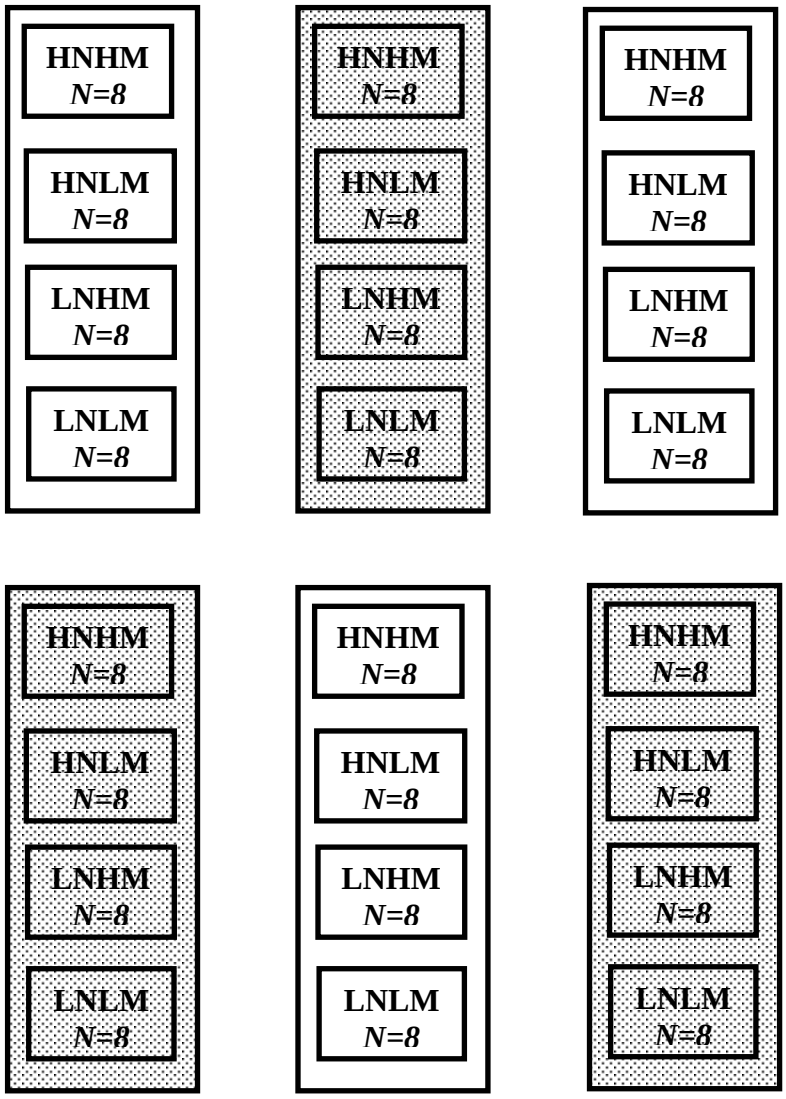
Table 2.2: Analysis of variance (ANOVA) of growth variables measured for *Acorus calamus* grown under low and high light, nutrient and moisture treatments. The experiment was set up as a split plot design with nutrient and moisture nested within light treatments. ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Source	Light	Nutrient	Moisture	Nutrient x Moisture
d.f.	1	2	2	2
Total no. of buds	0.19	3.63*	27.05***	2.61
Mature buds	2.80	10.32***	5.02**	2.11
Total no. of leaves	17.55***	11.60***	20.97***	3.77*
Leaf area	20.40***	33.30***	13.50***	0.36
Leaf biomass (g)	0.05	20.50***	30.94***	5.87**
Rhizome length (cm^2)	7.81**	1.85	21.77***	1.64
Rhizome biomass	0.02	6.50**	26.61***	3.18*
Root biomass	1.51	17.99***	4.54*	1.16
Total biomass	3.10	29.97**	317.82***	12.43
Specific Leaf Area	10113.57**	7861.65*	1095.33	9901.43*
Shoot – root ratio	141.48**	768.81***	112.70	112.86

Table 2.3: Means ± standard error, minimum and maximum values for growth variables in *Acorus calamus* rhizomes after being subjected to high and low light, nutrient and moisture treatments.

	Mean	± S.E.	Minimum	Maximum
Total no. of buds	4.104	0.138	1.000	10.000
Mature buds	2.176	0.088	1.000	6.000
Total leaf	12.692	0.464	3.000	35.000
Leaf area	131.885	5.833	12.260	405.000
Leaf biomass (g)	2.723	0.089	0.185	5.323
Rhizome length	5.564	0.178	1.200	15.000
Rhizome biomass (g)	2.358	0.085	0.070	5.500
Root biomass (g)	1.931	0.093	0.100	7.800
Total biomass (g)	7.013	0.210	0.385	14.519
Specific Leaf Area (SLA)	53.007	2.596	4.152	272.378
Specific Rhizome length (SRL)	2.99	0.211	0.412	32.857
Shoot-root ratio	4.068	0.367	0.395	45.773

Figure 2.1 Design of experiment examining the influence of light, water and nutrients on growth of rhizomes of *Acorus calamus* L. The experiment was set up as a split plot design with nutrient and moisture nested within light treatments. Large rectangles represent shadehouses with light treatments: open rectangles- full sunlight; shaded rectangles-fifty percent shade. Smaller rectangles represent nutrient and moisture treatment combinations within a shadehouse: HN-high nitrogen; HM-high moisture; LN-low nitrogen; LM-low moisture


HNHM
N=8
HNLM
N=8
LNHM
N=8
LNLM
N=8
HNHM
N=8
HNLM
N=8
LNHM
N=8
LNLM
N=8
HNHM
N=8
HNLM
N=8
LNHM
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LNLM
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HNHM
N=8
HNLM
N=8
LNHM
N=8
LNLM
N=8
HNHM
N=8
HNLM
N=8
LNHM
N=8
LNLM
N=8

Figure 2.2: Means ± S.E. of total plant biomass of *Acorus calamus* L. grown under low and high light, nutrient and moisture treatments. Means with same letter are not significantly different from each other ($P > 0.05$). Portion above upper horizontal line indicates mean leaf biomass, portion below upper horizontal line indicates mean rhizome biomass and portion below lower horizontal line indicates mean root biomass.

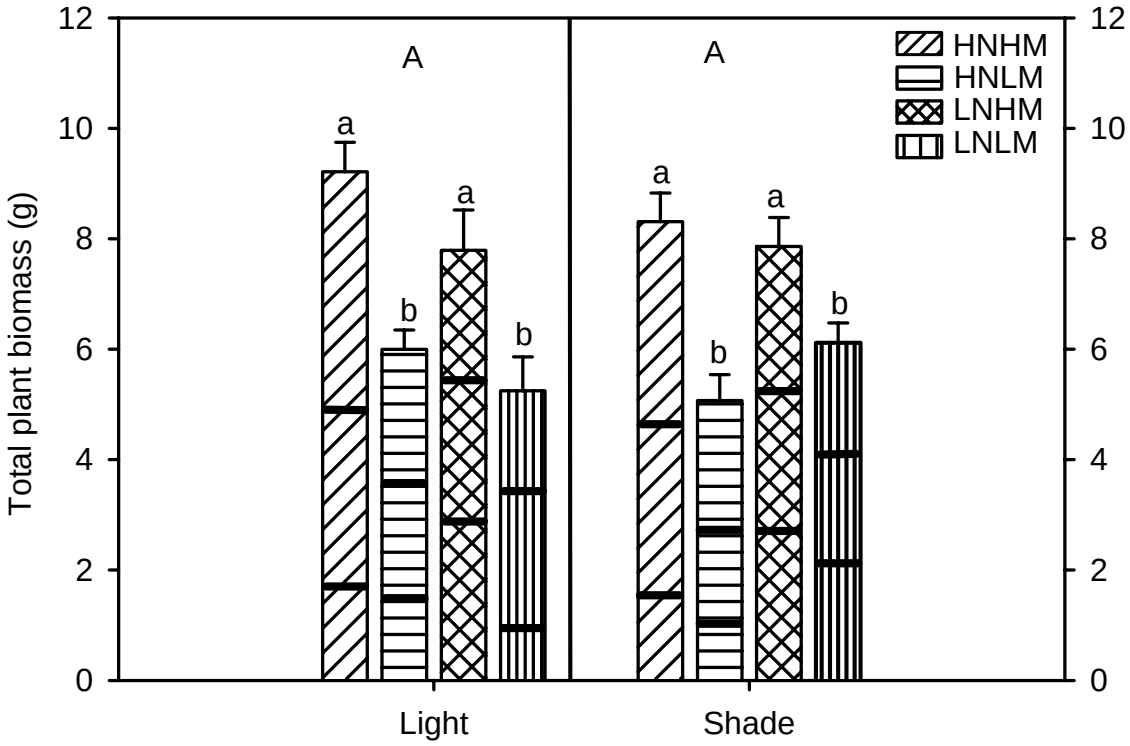

Total plant biomass (g)
12
10
8
6
4
2
0
A
A
a
b
a
b
a
b
a
b
HNHM
HNLM
LNHM
LNLM
Light
Shade

Figure 2.3: Means ± S.E of aboveground variables in *Acorus calamus* L. rhizomes grown under low and high light, nutrient and moisture treatments. Means with same letter are not significantly different from each other ($P > 0.05$).

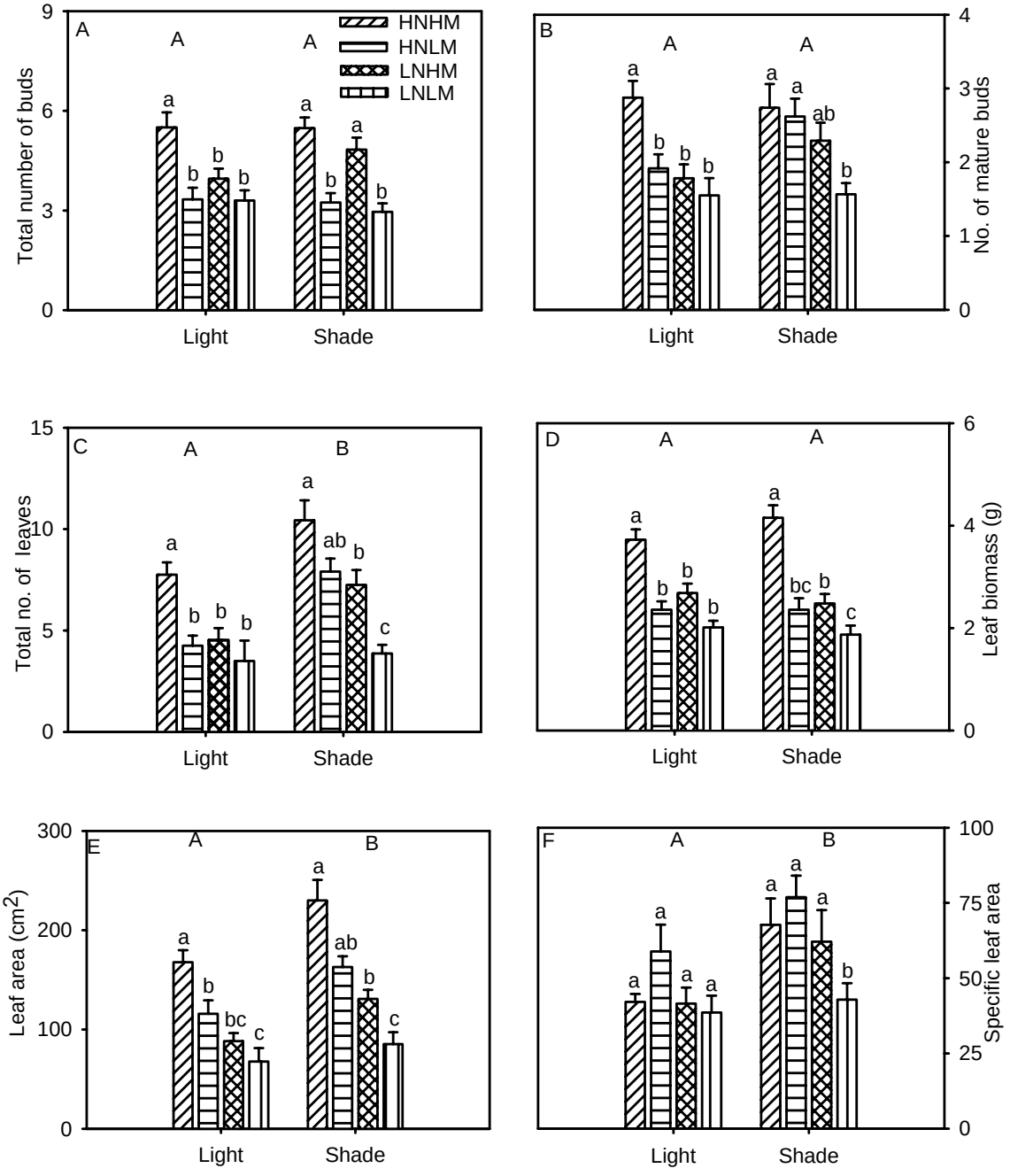
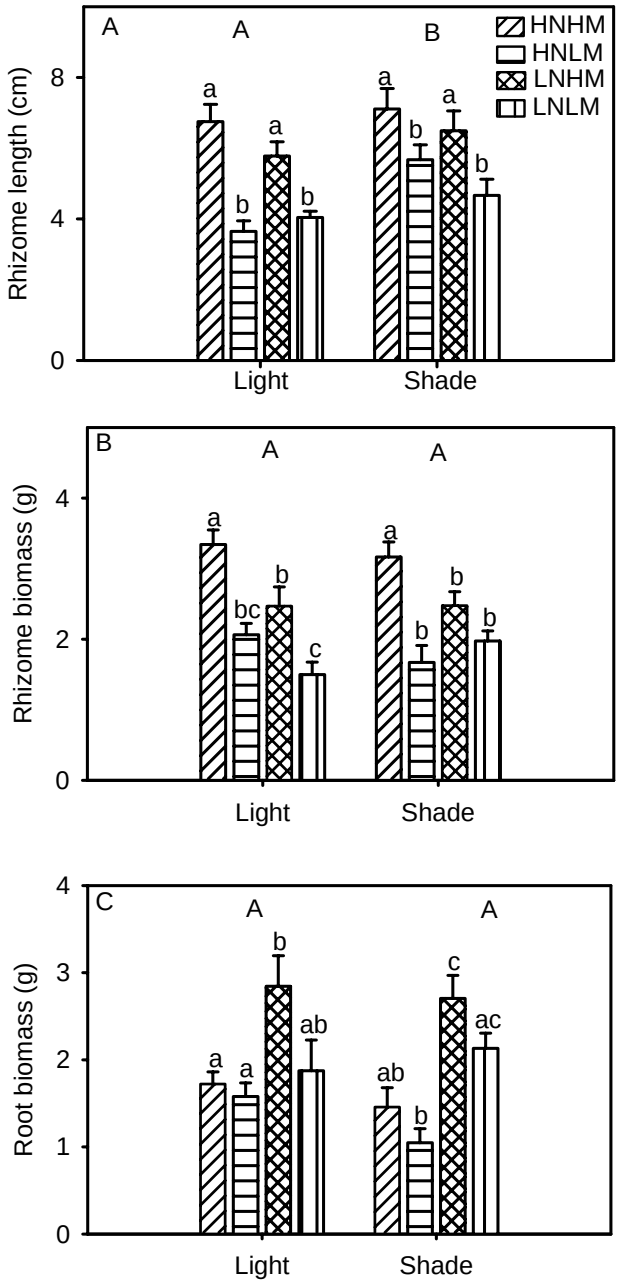

A
B
C
D
E
F
HNHM
HNLM
LNHM
LNLM
Total number of buds
No. of mature buds
Total no. of leaves
Leaf biomass (g)
Leaf area (cm2)
Specific leaf area
Light
Shade

Figure 2.4: Means ± S.E of belowground variables in *Acorus calamus* L. rhizomes grown under low and high light, nutrient and moisture treatments. Means with same letter are not significantly different from each other ($P > 0.05$).


A
A
B
HNHM
HNLM
LNHM
LNLM
Rhizome length (cm)
8
4
0
a
b
a
b
a
b
a
b
Light
Shade
B
A
A
Rhizome biomass (g)
4
2
0
a
bc
b
c
a
b
b
b
Light
Shade
C
A
A
Root biomass (g)
4
3
2
1
0
a
a
b
ab
ab
b
c
ac
Light
Shade

Chapter 3: Genetic variation in populations of *Acorus calamus* L. in southeast Ohio.

INTRODUCTION

Acorus calamus L. (Sweetflag) is an emergent aquatic macrophyte with a ubiquitous global distribution in temperate and subtropical Asia, North America, Europe and South Africa. The rhizome of this geophyte is of economic and ethnobotanical importance to several cultures and traditional systems of medicine over the world (Motley 1994, Moerman 1998, McGaw 2002). Thought to have originated in the Indian subcontinent, its widespread distribution is attributed to intentional introduction by humans along trade and colonization routes (Motley 1994). The plant is being promoted as a crop on marginal lands (Lokesh and Chandrakanth 2002). However populations of the plant have been reported as threatened in the Indian subcontinent and Europe and declining in North Americak. Reasons attributing to population decline include over harvest of the rhizome and habitat loss (IIFM 1998, Lange 1998 Robbins 1998).

On the North American continent the plant is a locally abundant helophyte found in swampy, marshy areas along the edges of ponds, streams and lakes (Braun 1921, Buell 1935a, Jervis and Buell 1964, Packer and Ringius 1984). During summers in North America, Sweetflag has long swordlike foliage with equitant leaf bases arising from a pinkish white sympodial rhizome found near the surface of the ground. During winters the shoots die though the terminal bud persists. A high degree of anoxia tolerance (Weber and Braendle 1996, Schluter and Crawford 2001, Joly and Braendle 1995) enables the plant to tide over

prolonged periods of flooding and snow. Both triploid and diploid varieties of the plant are found in North America (Love and Love 1957); the former being referred to as *Acorus calamus* L. var. *americanus*, or sometimes even *Acorus americanus.* The sterile triploid variety is thought to have been introduced from Europe and is now been naturalized to the North America (Buell 1935b, Packer and Ringius 1984). Evolutionarily, the genus is considered the most primitive extant monocot (Duvall et al. 1993).

Though the plant produces both sexually by seed and asexually by rhizome, North American populations are thought to be generally derived from rhizome stock planted by humans. Early settlers (Motley 1994, Les and Mehroff 1999) planted the rhizome around settlements while Native Americans distributed the plant along hunting trails (Gilmore 1930). Scarcely any seedlings have been reported in natural populations (Buell 1935b). Even though *Acorus calamus* has been recorded as part of the emergent above ground vegetation in wetlands, there has been no record of it being present in the seed bank (Leck and Simpson 1987). These observations suggest that populations of *Acorus calamus* in North America may be primarily clonal in nature though no empirical evidence has been provided. It is possible that population patches of *A. calamus* are all derived from a single rhizome serving as a diaspore. The tight spatial packing and intertwining of ramets in populations makes it difficult to differentiate genets. Further, genets in a population may be the result of clone fragmentation and thus may be members of the same genotype.

Despite its ethnobotanical and evolutionary significance, genetic variability of Sweetflag has not been studied. Ascertaining genetic diversity in populations is a means to gauge if populations are clonal in nature. I estimated the extent of clonal growth, and intra and inter population genetic diversity in populations of *Acorus calamus* L. populations in southeast Ohio.

It was initially thought that plants reproducing primarily through clonal reproduction (Silander 1985) have low genotypic diversity. Later, information compiled on genetic diversity surveys of several clonal plants indicate that these species often have moderate to high levels of both intra- and interpopulation diversity (Ellstrand and Roose 1987, Hamrick and Godt 1990). Recent studies on clonal plants have given further evidence to their findings (Sherman-Broyles et al. 1992, Piquot et al. 1998, Szmidt 2002) though most of these genotypic patterns have been largely confined to terrestrial plant species (Ellstrand and Roose 1987, Hamrick and Godt 1990, Sherman-Broyles et al. 1992, Parker and Hamrick 1992, Tecic et al. 1998, Godt and Hamrick 1998). Studies on submerged aquatic macrophytes indicate that they have primarily monoclonal populations with low levels of genetic diversity (Laushman 1993, Hollingsworth et al. 1998, Iida and Kadono 2001, Hofstra et al. 1995, Aspinwall and Christian 1992). Genotypic patterns in emergent macrophytes are less clear. While some studies suggest that emergent macrophytes have moderate levels of genetic diversity (Barrett et al. 1993, Stenstorm et al. 2001), others hint that monoclonal populations may be more common (Mymudes and Les 1993, Keller 2000, Piquot

et al. 1998, Pellegrin and Hauber 1999). More studies on genetic diversity of emergent macrophytes are needed before a general pattern can be concluded.

METHODS

DNA markers are useful tools to assess genetic variation in populations of organisms. I utilized Inter Simple Sequence Repeats (ISSRs), a polymerase chain reaction (PCR) based neutral DNA marker for genetic analysis. ISSRs access variation in genomic DNA in the region between palindrome microsatellite DNA sequences (see Wolfe and Liston 1998 for details on microsatellites). Single primers specific to microsatellite regions are used to amplify ISSRs. The primers consist of di- and trinucleotide repeats with additional 1-3 nucleotides at the 5' or 3' end to serve as an anchor. ISSR techniques are nearly identical to RAPD techniques except that ISSR primer sequences are designed from microsatellite regions and the annealing temperatures used are higher than those used for RAPD markers. No prior knowledge of the target sequence is required (Godwin et al. 1997). Like RAPDs, ISSRs are dominant markers (they cannot provide information on heterozygosity of loci). ISSRs have several properties that make them ideal DNA markers for population studies. They are highly polymorphic and hypervariable (as compared to RAPDs), occur frequently in plant genomes, are relatively cheap (as compared to AFLPs or microsatellite

markers) and easily reproducible. Information generation is more efficient than allozyme markers (Esselman et al. 1999).

Population level information generated by ISSRs concurs with that generated by other dominant markers such as RAPDs (Adams et al. 2003, Archak et al. 2003) and AFLPs (Nybom 2004). ISSRs are increasingly being used to answer questions of genetic diversity at the population level: ecogeography of populations (McCauley and Ballard 2002, Meekins et al. 2001 Jian et al. 2004); genetic diversity of weeds (Ash et al. 2004, Ye et al. 2004, Meekins et al. 2001); and, hybridization between closely related species (Wolfe et al. 1998, Cortes-Palomec 2001). Their predominant use in studying cultivars and genotyping germplasms (Tsumura et al. 1996, Fang and Roose 1997, Nagaoka and Ogihara 1997, Blair et al. 1999) also indicates their usefulness in studying patterns in clonally reproducing organisms (Li and Ge 2001, Liston et al. 2003).

Study Area and Sampling

I surveyed ten populations of *A. calamus* (Table 1) in the unglaciated Allegheny Plateau of southeast Ohio, U.S.A. Watersheds in the area lie within the northern Ohio River drainage system. Ohio has lost over 90% of its natural wetlands owing to farmland conversion and development activities (Gordon 1969, Cusick and Silberhorn 1977, Ohio Department of Natural Resources 2002). Wetlands that remain in southeast Ohio are chiefly human- made consisting of ephemeral streams, vernal pools, and human-made impoundments

(Boreman 2001). Leaf tissue was randomly sampled from sixteen individuals of each population. Each individual sample was placed in a labeled bag along with Silica gel, a desiccant and then stored at -20^{o} C till DNA could be extracted.

DNA Extraction and ISSR Amplification

DNA was extracted from the leaf tissue using a modified chloroform isoamyl alcohol extraction procedure (Ballard et al. 1998) adapted from the SDS buffer protocol of Edwards et al. (1991). The extracted DNA in each sample was quantified using a BioPhotometer (©1998 Eppendorf). Samples were standardized to about 50 µg DNA per sample for consistency. The extracted DNA was stored at - 20^{o} C till PCRs were conducted on them. I screened primers listed by Wolfe et al. (1998) and used three that produced clear bands (Table 2).

For each primer and sample, a 25 µl of a standard PCR master mix was made with 1.5 µl of sample DNA, 9 µl of distilled water, 5 µl $MgCl_2$ (25 mM, Eppendorf), 2.5 PCR buffer (1.5mM Mg^{2+}, Eppendorf), 4 µl dNTP (10 mM, Fisher), 2.5 µl BSA (bovine serum albumin, 4 mg/ml, Fisher) and 0.5 µl of TAQ polymerase (Eppendorf). PCR (Mullis et al. 1986) was performed using a Stratagene Robocycler® temperature cycler. The amplification program used was denaturation at 94º C for 2 min; followed by denaturation for 40 cycles of 94ºC for 30 sec, annealing at 44ºC for 40 sec and, extension at 72ºC for 1 min 30 sec; and, a final extension at 72ºC for 20 min.

After amplification 7 µl of the PCR product was mixed with 3 µl of loading dye and run on a 1.3% Agarose gel stained with Ethidium Bromide (Fisher). A 250 base-pair ladder (GIBCO) was also run on the gel to allow for quantification of the fragments. The gel was visualized, photographed and scored using Quantity One © (Bio-Rad Laboratories , Hercules, CA, USA). Loci with distinct bands were scored as present '1' other wise being scored as '0' if absent in a sample.

Genetic Diversity and Population Structure

Genetic diversity within populations was estimated based on the frequency of occurrence of ISSR bands using the Shannon's diversity and eveness index (see Magurran 1988 for details), Nei's estimate of gene diversity (Nei 1978), percentage polymorphic loci, and, expected heterozygosity assuming the populations to be in Hardy Weinberg equilibrium (see Nei 1987). Since ISSRs are dominant markers (hence I cannot eveluate heterozygosity), the estimation procedure suggested by Weir (1990) was used wherein the frequency of the recessive allele is treated as the square root of the absences seen at a locus. The Shannon's index also yielded a measure of "evenness" or homogeneity of genetic variation among populations.

Genetic differentiation both within and among populations was estimated using an Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992). AMOVA is a non parametric method of estimating genetic variation among

populations using Euclidean distances derived from vectors of 1s and 0s. Pairwise binary genetic distances were computed based on the Huff et al. (1993) method for dominant markers. A null distribution is computed by resampling of data. AMOVA extracts variance components among and within populations and generates a Φ statistic (an F-statistic analog). One way AMOVAs were also permuted between each pair of populations. Variance estimates were based on 1000 permutations. I calculated Nei's unbiased measure of genetic distance and identity (Nei 1978) between each pair of populations and G_{ST}, a coefficient of genetic differentiation and N_m a measure of gene flow among all the populations (Nei 1987).

A cluster analysis using the unweighted pair group method (UPGMA) based on Jaccard's similarity coefficient and a Principal Coordinates Analysis (PCoA) based on Dice's similarity coefficient was conducted to examine similarity among populations. Both Dice and Jaccard's coefficients use only information on the presence of loci in populations and ignore their absence.

Shannon's diversity and evenness index was computed using MVSP Plus Version 3.0 (© Kovach Computing Services, Wales, U.K.). Percent polymorphic loci and expected heterozygosity were calculated using Tools for Population Genetic Analysis (TFPGA) 1.3 (Miller 1997). Nei's measures of genetic identity and distance were computed using POPGene 1.32 (Yeh et al. 1999). AMOVA was conducted using GenALEx V5 (Peakall and Smouse 2001). UPGMA and PCoA were conducted using NTSYS-pc 2.02J (Rohlf 1999).

RESULTS

Two hundred bands were scored among the populations of *A. calamus* using three selected primers. The number of fragments generated by each primer varied between 100 bp to 5540 bp (Table 2). Shannon's Index varied among populations indicating that there was varying amounts of genetic diversity among populations. The index for evenness of genetic diversity within populations was high (> 0.90) for all populations indicating that individuals within a populations shared many of the same loci. This implied that though genetic diversity varied considerably among populations, it remained homogenous among individuals within a population. Nei's estimate of genetic diversity and the measures of expected heterozygosity and percentage of polymorphic loci was low varying from 6% to 16.5% among populations (Table 3). All the measures indicated that individuals within populations were homogenous in terms of their genetic composition and there was very little within population diversity.

The AMOVA indicated that most of the variation (58%) is among populations. There is less variation (42 %) within populations (Table 4). The Φ_{ST} was 0.581 and was significantly different from zero ($P < 0.001$) indicating that there was a significant amount of variation among populations rather than within populations. The measure of evenness of genetic diversity within populations derived from the Shannon index was high (> 0.90) that individuals within a populations had many of the same loci implying little genetic variation within a

population. The pairwise population Φ_{ST} (Table 5) indicated that all populations varied significantly ($P < 0.001$) from each other. The overall coefficient of genetic variation among populations ($G_{st} = 0.7188$) and gene flow ($N_m = 0.1956$) indicated a large amount of differentiation among the populations with very little gene flow between them. Estimates of genetic identity between all pairs of populations, even geographically close populations, were high and hence genetic distance was also high (Table 6).

The UPGMA cluster analysis grouped individuals from the same population together for all populations. The populations themselves grouped into two major dissimilar (0.02) clusters (figure 1). HFNF, OA3 and PSR formed one distinct cluster and the remainder seven populations composed the other cluster. The clustering together of the populations did not show relation to geographic proximity. The UPGMA also showed that there was a moderate amount of variation (about 25%) within populations. PCoA also showed similar results (figure 2). Individuals from the same population mostly formed tight clusters. Some populations (viz. GBR and OARR and, HFF and RID) were located close to each other in ordination space, however these were not populations that were situated close to each other geographically. In fact, in both the UPGMA cluster analysis and PCoA, OAS and OA3 which are geographically neighboring populations are genetically very dissimilar.

DISCUSSION

All the populations of *A. calamus* that were surveyed in southeast Ohio have extremely low measures of estimated heterozygosity, poplymorphic loci and genetic diversity. Genetic variation within a population is homogenous. The pattern of low genetic diversity within populations of *Acorus calamus* L. in southeast Ohio indicates that they are monoclonal. The diaspore initiating a population could have been either a seed or a rhizome. Subsequent growth and expansion of a population patch occurs due to clonal reproduction of ramets by the rhizome.

Establishment of populations from a single propagule results in a substantial founder effect that is characterized by very low diversity within populations. ISSRs are hypervariable as compared to other tools that access genetic variation such as allozymes and RAPDS (Nybom 2004). This makes them ideally suited to studying clonally propagated populations as have been observed in *Acorus calamus*.

Clonal propagation is common among monocots and emergent macrophytes. It is characteristic of plants growing in resource poor, stressful environments. Though ramets can exist independently, physiological integration within a genet facilitates translocation of photoassimilates, water and minerals. Clonal reproduction promotes persistence of successful genotypes, higher survival of nascent ramets, overall growth of the genet and rapid regeneration

after local damage (van Groenendael et al. 1997, Pennings and Callaway 2000, Jonsdottir and Watson 1997, Hutchings and Wijesinghe 1997) thus increasing fitness. In aquatic plants rhizomes also act as propagules when they fragment and pieces of the genet along with “bud banks” are dispersed (Grace 1993).

Several other perennial helophytes and halophytes display similar patterns of low within population genetic diversity and high among population diversity and differentiation (Barrett et al. 1993) as seen in *A. calamus*. Rhizomatous growth coupled with inbreeding has attributed to the high levels of homozygosity observed in North American *Typha* populations (Mashburn et al. 1978, Sharitz et al. 1980). Clonal propagation and dispersal of vegetative propagules is thought to be responsible for the uniformity of genetic diversity within populations of the weedy *Phragmites australis* (Pellegrin and Hauber 1999). Bottlenecking and the loss of heterozygosity is disadvantageous in rapidly changing environments. However, the shift towards asexuality in emergent macrophytes may reflect an evolutionary shift in preserving adaptive gene complexes in stable aquatic environments (Santamaria 2002).

The patterns of genetic variation in this study indicated a high amount of genetic variation and divergence among populations of *A. calamus* in southeastern Ohio. Moderate variation also existed within the populations. Establishment of populations through the narrow genetic bottleneck of a single diaspore results in genetic drift. Genetic drift catalyzes population differentiation. However

populations that have multiple founder events can show some variation. This might be the case with *A. calamus* populations.

Very few alleles from the parent population are represented and alleles getting fixed in the new population may be infrequent in the parent population. The genetic mosaic hypothesis (Gill et al. 1995) states that clonal organisms accumulate spontaneous mutations that are preserved in the population through somatic cell lines. The rate of such mutations is very low and over time can contribute to some variation in the population. Fixing of these mutations in a population can further contribute to its random genetic drift. Population differentiation is a common phenomenon in emergent macrophytes and is thought to be an adaptive strategy to local variation in flooding, nutrients and water level resulting in the small scale segregation of ecotypes (Santamaria 2002).

Loveless and Hamrick (1984) reviewed genetic variation in angiosperm populations and indicated that much of population structuring depends on the life history of the species. Similarly genetic variation in many emergent macrophyte populations is structured by their breeding systems and life history. Patterns of genetic variation in *A. calamus* are akin to those observed in submerged hydrophilous angiosperms. In these species, widespread monomorphism and weak population differentiation is catalyzed by inefficient pollen transfer, reduced sexual reproduction, diminished seed production and widespread clonal growth (Les 1988).

Values for heterozygosity, polymorphic loci and Nei's measure of gene diversity in populations of *A. calamus* are lower than in several species of submerged hydrophytes (see Laushman 1993 for comparison). Presently, very little is known about the life history stages of *A. calamus*: the phenology of flowering, seed germination and seedling survival has yet to be studied. Even occasional recruitment of seedlings would have been reflected by the occurrence of multiple clones within populations, however, this was absent in the populations that were sampled. With a non persistent seedbank (Leck and Simpson 1995), poor soil nutrients and frequent inundation, seed germination and seedling survival of the species maybe precarious.

There is a possibility that all the populations that were sampled in south eastern Ohio are sterile triploids and hence pollen and seed limited. Polyploidy is a common occurrence in aquatic angiosperms and has long term evolutionary consequences often providing the opportunity for sympatric speciation (Les and Philbrick 1993). Packer and Ringius (1984) used herbarium records to survey *Acorus calamus* specimens from herbaria in Canada and concluded that they were mainly diploid fertile populations.

The distribution of sterile and fertile populations in the United States is less clear. Eckert et al. (2003) had compared genetic variation in triploid sterile populations with diploid sexually reproducing populations of the aquatic macrophyte *Butomus umbellatus*. Contrary to expectations, sexually fertile populations did not exhibit higher genetic diversity than sterile populations.

Populations were monomorphic with predominantly clonal mode of reproduction. Seedlings were out-competed for safe sites by bulbils during establishment. Often even when plants with both asexual and sexual reproduction are self compatible, clonal forms of growth can interfere with the sexual breeding system (Handel 1985). Drastic variation in seed production can result in a concurrent difference in genetic variation as has been observed among populations of the aquatic plant *Decodon verticillatus* (Eckert 2002). Patterns of genetic variation suggest that there might be some sexual reproduction occurring though this has to be further studied.

ISSRs are neutral markers that target hypervaraible regions of the genome. It is important that other techniques such as AFLPs also be used to further verify and patterns of genetic variation that have been observed in these populations. Multiple techniques are often used when studying cultivars (Archak et al 2003).

Species ranges depend on the vagility of the clonal diaspore. In the case of *A. calamus* in southeast Ohio, genetically similar populations do not show any geographical proximity. There is no known non-human biotic vector of the rhizome. The rhizome has economic potential and is horticulturally important. All the wetlands where the populations are located are human – made. These factors and the wide dispersal of genetically similar populations suggest that rhizomes of *A. calamus* may have been introduced and spread in south eastern Ohio by humans.

Humans have been responsible for introduction and dispersal of many plant species. The case of weedy plant species is well documented (Baker 1986). Intentional dispersal of several economically and horticulturally important plant species by humans (Nabhan 2000, Dehnen-Schmutz 2004) is a phenomenon common to settlements and cultures. While food crops (e.g., *Mannihot* spp.) and plantation crops (e.g., *Hevea* spp) are farmed on a large scale, incipiently cultivated ornamental and horticultural species may be distributed more randomly.

The extent of genetic homogeneity within populations implies that if *A. calamus* in Ohio were to be commercially exploited in the future, it would be easy to sample and harvest rhizomes from populations that yield the best quality of essential oils and medicinal products. Also, it will be cost effective to obtain a consistent quality of the rhizome constituents in wild and cultivated populations of the plant.

LITERATURE CITED

Adams R.P, A.E. Schwarzbach, R.N. Pandey. 2003. The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from *Juniperus*. Biochemical Systematics and Ecology. 31:375.

Archak S., A.B. Gaikwad, D. Gautam, E.V.V.B. Rao, K.R.M. Swamy, J.L. Karihaloo. 2003. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome 46:362-369.

Aspinwall N., C. Terry. 1992. Clonal structure, genotypic diversity, and speed production in populations of *Filipendula rubra* (Rosaceae) from the North Central United States. American Journal of Botany 79:294 – 299.

Ash G.J., R. Raman, N.S. Crump. 2003. An investigation of genetic variation in *Carthamus lanatus* in New South Wales, Australia, using intersimple sequence repeats (ISSR) analysis. Weed Research 43:208-213.

Baker, H.G. 1986. Patterns of plant invasion in North America Pages 44-57 *in*: Mooney, H., A. Drake, A. James eds. Ecology of biological invasions of North America and Hawaii. Ecological Studies 58. New York: Springer-Verlag. 321 pp.

Ballard Jr., H.E., K.J. Sytsma, R.R. Kowal. 1998. Shrinking the violets: Phylogenetic relationships of infrageneric groups in *Viola* (Violaceae) based on Internal Transcribed Spacer DNA sequences. Systematic Botany 23:439-458.

Barrett, S.C.H., C.G. Eckert, B.C. Husband. 1993. Evolutionary processes in aquatic plant populations. Aquatic Botany 44:105-145.

Belanger, A., F. Boudreau, N. Grondin. 2000. Essential oil from *Acorus calamus*. Research Summary Volume 27, Horticultural Research and Development

center, Agriculture and Agri-food Canada, Quebec Canada. 34 pp. http://res2.agr.ca/stjean/publication/resume9900/resume9900_e.pdf

Blair M.W., O. Panaud, S.R. McCouch. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 98:780-792.

Boreman, J. 2001. Ohio Chapter protects and restores threatened wetlands. Ohio Landscapes. The Nature Conservancy. "Fall" pp 8-11.

Braun, L.E. 1921. Composition and source of the flora of the Cincinnati region. Ecology 2:161-180.

Buell, M. 1935. *Acorus calamus* in America. Rhodora 37:367-369.

Buell, M. 1935 Seed and seedling of *Acorus calamus*. Botanical Gazette 96:758-765.

Cusick, A.W., G.M. Silberhorn. 1977. The Vascular plants of unglaciated Ohio. Bulletin of the Ohio Biological Survey, Volume 5, Number 5. Ohio State University, Columbus, OH, USA.

Cortés-Palomec, A.C. 2001. Isolation mechanisms and gene flow between co-occurring species of Mexican *Viola* (Violaceae). MS Thesis. Ohio University, OH.

Dehnen-Schmutz, K. 2004. Alien species reflecting history: Medieval castles in Germany. Diversity and Distributions. 10:51-57.

Duvall, M.R., G.H. Learn, L.E. Eguiarte, M.T. Clegg. 1993. Phylogenetic analysis of rbcl sequences identifies *Acorus calamus* as the primal extant monocotyledon. Proceedings of the National Academy of Sciences. 90:4641-4644.

Eckert, C.G. 2002. The loss of sex in clonal plants. Evolutionary Ecology. 15:501 – 520.

Edwards, K., C. Johnstone, and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research. 19:13-49.

Ellstrand, N.C., M.L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. American Journal of Botany. 74:123-131.

Esselman E.J., L. Jianqiang, D.J. Crawford, J.L. Winduss, A.D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. Molecular Ecology. 8:443-451.

Excoffier, L., P.E. Smouse, J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131:479-491.

Fang D.Q., M.L. Roose. 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theoretical and Applied Genetics 95:408-417.

Gill D.E., L. Chao, S.L. Perkins, J.B. Wolf. 1995. Genetic mosaicism in plants and clonal animals. Annual Review Ecology and systematics 26:423 – 444.

Gilmore, M.R. 1930. Dispersal by Indians as a factor in the extension of discontinuous distribution of certain species of native plants. Papers of the Michigan Academy of Science, Arts and Letters. 13:89-94.

Godt, MJW, JL Hamrick. 1998. Allozyme diversity in the grasses, pp. 11-29 *in* GP Cheplick ed. Population Biology of Grasses. Cambridge University Press, Cambridge Mass.

Godwin, I.D., E.A.B. Aitken, and L.W. Smith. 1997. Application of inter-simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis. 18:1524-1528.

Gordon, R.B. 1969. The natural vegetation of Ohio in pioneer days. Bulletin of the Ohio Biological Survey Volume III Number 2. Ohio State University, Columbus, OH, USA.

Grace, J.B. 1993. The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. Aquatic Botany 44:159-180.

Hamrick, J.L., M.J.W. Godt. 1990. Allozyme diversity in plant species. Pages 43 – 63 *in* AHD Brown, MT Clegg, AL Kahler, BS Weir, eds. Plant population genetics, breeding and genetic resources. Sinauer Associates Inc., Sunderland.

Handel, SN 1985 The intrusion of clonal growth patterns on plant breeding systems. American Naturalist 125. 367-384.

Hofstra D.E., K.D. Adam, J.S. Clayton. 1995. Isozyme variation in New Zealand populations of *Myriophyllum* and *Potamogeton* species. Aquatic Plants. 52:121-131.

Hollingsworth, P.M., M. Tebbitt, K.J. Watson, R.J. Gornall. 1998. Conservation genetics of an arctic species, *Saxifgra rivularis* L., in Britain. Botanical Journal of the Linnean Society. 128:1-14.

Huff, D.R. 1997. RAPD charactrization of heterogeneous perennial ryegrass cultivars. Crop Science. 37:557–564.

Huff, D.R., R. Peakall, and P.E .Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalo grass [*Buchloë dactyloides* (Nutt.) Engelm.]. Theoretical and Applied Genetics. 86:927-934.

Iida S., Y. Kadano. 2001. Population genetic structure of *Potamogeton anguillanus* in Lake Shinji, Japan. Limnology. 2:51-53.

Indian Institute of Forest Management. 1998. Conservation assessment and management planning of non timber forest products in Madhya Pradesh. IIFM, Nehru Nagar, Bhopal, India.

Jervis, R.A., M.F. Buell 1964 *Acorus calamus* in New Jersey. Torreya 91:335-336.

Joly, C.A., R. Braendle. 1995. Fermentation and adenylate metabolism of *Hedychium coronarium* JG Koenig (Zingiberaceae) and *Acorus calamus* L (Araceae) under hypoxia and anoxia. Functional Ecology. 9:505-510.

Kumar, V.S., R.K. Srivastava, A. Krishna, V.K.S. Tomar, A.K. Singh, S. Kumar 2000. Cultivation, chemistry, biology and utilization of bach (*Acorus calamus*): a review. Journal of Medicinal and Aromatic Plant Sciences 22:338-348.

Jian, S., T. Tang, Y. Zhong, and S. Shi. 2004. Variation in inter-simple sequence repeat (ISSR) in mangrove and non-mangrove populations of *Heritiera littoralis* (Sterculiaceae) from China and Australia. Aquatic Botany. 79:75-86.

Jonsdottir, I.S. and M.A. Watson. 1997. Extensive physiological integration: an adaptive trait in resource-poor environments? Pages 109-136 In H. de Kroon and J. van Groenendael editors. The ecology and evolution of clonal plants. Backhuys publishers, Lieden, The Netherlands.

Keller B.E.M 2000. Genetic variation among and within populations of *Phragmites australis* in the Charles River watershed. Aquatic Botany. 66:195-208.

Lange, D. 1998. Europe's Medicinal and Aromatic Plants: Their Use, Trade and Conservation TRAFFIC International, UK.

Laushman, R.H. 1993. Population genetics of hydrophilous angiosperms. Aquatic Botany. 44:147-158.

Leck, M.A., R.L. Simpson 1995 Ten year seed bank and vegetation dynamics of a tidal freshwater marsh. American Journal of Botany 82:1547-1557.

Les, D.H. 1988. Breeding systems, population structure, and evolution in hydrophilous angiosperms. Annals of the Missouri Botanical Garden. 75:819-835.

Les, D.H., C.T. Philbrick. 1993. Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. Aquatic Botany. 44:181-228.

Li A., S. Ge. 2001. Genetic variation and clonal diversity of *Psammochloa villosa* (Poaceae) detected by ISSR markers. Annals of Botany. 87:585-590.

Liston A., B.L. Wilson, W.A. Robinson, P.S. Doescher, N.R. Harris, T. Svejcar. 2003. The relative importance of sexual reproduction versus clonal spread in an aridland bunchgrass. Oecologia. 137:216.

Lokesh, GB, MG Chandrakanth 2002 A study for commercial cultivation of sweet flag (*Acorus calamus*) in Tumkur district of Karnataka. Journal of Medicinal and Aromatic Plant Sciences 24:984-989.

Love, A., D. Love. 1957. Drug content and polypoidi in *Acorus*. Proceedings of the Genetic Society of Canada.

Loveless, M.D., and J.L. Hamrick. 1984. Ecological determinants of genetic structure of plant populations. Annual Review of Ecology and Systematics. 15.65-95.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, NJ.

Mashburn, S.J., R.R. Sharitz, and M.H. Smith. 1978. Genetic variation among *Typha* populations of the southeastern United States. Evolution 32:681-685.

McCauley, R.A., H.E. Ballard Jr. 2002. Inferring nativity and biogeographic affinities of central and marginal populations of *Froelichia floridana* (Amaranthaceae) from Inter-simple sequence repeat (ISSR) markers. Journal of the Torrey Botanical Society 129:311-325.

McGaw, L.J., A.K. Jager, J. van Staden. 2002. Isolation of beta-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* in South Africa. South African Journal of Botany 68:31-35.

Meekins J.F., H.E. Ballard Jr., B.C. McCarthy. 2001. Genetic variance and molecular biogeography of a North American invasive plant species (*Alliaria petiolata*). International Journal of Plant Sciences. 62:161-169.

Miller, M.P. 1997. Tools For Populations Genetic Analysis (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. http://bioweb.usu.edu/mpmbio/index.htm.

Moerman, D.E. 1998. Native American ethnobotanyTimber Press, Portland, Oregon.

Motley, T.J. 1994. The ethnobotany of Sweetflag *Acorus calamus* (Araceae). Economic Botany 48:397-412.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, H. Erlich. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symposium on Quantitative Biology. 51:263-273.

Mymudes, M.S., D.H. Les. 1993. Morphological and genetic variability in *Plantago cordata* (Plantaginaceae), a threatened aquatic plant. American Journal of Botany. 80:351-359.

Nabhan, G.P. 2000. Cultural dispersal of plants and reptiles to the Midriff Islands of the Sea of Cortes: integrating indigenous human dispersal agents into island biogeography. Journal of the Southwest. 42:545-558.

Nagaoka T., Y. Ogihara 1997 Applicability of intersimple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theoretical and applied genetics. 94:597-602.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89:583-590.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY. 512 pp.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology. 13:1143-1155.

Ohio Department of Natural Resources. 2002. A history of Ohio wetlands. URL:http://www.dnr.state.oh.us/wetlands/history.htm.

Packer, J.G., G.S Ringius. 1984. The distribution and status of *Acorus* (Araceae) in Canada. Canadian Journal of Botany. 62:2248-2252.

Parker K.C., J.L. Hamrick 1992 Genetic diversity and clonal structure in a columnar Cactus, *Lophocereus schottii*. American Journal of Botany. 79:86-96.

Peakall, R., and Smouse, P.E. 2001. GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.edu.au/BoZo/GenAlEx/

Pellegrin D., D.P. Hauber. 1999. Isozyme varaition among populations of the clonal species *Phragmites australis* (Cav.) Trin.ex Steudel. Aquatic Botany 63:241-259.

Pennings, S.C. and R.M. Callaway. 2000. The advantages of clonal integration under different ecological conditions: a community wide test. Ecology. 81:709-716.

Piquot Y., P. Saumitou-Laparde, D. Petit, P. Vernet, J.T. Epplen. 1998. Genotypic diversity revealed by allozymes and oligonucleotide DNA fingerprinting in French populations of the aquatic macrophyte *Sparganium erectum*. Molecular Ecology. 5:251-258.

Robbins, C. 1999. Medicine from US Wildlands: An assessment of native plant species harvested in the United States for medicinal use and trade. Evaluation of the conservation and management implications. Report prepared for The Nature Conservancy. TRAFFIC North America. http://www.nps.gov/plants/medicinal/pubs/traffic.htm

Rolhf, F.J. 1998 NTSYS-pc. Numerical taxonomy and multivariate analysis system, Version 2.01. Applied Biostatistics, New York.

Santamaria, L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. Acta Oecologica. 23. 137-154.

Schluter, U., R.M.M. Crawford. 2001. Long term anoxia tolerance in leaves of *Acorus calamus* L. and *Iris pseudacorus* L. Journal of Experimental Botany. 52:2213-2225.

Sharitz, R.R., S.A. Wineriter, M.H. Smith, and E. Liu. 1980. Comparison of isozymes among Typha species in the eastern United States. American Journal of Botany 67:1297-1303.

Sherman-Broyles S.L., J.P. Gibson, J.L. Hamrick, M.A. Bucher, M.J. Gibson 1992. Comparisons of Allozyme Diversity Among Rare and Widespread Rhus Species. Systematic Botany. 17:551-559.

Silander, J.A. Jr. 1985. Microevolution in clonal plants. Pages 107 – 152 *in* J.B.C. Jackson, L.W. Buss, R.E. Cook eds. Population biology and evolution of clonal organisms. Yale University Press, New Haven, CT, USA.

Szmidt A.E., M.C. Nilsson, E. Briceno, O. Zackrisson, X.R. Wang. 2002. Establishment and genetic structure of *Empetrum hermaphroditum* populations in northern Sweden. Journal of Vegetation Science. 13:627-634.

Tecic D.L., J.L. McBride, M.L. Bowles, D.L. Nickrent. 1998. Genetic variability in the federal threatened Mead's Milkweed, *Asclepias meadii* Torrey (Asclepiadaceae), as determined by allozyme electrophoresis. Annals of the Missouri Botanical Garden. 85:97-109.

Tsumura, Y., K. Ohba, S.H. Strauss. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and Sugi (*Cryptomeria japonica*). Theoretical and Applied Genetics 92:40-45.

Van Groenendael, J.M., L.M. Klimes, J. Klimesova and R.J.J. Hendriks 1997 Comparative ecology of clonal plants. p 191-209. *In*: Silvertown, J., M. France and J.L. Harper (eds.). Plant life histories: ecology, phylogeny and evolution. Cambridge University Press, Cambridge, United Kingdom.

Weber, M., R. Brandel. 1996. Some aspects of the extreme anoxia tolerance of Sweet Flag, *Acorus calamus* L. Folia Geobotanica Phytotaxa. 31:37-46.

Weir, B.S. 1990. Genetic Data Analysis: Methods for discrete population genetic data. Sinauer Associates Inc. Sunderland, MA, USA 377 pp.

Wolfe, A.D., Q. Xiang, S.R. Kephart. 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. Molecular Ecology. 7:1107–1125.

Yeh, F.C., R.C. Yang, T.J.B. Boyle, Z.H. Ye, J.X. Mao. 1999. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

Table 3.1: Location of populations of *Acorus calamus* L. in southeast Ohio sampled for ISSR analysis. Leaf tissue was gathered from sixteen individuals of each population.

Site	Location	Coordinates
LSNP	Lake Snowden, Athens County	N 39° 14.536' W 82° 11.371'
LSNF	Lake Snowden, Athens County	N 39°14.516' W 82° 11.362'
OARR	Lavelle Road, Athens, Athens County	N 39° 19.460' W 32° 8.224'
OA3	Lavelle Road, Athens, Athens County	N 39° 19.460' W 30° 6.343'
OAS	Lavelle Road, Athens, Athens County	N 39° 19.468' W 32° 8.265'
GBR	Route 56, Athens County	N 39° 20.791' W 82° 14.670'
RID	Ridges, Ohio University, Athens County	N 39° 18.920' W 82° 06.959'
HFF	Rt. 33, Athens County	N 39° 23.450' W 82° 09.140'
HFNF	Rt. 33, Athens County	N 39° 23.432' W 82° 09.159'
PSR	United Plant Savers, Meigs County	N 39° 04.808' W 82° 10.469

Table 3.2: ISSR prime sequences used in estimating genetic variation within and among populations of *Acorus calamus* L. in southeast Ohio. The number of bands and range of fragments refers to fragments generated during this study.

Primer sequence	No. of scorable bands	Range of fragments (bp)
$(GA)_8C$	67	100-3150
$(GA)_8YT$	69	100-5540
$(CT)_8RG$	64	130-2920

Table 3.3: Estimates of genetic diversity for ten populations of *Acorus calamus* L. in southeast Ohio assayed with ISSR markers.

Sample	No. of marker bands	Shannon Index	Evenness	Nei's estimate (± S.E.)	Expected Heterozygosity	% polymorphic loci
GBR	33	3.326	0.951	0.0585 ± 0.15	0.0588	16.50
HFF	27	3.145	0.954	0.0416 ± 0.13	0.0412	11.00
HFNF	27	3.14	0.953	0.0350 ± 0.12	0.0349	10.00
LSNF	18	2.801	0.969	0.0162 ± 0.08	0.0163	4.50
LSNP	23	2.933	0.936	0.0184 ± 0.08	0.0185	6.50
OA3	23	3.016	0.962	0.0248 ± 0.10	0.0249	7.00
OARR	24	3.108	0.978	0.047 ± 0.13	0.0445	11.50
OAS	17	2.678	0.945	0.0255 ± 0.11	0.0257	6.03
PSR	19	2.851	0.968	0.0143 ± 0.08	0.0143	4.00

Table 3.4: Analysis of Molecular Variance (AMOVA) for eleven populations of *Acorus calamus* L. in southeast Ohio. P values calculated based on 999 permutations.

Source	df	SS	MS	Estimated Variation	%age Variation	Φ_{PT}	*P*
Among Pops.	9.00	244.68	27.19	1.63	58	0.58	0.001
Within Pops.	150.00	176.17	1.17	1.17	42		

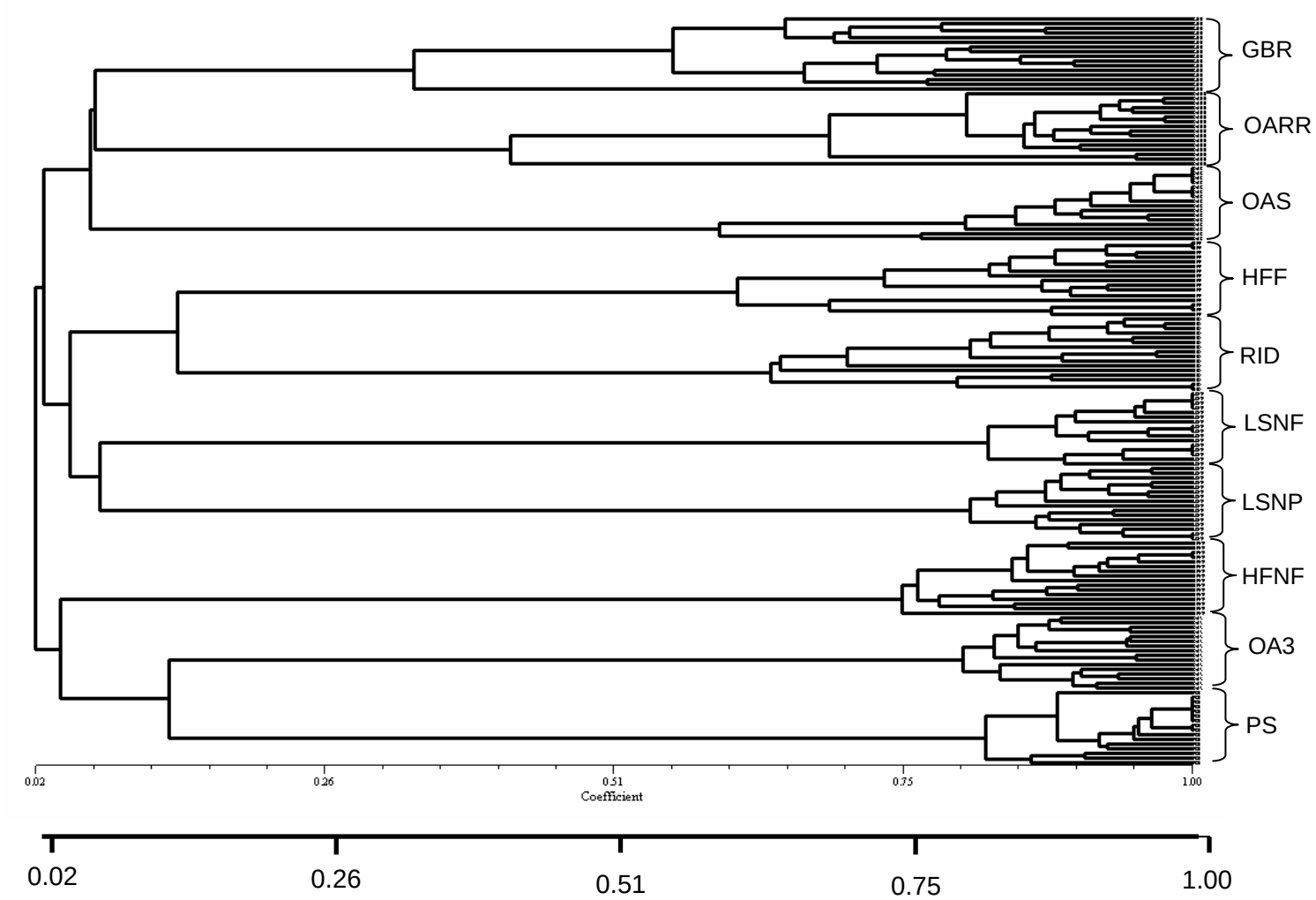
Table 3.5: Pairwise population Φ_{PT} (below diagonal) values for ten populations of *Acorus calamus* L. in southeast Ohio. Probability values based on 999 permutations shown above diagonal.

Pop	GBR	HFF	HFNF	LSNF	LSNP	OA3	OARR	OAS	PSR	RID
GBR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HFF	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HFNF	0.47	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSNF	0.53	0.58	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSNP	0.49	0.57	0.59	0.63	0.00	0.00	0.00	0.00	0.00	0.00
OA3	0.49	0.55	0.58	0.63	0.61	0.00	0.00	0.00	0.00	0.00
OARR	0.46	0.54	0.56	0.62	0.59	0.57	0.00	0.00	0.00	0.00
OAS	0.51	0.58	0.61	0.66	0.62	0.61	0.59	0.00	0.00	0.00
PSR	0.56	0.63	0.64	0.71	0.68	0.64	0.63	0.69	0.00	0.00
RID	0.46	0.49	0.55	0.59	0.57	0.55	0.55	0.58	0.62	0.00

Table 3.6: Pairwise population estimates of Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) for ten populations of *Acorus calamus* L. in southeast Ohio.

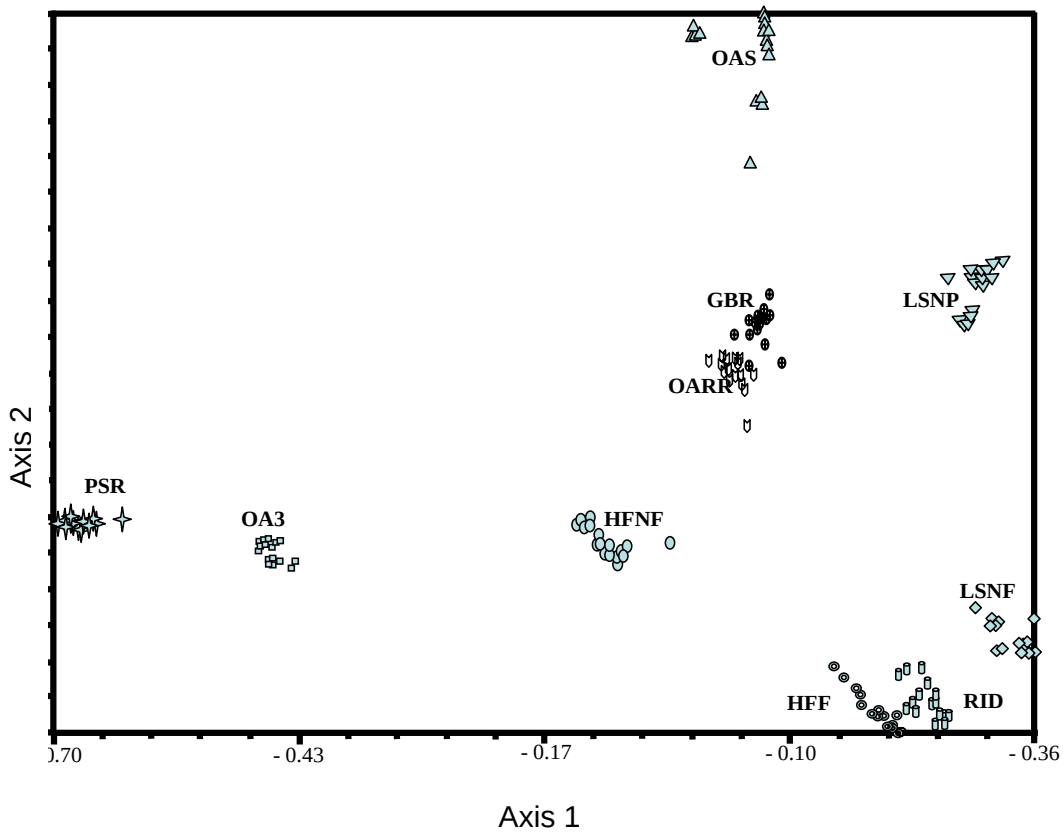
Pop	GBR	HFF	HFNF	LSNF	LSNP	OA3	OARR	OAS	PSR	RID
GBR	****	0.93	0.93	0.92	0.93	0.92	0.94	0.94	0.91	0.93
HFF	0.07	****	0.91	0.91	0.90	0.90	0.91	0.92	0.90	0.93
HFNF	0.08	0.10	****	0.89	0.89	0.89	0.91	0.90	0.90	0.90
LSNF	0.08	0.09	0.11	****	0.90	0.89	0.91	0.91	0.90	0.91
LSNP	0.08	0.10	0.12	0.11	****	0.88	0.90	0.91	0.90	0.90
OA3	0.09	0.11	0.12	0.12	0.12	****	0.90	0.90	0.90	0.90
OARR	0.06	0.09	0.09	0.10	0.10	0.10	****	0.93	0.91	0.91
OAS	0.07	0.09	0.10	0.10	0.10	0.11	0.08	****	0.90	0.91
PSR	0.09	0.11	0.12	0.12	0.13	0.11	0.09	0.11	****	0.90
RID	0.07	0.08	0.11	0.10	0.11	0.10	0.09	0.09	0.106	****

Figure 3.1: Cluster analysis using unweighted pair-group mean analysis (UPGMA) based on Dice's similarity coefficient for individuals sampled from ten populations of *Acorus calamus* L. in southeast Ohio. Simlarity calculated from presence/absence of specific inter simple sequence repeat (ISSR) fragments.



Jacard's coefficent

Figure 3.2: Principal Coordinates Analysis (PCoA) of 10 populations of *Acorus calamus* L. sampled from southeast Ohio based on inter-simple sequence repeat (ISSR) fragments.


OAS
GBR
LSNP
OARR
PSR
OA3
HFNF
LSNF
HFF
RID
Axis 2
Axis 1
0.70
- 0.43
- 0.17
- 0.10
- 0.36

Chapter 4: The influence of environmental factors and storage on the germination response of *Acorus calamus* seeds

INTRODUCTION

Regeneration of plant communities from seed banks, as well as broadcast of seed stock is an effective management technique in wetland mitigation and construction projects (Mitsch and Gosselink 2000). Seeds offer genetic variation and, ease of portability and storage as compared to vegetative propagules. Seeds also offer resilience to wetland communities in times of drought (Brock et al. 2003). Successful population establishment depends on the life history characteristics of candidate species (Keddy 2000) and their ability to withstand environmental conditions at the site (Kentula et al. 1992). Hence, wetland managers need to carefully scrutinize and screen species that chosen for proliferation at a wetland restoration projects (Cronk and Fenessy 2001).

While many species are considered suitable for wetland mitigation in North America (Mitsch and Gosselink 2000), the germination response of the seeds of these species can vary depending upon local environmental conditions (Leck and Brock 2000, Smith et al. 2002, Combroux et al. 2002, Combroux and Bornette 2004). Seed germination in temperate wetland species can be influenced by several environmental factors including ambient diurnal temperatures (Baskin and Baskin 1998, Budelsky and Galatowitsch 1999, Brandel 2004), water inundation (Evans and Ethrington 1990, Boedeltje et al. 2002, Crossle and Brock 2002), sedimentation (Spencer and Ksander 2002), disturbance (Barry et al. 2004), salinity (Khan and Ungar 1984, Nielsen

et al. 2003), pH (Cachlis and Reddy 2000) and the interaction of various environmental factors (Xiong et al. 2003).

Information on the germination ecology of wetland species is a useful tool that allows for the prediction of succession (Stockey and Hunt 1994) and hence the strategic selection of species during wetland conservation (Keddy et al. 1989). However, wetland managers are hampered by the lack of information on germination traits of several candidate species (Leck 1989, Keddy et al. 1989).

Acorus calamus L. (Sweetflag, family Acoraceae), an emergent aquatic macrophyte, is considered a potential candidate for use in wetland restoration and phytoremediation projects in North America (Mitsch and Gosselink 2000, U. S. Department of Defense 1999, Pivetz 2001, Wilson et al. 2001). The species has a ubiquitous global distribution in tropical and temperate regions of Asia, North America and Africa. It is locally abundant in North America (Braun 1921, Buell 1937, Jervis and Buell 1964, Packer and Ringius 1984) often occurring during succession in wetlands (Van der Walk and Bliss 1971). Populations of the plant occur in variety of nutrient poor soils (Pai and McCarthy *in press*). During clonal propagation, ramets are produced from lateral buds emerging from a sympodial rhizome. The species has high anoxia tolerance (Weber and Braendle 1996, Schluter and Crawford 2001) enabling its populations to withstand prolonged flooding.

The species is of medicinal, cultural and economic value. It is used as a stomachic and hallucinogen in several traditional systems of medicine in

Asia, Africa, Europe and North America (Motley 1994, van Wyke et al. 1997, Moerman 1998, Kumar et al. 2000) and is traded as a garden ornamental in the North American horticultural industry (Winterrowd 1990). In fact, the widespread distribution of *A. calamus* is often attributed to intentional introduction by humans (Motley 1994, Gilmore 1930, Les and Mehroff 1999). Essential oils from the rhizome are traded in the global pharmaceutical and perfume industry and are being investigated for pesticidal properties (Rahman and Schmidt 1999). Market demand has even prompted cultivation of the plant in India (Lokesh and Chandrakanth 2002, Oudhia 2002). Its socioeconomic importance, indigenous nature and suitability for wetland habitats make *A. calamus* an appropriate species for wetland restoration.

Both triploid and diploid varieties of the species are found in North America, only the latter is capable of producing viable seeds. In North America the plant blooms in spring and fruits mature midsummer. Fruit fall to the ground when leaves from which they are subtended die back during late summer. Fruits are trilocular berries with axillary placentation and can contain as many as 9 seeds (Buell 1935). Germinability of the species has been recorded in the lab (Buell 1935, Muenscher 1936, Keddy and Constabel, 1986) but seedlings are conspicuously absent in areas where mature field populations occur (Jervis and Buell 1964, Leck 1987, Pai *pers. obsv.*). Seed have primary innate dormancy and require cold stratification for at least thirty days before germination (Muenscher 1936). They are insensitive to sand particle size during germination (Keddy and Constabel 1986).

In natural systems, it is possible that local environmental conditions or storage in the soil over a long period of time hinder the germination and influence the viability of *A. calamus* seeds. I examined germination of *A. calamus* seeds for use in wetland restoration by subjecting them to experimental manipulations of light, water, pH and temperature. I also investigated if seed viability was influenced by storage. Findings from the study would help ascertain the suitability of *Acorus calamus* seed as a material for use in wetland restoration projects.

METHODS

Laboratory treatments

Fruits of *Acorus calamus* were procured from the Prairie Moon Nursery, Kentucky. Fruits had been collected from populations located at Harper's Ferry, Iowa and immediately shipped so that they arrived at the lab within a week from collection. On arrival, seeds were stored at $4^{o}C$ in the dark for three months and then subjected to experimental manipulations of light, water, pH and temperature. Fruits contained a mean of 4 seeds per fruit.

A 2 x 4 x 2 x 3 factorial design was used to assess the effect of light, temperature, moisture and pH, respectively on the germination of *Acorus calamus* seeds. After the cold stratification treatment, seeds were transferred into Gelman plastic petri plates (Fisher Scientific Co.) lined with Whatman # 5 filter paper.

pH solutions consisted of 1 M of a wide ranging phosphate buffer mixed with concentrated HCl and KOH. The acid and alkali were titrated into the solution using a pH meter to achieve the desired pH. Solutions were made upto a ph of 4.5 (acidic), 7 (neutral) and 8 (alkaline), respectively. The pH range is representative of that measured in soils at population patches of *A. calamus* (Pai and McCarthy *in press*) in southeast Ohio. Moisture treatments consisted of 1.5ml (moist) and 5 ml (submerged) of the buffer solution placed into the petri plates. The moist treatment replicated a damp surface resulting from waterlogged soil while the submerged treatment replicated inundation due to flooding. Petri plates containing 10 fruits (40 seeds) were placed in each of four alternating 12h night/12 h day temperature regimes (5/15 ^{o}C, 5/25 ^{o}C, 15/25 ^{o}C and 25/35 ^{o}C) with a 12 h dark/12 h light photoperiod (20 µmol m^{-2} s^{-1}) for thirty days. Temperature regimes replicated those that are observed throughout the range of *A*. *calamus* in North America. For dark treatments (24 hour dark), an additional set of petri plates was covered with aluminum foil. Replicate sets of petri plates containing seeds were prepared for each time of observation. There were five replicates for each treatment combination with a total of 120 petri plates in the light treatments and 600 petri plates in the dark treatments. Germination was said to have occurred when emergence of the radicle was noticed.

Germination readings were recorded at five day intervals over thirty days. The aluminum foil was removed from petri plates that had been subjected to dark treatments to examine germination. Once exposed to light,

petri plates in the dark treatment were discarded. Before discarding, petri plates were left uncovered in the germination chambers to observe recovery of ungerminated seeds.

To examine seed viability over time, fruits were stored under distilled water in the dark. Every six months, ten fruits (forty seeds) from storage were placed in each of five replicate petri plates lined with Whatman # 5 paper and 5 ml of distilled water. The petri plates were then placed in alternating 12h night/12 h day temperature regime of 15/25 ^{o}C with a 12 h dark/12 h light photoperiod (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for thirty days. The influence of storage was examined over a period of twenty four months.

Statistical Analyses

Percent germination was examined using a four-way fixed effects general linear model (GLM) ANOVA. Since none of the seeds germinated in the dark, a four-way GLM ANOVA could not be performed and a three-way fixed effects GLM ANOVA was used instead. Germination velocity (G) was calculated using a modified Timson's Index (Khan and Ungar 1984) as follows

$$G = \sum \frac{\% \, seeds \, germ.}{total \, germ \, period}$$

A *G* of zero indicated that no seeds germinated while values at one hundred indicated that all seeds germinated within the first five days. Data for germination velocity was also subjected to a three-way ANOVA. A Tukey-

Kramer multiple comparison procedure was used to compare individual means across treatment combinations for both percent germination and germination velocity. A regression analysis was used to examine the trend in percent germination of seeds subjected to the storage treatment.

All data were subjected to standard tests for normality, equal variance, and equality of covariance using the D'Agostino Omnibus test, Modified Levene equal variance test, and Box's M test (Hintze 2000). Data that did not meet assumptions of normality were appropriately transformed before further analyses. The GLM ANOVA and tests for assumptions were conducted using NCSS 6.0 (Hintze 2000).

RESULTS

In the light experiment, seed germination was noticed in some treatments at five days. Most of the seeds germinated within twenty days from the start of the experiment. None of the seeds germinated in the dark. However, recovery experiments indicated that on exposure to light, seeds from the dark treatments initiated germination within 5-10 days, similar to seeds in the light.

Only the seeds placed in the light treatments responded to any of the other experimental factors. GLM ANOVA indicated that there was a significant effect of ambient temperature ($F = 8.42$, $P < 0.0001$) on percentage germination. The percentage of seeds that germinated in the 15/25 ^{o}C and

25/35 ^{o}C temperature regimes were similar to that in the 5/25 ^{o}C but were different from the seeds that germinated at 5/15 ^{o}C. More seeds germinated at warmer temperatures especially the late spring and summer temperatures. Moisture also significantly ($F = 24.57$, $P < 0.0001$) influenced the percentage of seeds that germinated. More seeds germinated under submerged conditions compared to moist conditions. pH did not influence ($F = 8.42$, $P > 0.1$) seed germination and similar numbers of seeds germinated in all three pH treatments at the end of thirty days (figure 1).

Germination velocity, an indicator of the rate of germination of seeds, was significantly influenced by temperature ($F= 24.9$, $P = 0.00$), moisture ($F= 19.62$, $P < 0.0001$) and pH ($F = 15.36$, $P. >0.0001$). In addition there were significant moisture x temperature ($F = 8.38$, $P. >0.0001$), temperature x pH ($F = 2.38$, $P. >0.0001$) and moisture x pH ($F = 15.36$, $P. >0.0001$) interactions. Seeds germinate faster in the acidic and neutral conditions in the spring/early summer temperature regimes (15/25^{O}C, 5/25^{O}C), with radicles starting to emerge ten days of treatments. In the 5/15^{O}C temperature regime, seeds took more time (fifteen days) before germination commenced (figure 2). Further, no additional seeds germinated over time in this treatment. In the 15/25^{O}C temperature regime, seeds germinated fastest in the under moist acidic conditions. Germination velocity in all cases was higher in the neutral and acidic conditions as compared to alkaline conditions (table 1). However over time, the germination of moist treatments slowed down and but that in the submerged treatments continued (figure 2).

A steady decrease in the percentage of seeds that germinated occurred with an increase in storage time (figure 3). However, the percentage germination was not significantly different ($F= 2.11$, $P > 0.1$) over two years. Seeds remained viable even at the end of two years.

DISCUSSION

Our findings suggest that seeds of *Acorus calamus* are able to germinate under a wide set of environmental factors, though germination response varies with each set of conditions. Seed germination responses to environmental conditions are thought to be bet hedging strategies that involve tradeoffs between seedling survival and seed persistence (vs. deterioration) in the soil (Probert 2000). The requirement for cold stratification to break seed dormancy in *Acorus calamus* is also noticed in several temperate species (Baskin and Baskin 1998). Seed dormancy is thought to be an adaptation that allows greater seedling survival by avoiding exposure to arid conditions and low freezing temperatures. Since seeds of *Acorus calamus* fall to the ground in late summer, the need for cold stratification at winter temperatures allows the seeds to persist through fall without germinating even if moist conditions are met (Thompson 2000). Thus, the species avoids exposing seedlings to freezing frost and snow.

Ambient temperature provides an important cue for germination (Probert 2000). *A. calamus* germinates from early spring to summer

temperatures. Some seed germination is noticed in the fall temperature (5/15 ^{o}C), however, the amount of seeds that germinate is substantially less than in the spring and summer temperatures. The availability of light and moisture may induce some seeds to germinate but the low temperature regime deters greater germination velocity and total germination.This provides *A. calamus* a long germination window, enabling it to capitalize on favorable conditions of moisture whenever it occurs. The germination velocity is greatest in spring rather than in summer temperatures, indicating that these may be ideal conditions for germination of *Acorus calamus* seeds. However, being equipped with the ability to germinate over several seasons implies that *A. calamus* seeds can opportunistically germinate if other requirements such as moisture and light are suitable.

Though germination velocity is greater in the moist environments, water logging and inundation favor overall percentage of seeds that germinate. Aquatic macrophytes grow in frequently flooded environments and submergence may not be ideal for seedling growth as compared to a moist waterlogged microenvironment (Boedeltje et al. 2002). However, summer temperatures, coupled with waterlogged conditions may cause deterioration of the seed (Probert 2000), hence inducing then to germinate in larger numbers. Another reason for high percentage germination in *A.calamus* seeds when submerged may have to do with its fruit wall. Seeds in the dry fruit are hard to separate from the walls of the almost achene like berry. Submergence may

cause disintegration of the fruit wall allowing for more radicles to successfully emerge thus resulting in higher percentage of germination.

Light is a primary limiting factor to the germination of *A. calamus* seed germination. Phytochromes in the seed detect light and cue seeds to environmental disturbances. If buried under detritus, loam or humus, the seeds are subjected to perpetual darkness (Pons 2000) and will not germinate at all. Mild disturbances through flooding that wash away detritus may expose seeds to light and signal the initiation of germination. If the right temperature conditions are met and moisture is available, seeds should germinate after a flooding event.

The total seed germination did not vary among pH treatments. Pollution from anthropogenic sources and frequent flooding can cause fluctuations in the pH regime of a wetland making it vary from alkaline or acidic. *A. calamus* seeds are capable of germinating in a wide range of pHs. This implies that *A. calamus* is capable of germinating in a variety of wetlands; even those influenced by anthropogenic pollution. Germination velocity of *A. calamus* seeds is greater in acidic environments. The seeds are adapted to germinate on natural wetland soil which is often waterlogged and highly reduced. The storage experiment indicates that *A. calamus* seeds can remain viable in the soil for at least two years. There may be loss of viability over longer periods of time.

Much of the germination requirements for *Acorus calamus* seeds are similar to other herbaceous wetland species capable of creating persistent

seed banks: they respond positively to light and alternating temperatures (Leck 1998). Our study demonstrates that seeds of *Acorus calamus* have wide ecological amplitude with respect to moisture, pH and temperature and can be used in a variety of wetland zones. If used in wetland mitigation or construction projects, they are best used at the very onset of the project when there is minimal vegetation and high light availability. Also ambient water conditions will be more acidic and more suitable for faster germination of *A. calamus*. Rate of seed germination may be increased by seeding on moist rather than inundated areas.

A. calamus creates a viable seed bank till optimal conditions are available for germination. However, the seeds are limited by light. The seeds are best used during the initial stages of wetland construction, when soil is sandy rather than loamy or humic. Periodic flooding events that turnover the soil will induce germination of buried seeds.

Over time as vegetation communities grow, and organic matter debris builds up, seeds will be buried and unable to germinate. At this time, the seed may become a seed bank fugitive (sensu Leck 1998) that is capable of germinating only after some large scale perturbation such as a drawdown.

LITERATURE CITED

Acuna, U. M., D. E. Atha, J. Ma, M. H Nee, E. J. Kennelly. 2002. Antioxidant capacities of ten edible North American plants. Phytotherapy Research. 16: 63-65.

Barry, M.J., R. Bowers and F.A. De Szalay. 2004. Effects of hydrology, herbivory and sediment disturbance on plant recruitment in a Lake Erie Coastal Wetland. American Midland Naturalist 151: 217-232.

Baskin, C. C. and J. M. Baskin. 1998. Seeds - ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, CA, USA.

Boedeltje, G., G. N. J. ter Heerdt and J. P. Bakker. 2002. Applying the seedling-emergence method under waterlogged conditions to detect the seed bank of aquatic plants in submerged sediments. Aquatic Botany 72: 121-128.

Brändel, M. 2004. The role of temperature in the regulation of dormancy and germination of two related summer-annual mudflat species. Aquatic Botany 79: 15-32.

Braun, L. E. 1921. Composition and Source of the Flora of the Cincinnati Region. Ecology 2:161-180.

Buell, M. 1935. Seed and seedling of Acorus calamus. Botanical Gazette 96: 758-765.

Buell, M. F. 1937. *Acorus calamus* in America. Rhodora 37: 367-369.

Brock, M. A., D. L. Nielsen, R. J. Shiel, J. D. Green and J. D. Langley. 2003. Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. Freshwater Biology. 48: 1207-1218.

Budelsky, R. A. and S. M. Galatowitsch. 1999. Effects of moisture, temperature, and time on seed germination of five wetland *Carices*: implications for restoration. Restoration Ecology 7: 86-97.

Cachlis, D. and K. N. Reddy. 2000. Factors affecting *Campis radicans* seed germination and seedling emergence. Weed Science 48:212-216.

Combroux, I. C. S. and G. Bornette. 2004. Propagule banks and regenerative strategies of aquatic plants. Journal of Vegetation Science. 15: 13-20.

Combroux, I. C. S., G. Bornette and C. Amoros. 2002. Plant regenerative strategies after a major disturbance: the case of a riverine wetland restoration. Wetlands 22: 234-246.

Cronk, J. L. and M. S. Fenessy. 2001. Wetland Plants*:* Biology and Ecology. Lewis Publishers, Boca Raton, FL, USA.

Crosslé, K and M. A. Brock. 2002. How do water regime and clipping influence wetland plant establishment from seed banks and subsequent reproduction? Aquatic Botany. 74: 43-56.

Evans, C. E. and J. R. Etherington. 1990. The effect of soil water potential on seed germination of some British plants. New Phytologist. 115: 539-548.

Gilmore, M. R. 1930. Dispersal by Indians as a factor in the extension of discontinuous distribution of certain species of native plants. Papers of the Michigan Academy of Science, Arts and Letters. 13: 89-94.

Hintze, J. L. 2001. Number Cruncher Statistical Systems (NCSS), Kaysville, Utah, USA.

Jervis, R. A. and M. F. Buell, 1964. *Acorus calamus* in New Jersey. Torreya. 91: 335-336.

Keddy, P. A. 2000. Wetland ecology: principles and conservation. Cambridge University Press, Cambridge UK.

Keddy, P.A.and P. Constabel, 1986. Germination of ten shoreline plants in relation to seed size, soil particle size and water level: an experimental study. Journal of Ecology. 74: 133-141.

Keddy, P. A., I. C. Wisheu, B. Shipley and C. Gaudet. 1989. Seed banks and vegetation management for conservation: toward predictive community ecology. Pages 347-363 in M. A. Leck, V. T. Parker, R. L. Simpson eds. Ecology of soil seed banks. Academic press, New York, NY, USA.

Kentula, M. E., R. P. Brooks, S. E. Gwin, C. C. Holland, A. D. Sherman and J.C. Sifneos. 1992. An approach to improving decision making in wetland restoration and creation. Edited by A.J. Hairston. U. S. Environmental; Protection agency, Environmental Resaerch Laboratory, Corvallis, OR, USA.

Khan, M. A. and I. A. Ungar. 1984. The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. American Journal of Botany. 71: 481-489.

Kumar, V. S., R. K. Srivastava, A. Krishna, V. K. S. Tomar, A. K. Singh and S. Kumar. 2000. Cultivation, chemistry, biology and utilization of bach (*Acorus calamus*): A review. Journal of Medicinal and Aromatic Plant Sciences. 22: 338-348.

Leck, M. A. 1998. Wetland seed banks. Pages 283-305 in M. A. Leck, V. T. Parker, R. L. Simpson eds. Ecology of soil seed banks. Academic press, NewYork, NY, USA.

Leck, M. A. and M. A. Brock. 2000. Ecologocal and evolutionary trends in wetlands: evidence from seeds and seed banks in New South Wales and, Australia and New Jersey, USA. Plant Species Biology 15: 97-112.

Leck, M. A. and R. L. Simpson. 1987. Seed bank of a freshwater tidal wetland: turnover and relationship to vegetation change. American Journal of Botany. 74: 360-370.

Les, D. H. and L. J. Mehrhoff. 1999. Introduction of nonindigenous aquatic vascular plants in southern New England: a historical perspective. Biological Invasions. 1: 281-300.

Lokesh, G. B., M. G. Chandrakanth. 2002. A study for commercial cultivation of sweet flag (*Acorus calamus*) in Tumkur district of Karnataka. Journal of Medicinal and Aromatic Plant Sciences. 24: 984-989.

Mitsch, W.J. and J. G. Gosselink. 2000. Wetlands. 3rd edition. John Wiley and Sons, NY, USA.

Moerman, D. E. 1998. Native American Ethnobotany. Timber Press, Portland, OR, USA.

Motley, T. J. 1994. The ethnobotany of Sweetflag *Acorus calamus* (Araceae). Economic Botany. 48:397-412.

Muenscher, W.C., 1936. Storage and germination of aquatic plants. Cornell University Agricultural Experiment Station, Ithaca, New York, USA 17pp.

Nielsen, D.L., M.A. Brock, K. Crosslé, K. Harris, M. HealeyandI. 2003. The effects of salinity on aquatic plant germination and zooplankton hatching from two wetland sediments. Freshwater Biology 48: 2214-223.

Oudhia, P. 2002. Rice-acorus intercropping: a new system developed by innovative farmers of Chhattisgarh (India) International Rice Research Notes. 57:56-57. http://www.irri.org/publications/irrn/pdfs/vol27no1/irrn27%2D1notes.pdf

Packer, J. G. and G. S. Ringius. 1984. The distribution and status of *Acorus* (Araceae) in Canada. Canadian Journal of Botany. 62:2248-2252.

Pai, A. and B.C. McCarthy. in press. Variation in shoot density and rhizome biomass of *Acorus calamus* L. with respect to environment. Castanea.

Pivetz, B. E. 2001 Phyto remediation of contaminated soil and groundwater at hazardous waste sites. United states Environmental Protection agency Groundwater Issue. EPA/540/S-01/500

Pons, T.L. 2000. Seed responses to light. Pages 237-260 in Editor M. Fenner, Seeds: The ecology of regeneration in plant communities, 2nd edition, CABI Publishing, Oxford, UK.

Probert, R. J. 2000. The role of temperature in the regulation of seed dormancy and germination. Pages 261-292 in Editor M. Fenner, Seeds: The ecology of regeneration in plant communities, 2nd edition, CABI Publishing, Oxford, UK.

Rahman, M. M. and G. H. Schmidt. 1999. Effect of *Acorus calamus* L. (Araceae) essential oil vapours from various origins on *Callosobruchus phaseoli* (Gyllenhal) (Coleoptra Bruchidae). Journal of Stored Product Research. 35: 285-295.

Schluter, U. and R. M. M. Crawford. 2001. Long term anoxia tolerance in leaves of *Acorus calamus* L. and *Iris pseudacorus* L. Jounal of Experimental Botany. 52: 2213-2225.

Smith, S. M., P. V. McCormick, J. A. Leeds and P. B. Garrett. 2002. Constraints of seed bank species composition and water depth for restoring vegetation in the Florida Everglades, U.S.A. Restoration Ecology 10: 138-145.

Spencer, D. F. and G. G. Ksander. 2002. Sedimentation disrupts natural regeneration of *Zannichellia palustris* in Fall River, California. Aquatic Botany. 73: 137-147.

Stockey, A. and R. Hunt. 1994. Predicting secondary succession in wetland mesocosms on the basis of autecological information on seeds and seedlings. Journal of Applied Ecology. 31: 543-559.

U. S. Department of Defense 1999. The use of constructed wetlands to phytoremediate explosives-contaminated groundwater at the Milan army ammunition plant, Milan, Tennessee. http://www.estcp.org/documents/techdocs/199520.pdf

Van der Valk, A. G. and Bliss, L. C. 1971. Hydrarch succession and net primary production of oxbow lakes in Central Alberta. Canadian Journal of Botany. 49:1177-1199.

Van Wyke, B., B. van Oudtshoorn and N. Gericke. 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa.

Weber, M. and R. B. Braendle. 1996. Some aspects of the extreme anoxia tolerance of the Sweet Flag, *Acorus calamus* L. Folia Geobotanica Phytotaxa. 31: 37-46.

Winterrowd, W. 1990. Just add water. Horticulture 68:24-25.

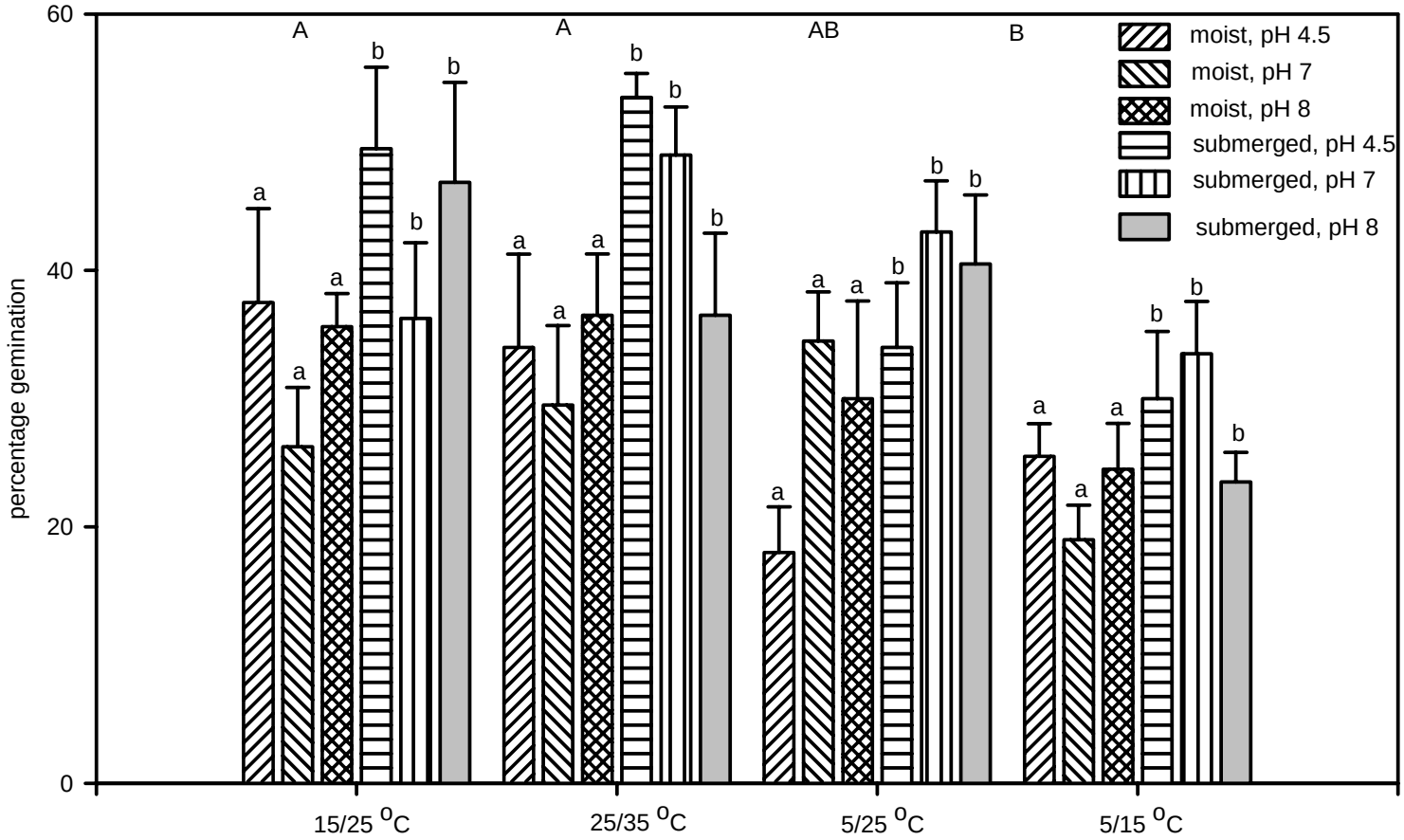
Wilson, P.C., T. Whitwell and S.J. Klaine 2001. Metalaxyl toxicity, uptake, and distribution in several ornamental plant species. Journal of Environmental Quality. 30: 411-417.

Xiong, S., M. E. Johansson, F. M. R. Hughes, A. Hayes, K.S. Richards and C. Nilsson, 2003. Interactive effects of soil moisture, vegetation canopy, plant litter and seed addition on plant diversity in a wetland community. Journal of Ecology. 91: 976-986.

Table 4.1: Germination velocity (Timson's Index) of *Acorus calamus* L. seeds germinated under different day/night (12h/12h) temperature, moisture (1.5ml moist, 5 ml submerged) and pH regimes.

	15/25 °C	**25/35 °C**	**5/15 °C**	**5/25 °C**
Moist x pH 4.5	19.940 (± 2.226)	7.843 (± 1.435)	12.417 (± 1.667)	20.450 (± 2.442)
Moist x pH 8	10.596 (± 1.288)	6.655 (± 1.368)	4.040 (± 0.305)	9.193 (± 1.253)
Moist x pH 7	10.421 (± 2.210)	8.602 (± 1.598)	4.667 (± 0.814)	8.410 (± 1.555)
Submerged x pH 4.5	8.467 (± 1.644)	8.670 (± 0.661)	3.703 (± 0.372)	9.562 (± 0.686)
Submerged x pH 8	6.775 (± 1.347)	11.377 (± 0.905)	3.310 (± 0.382)	7.147 (± 1.467)
Submerged x pH 7	7.913 (± 1.207)	8.585 (± 1.151)	4.547 (± 0.652)	8.710 (± 1.539)

Figure 4.1: Means (± S.E.) of percentage germination of *Acorus calamus* L. seeds exposed to four alternating temperature regimes, two moisture levels and three pH solutions. Different letters in upper case above bars indicate significant differences ($P < 0.001$) in means between temperature regimes. Different letters in lower case indicate significant differences ($P < 0.001$) in means between moisture and pH treatment combinations.


60
40
20
0
percentage gemination
A
A
AB
B
a
a
a
b
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b
a
a
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b
a
a
a
b
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b
a
a
a
b
b
b
moist, pH 4.5
moist, pH 7
moist, pH 8
submerged, pH 4.5
submerged, pH 7
submerged, pH 8
15/25 °C
25/35 °C
5/25 °C
5/15 °C

Figure 4.2: Rate of germination (means ± S.E.) of *Acorus calamus* L. seeds exposed to four alternating temperature regimes, two moisture levels and three pH solutions. Open symbols indicate moist treatments, closed symbols indicate submerged treatments. Circles indicate a pH of 4.5 (acidic), squares indicate pH of 7 (neutral) and inverted triangles indicate a pH of 8 (alkaline)

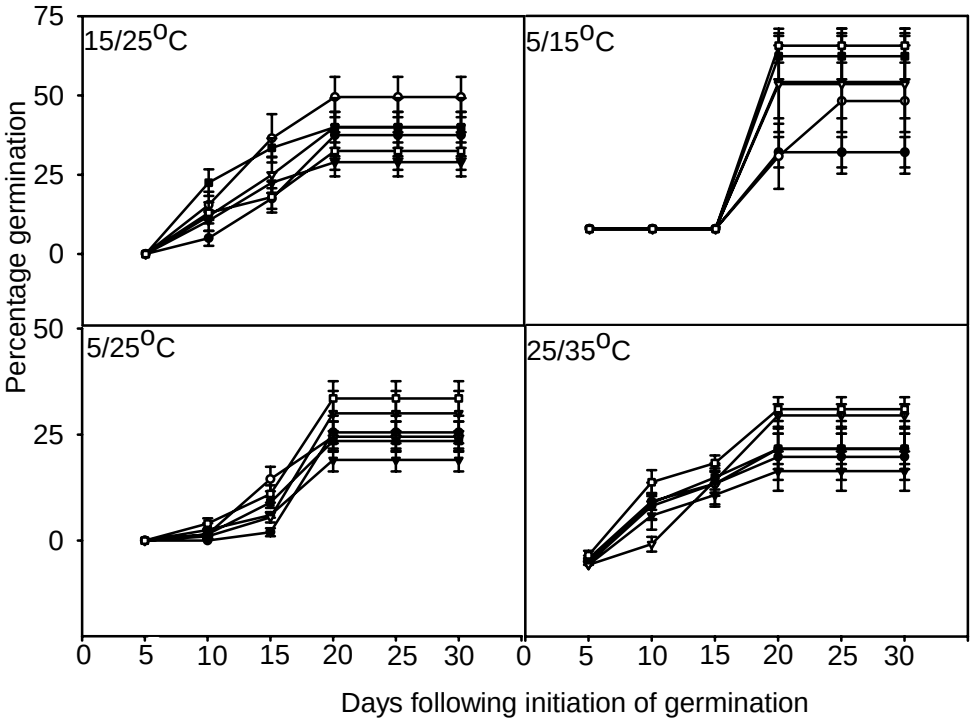
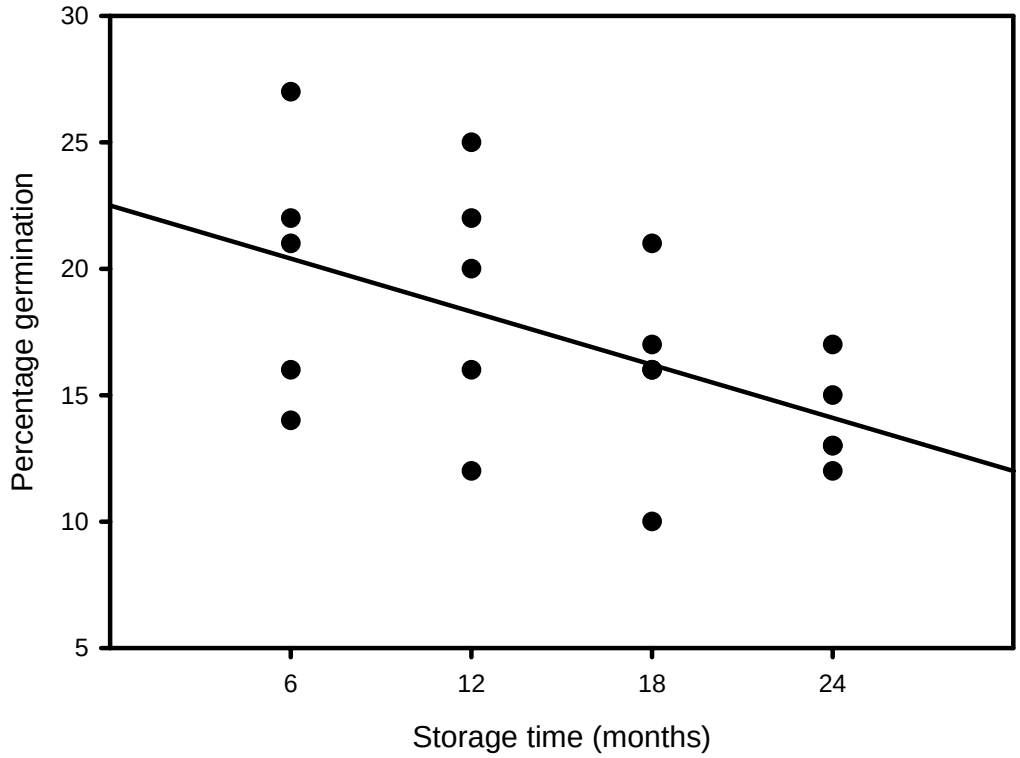

15/25°C
5/15°C
5/25°C
25/35°C
Percentage germination
75
50
25
0
50
25
0
0 5 10 15 20 25 30
0 5 10 15 20 25 30
Days following initiation of germination

Figure 4.3: Percentage germination of *Acorus calamus* L. seeds stored in distilled water and in the dark at 15 x 25^{o}C for 24 months. Seeds were germinated at intervals of six months.


30
25
20
15
10
5
Percentage germination
6
12
18
24
Storage time (months)