

Ecotypic differentiation for flood-tolerance and its morphological correlates in *Chamaecrista fasciculata*

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Abstract

Chamaecrista fasciculata is widespread in eastern North America and commonly found on dry sandy soils. In contrast, large seeded *C. fasciculata* var. *macrosperma* is restricted to fresh-water tidal marshes of the central coastal plain of Virginia, USA. Greenhouse experiments were conducted to determine if var. *macrosperma* represents a flood-tolerant ecotype and if flood tolerance is associated with decreased tolerance to dry conditions. Morphological measurements on root and shoot growth were made to determine correlates and possible mechanisms of tolerance to flood and dry conditions. Flood tolerance in var. *macrosperma* was confirmed. This ecotype had greater seedling survivorship and greater accumulation of biomass under flooded conditions than the upland form. Vegetative performance of the two types was comparable under well-watered but drained conditions. Flood tolerance is associated with increased seed mass, a two-fold greater rate of shoot elongation, and increased shallow lateral root growth. The evolution of flood tolerance does not result in a decrease in vegetative tolerance to dry conditions even under competitive conditions. The large seeded, flood-tolerant ecotype may be absent in drier habitats because of a trade-off between individual seed mass and seed number.

Keywords: Flood-intolerance; Root morphology; Seed mass; Shoot elongation

1. Introduction

The soil environment changes radically following flooding (Armstrong, 1979). The primary effect of flooding is the reduction of soil oxygen concentration due to

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respiration and to the slow diffusion of oxygen in water. A secondary consequence of anaerobic conditions is the generation of toxic metal ions by anaerobic microbial respiration (Crawford, 1989). A common response of terrestrial plants to flooded conditions is reduced growth rate and biomass (Jackson and Drew, 1984). Most terrestrial plants can tolerate low oxygen availability associated with root zone flooding for short periods of time. However, a suite of traits may be associated with increased flood tolerance seen especially in wetland plants including (1) shifting metabolism from root to shoot growth by increasing ADH activity and protein synthesis in shoots (Cobb and Kennedy, 1987), thereby increasing shoot elongation and elevating the photosynthetic apparatus above the water (Van der Sman et al., 1991), (2) ability to prevent post-anoxic injury from reactive forms of oxygen (Crawford, 1992), (3) formation of aerenchyma (interconnected gas-filled space which transfers oxygen from the leaves to the roots) (Justin and Armstrong, 1987), and (4) development of lateral roots at the expense of tap root formation, which improves the diffusion of the transferred oxygen to the rhizosphere and maintains the root zone nearer the soil surface (Blom and Voesenek, 1996).

The reinvasion of the aquatic environment by land plants appears to have occurred multiple times and is associated with the evolution of the numerous adaptive mechanisms cited above (Davy et al., 1990). However, we have an imperfect understanding of the initial evolutionary shifts of traits that allow terrestrial plants to expand their ranges back into the aquatic environment and the genetic changes underlying the evolution of these adaptive traits. Instead, our knowledge of plant adaptation to flooding is often based on the identification of suites of characters found in congeners and unrelated organisms (Justin and Armstrong, 1987). Investigations of intraspecific variation in flooding tolerance are needed to determine the initial mechanisms associated with the evolution of flooding. Studies of species which demonstrate variation in flood tolerance also offer great potential for understanding the genetic basis of flood tolerance through controlled crosses between flood-tolerant and flood-intolerant forms. Furthermore, it is necessary to determine if there are inherent constraints to the evolution of flood tolerance such as an association with decreasing ability to the tolerance of dry conditions. If either physiological or functional constraints are operating in the evolution of flood tolerance then we can expect negative genetic correlations, via negative pleiotropy, to influence the expression of those traits conferring flood and drought tolerance (Partridge and Sibly, 1991). Restriction of range via these negative trade-offs may be the driving force for subsequent speciation.

Relatively few studies have documented ecotypic differentiation for flood tolerance (reviewed in Davy et al., 1990, and Tiner, 1991), and little effort has been made to understand the genetic basis of intraspecific variation for flood tolerance, perhaps because many of these cases are for long-lived woody perennials, e.g. *Nyssa sylvatica* (Keely, 1979), and *Betula pedula/pubescens* (Davy and Gill, 1984). Furthermore, few studies have quantified morphological traits which are likely adaptations for the intraspecific evolution of flood tolerance. Exceptions include the demonstration by Linhart and Baker (1973) of extreme microgeographic variation for flood tolerance in the selfing annual *Veronica peregrina*, observations by Chan and Burton (1992) of different ADH alleles associated with within population genetic variation for flood tolerance in *Tri-*

folium repens and study by Keely (1979) which identified a number of traits associated with ecotypic variation for flood tolerance in *Nyssa sylvatica*.

As part of a larger program to understand the mechanisms and genetic basis of flooding tolerance, ecotypic differentiation for flood tolerance was investigated in the widespread annual *Chamaecrista fasciculata*. This species normally occurs on dry sandy soils but var. *macrosperma* is found in the water-saturated and intermittently flooded fresh water tidal marshes of the coastal plain of central Virginia, USA (Irwin and Barneby, 1982). The objectives of the study described here were three-fold: (1) greenhouse experiments were performed to quantify whether the occurrence of *C. fasciculata* var. *macrosperma* in flooded marshes represents a genetic and hence evolutionary response to flooding, (2) morphological features associated with flood tolerance were scored to better understand the possible mechanisms of flood tolerance and (3) a potential trade-off between tolerance to flooded and dry conditions in *C. fasciculata* var. *macrosperma* was quantified to determine the factors responsible for delimiting the ranges of the two forms of *C. fasciculata*. These objectives were addressed with experiments which quantified the effect of flooding and dry conditions on early seedling and later juvenile growth under non-competitive and competitive conditions.

2. Materials and methods

2.1. Sites and study organism

Chamaecrista fasciculata Michx. partridge pea (= *Cassia fasciculata*) (Irwin and Barneby, 1982), is a self-compatible, mostly outcrossing (Fenster, 1991, 1995) annual legume of eastern North America. It is found in open woodlands, old fields, roadsides, coastal and inland dunes, black oak- and pine-savanna, coastal and tall-grass prairie and, in its far western distribution, in sand-pockets and sandy gulches in short-grass prairie. The range of *C. fasciculata* is from southern Florida to Massachusetts and west to Minnesota and eastern Colorado. Throughout, it is generally found in dry, light soils or sands but may occasionally occur in wet rich soils and waterlogged clays of ditches and stream-banks (Irwin and Barneby, 1982).

In contrast, *C. fasciculata* var. *macrosperma* Fern. is restricted to the Tidewater region of southeastern Virginia, south of Richmond. It grows in freshwater tidal marshes where the water level is influenced daily by the tidal reach from Chesapeake Bay, 120 km away and the soil is permanently water-saturated, with extreme water levels as much as 80 cm deep (personal observation). The pods or fruit of var. *macrosperma* contain fewer ovules compared with *C. fasciculata*, but as the varietal designation suggests, the mature seed are between two- and five-fold heavier (21–58 mg vs. 4–16 mg). The only other clearly distinguishing feature of var. *macrosperma* in the field is a greater internodal distance (Irwin and Barneby, 1982). The pod and seed characteristics and internode lengths which differentiate var. *macrosperma* from upland *C. fasciculata* are also observed when both are grown in the greenhouse and segregate in hybrid zones in the field and in crosses between the two indicating that these traits have a heritable basis (C.B. Fenster, unpublished data, 1996).

Seeds were collected from three populations of *C. fasciculata*: (1) an upland population growing on a sandy disturbed site in early secondary succession approximately 1 mile north of Point of Rock Co. Park, on Point of Rock Road, in Chester Co. Virginia, (2) a smaller-seeded upland population in Konza Prairie, a tall-grass prairie preserve in the Flint Hills of Kansas and (3) a population of *C. fasciculata* var. *macrosperma* growing in Aston creek, a tributary of the Appomattox river in Point of Rock Co. Park, Chester Co., Virginia, approximately 2.5 km southeast of the upland Virginia population of *C. fasciculata* used in this study.

2.2. Experimental design to determine tolerance to flooded and dry conditions and their morphological correlates

Post-germination flood tolerance was investigated experimentally at different stages in the life-history of plants derived from flood-zone var. *macrosperma* and upland populations. Fully rounded seeds were induced to germinate by acid scarification (12 min in concentrated H₂SO₄), unless otherwise noted. To control for developmental stage, seedlings were used only if their emergent radicles were less than 1 cm long with their seed coats still intact. All plants were fertilized weekly with a 20:20:20 general purpose fertilizer 2 weeks after transplanting into 8 cm plastic pots in a 1:1 (v:v) mixture of commercial potting soil (peat, composted bark and Styrofoam particles) and surface (baked clay chips). The potting soil contained slow release fertilizer adequate for the first 2 weeks of growth. The traits measured included: allocation to roots vs. shoots, placement of roots in the soil column, lateral vs. tap root growth, and rate and duration of shoot elongation.

2.2.1. Seedling characters experiment 1

Newly germinated seedlings from flood-zone var. *macrosperma* and its neighboring *C. fasciculata* upland population (Virginia) were planted into the following treatments: lightly watered, well-watered, water-saturated and deep-flooding. Planting depth was also varied for all water treatments except the lightly watered treatment to determine if differences in seed mass were associated with differential ability to emerge from shallow (1 cm below the soil surface) or deep (5 cm below the soil surface) seed burial. The lightly watered treated seedlings were planted only at the shallow burial depth. For each of the water and burial treatments there were 30 plants with one seedling per pot. To induce water stress and to simulate dry conditions, the lightly watered seedlings were watered every second day in clear weather and every third day if cloudy. In the well-watered treatment, plants were watered twice daily and water was able to drain freely from the pots. Pots in the water saturated and deep-flood treatment were placed into dishpans and the water level was maintained at the level of the soil surface in the water-saturated treatment and 2 cm above the soil level in the deep-flood treatment. All 14 treatments (two populations × three water regimes × two planting depths, two populations × one water regime (lightly watered) × one planting depth (shallow)) were replicated across ten blocks on two greenhouse benches with three replicates per block (total number of plants = 14 treatments × 30 plants per treatment = 420 plants).

Plants were grown under 14-h day length using ambient light supplemented with high intensity sodium-vapor lights. These growing conditions approximate natural field conditions from mid-spring to late summer. Emergence above the soil and above the water surface was recorded. The experiment was initiated in February 1992 and terminated 61 days later when the plants were in exponential growth but not pot-bound. At harvest, hypocotyl length was measured, and plant biomass was separated into shoot and root mass components. In this and all subsequent experiments measurements were made on dry biomass by drying at 50°C for 4 weeks.

2.2.2. *Seedling characters experiment 2*

To approximate the tidal cycle which affects flood-zone var. *macrosperma*, 99 seedlings (with emergent radicles < 1 cm) from each of var. *macrosperma* and the neighboring upland population were planted into individual 8 cm plastic pots at a 1 cm burial depth, placed into dishpans and flooded intermittently (total = 198 seedlings). Dishpans were filled everyday at midday to 2 cm above the soil surface then lowered to the soil surface 6 h later. Seeds were planted in early May 1992, grown under ambient light and harvested 28 days later. Emergence date above the soil and water surfaces were recorded. Hypocotyl length was measured and shoots and roots were harvested at the termination of the experiment.

2.2.3. *Seedling characters experiment 3*

Duration and rate of hypocotyl growth and first internode growth in seedlings, as well as timing and frequency of germination were quantified to determine their association with flood tolerance. These traits were measured on 100, 100, and 107 scarified seeds from flood-zone var. *macrosperma*, the adjacent upland population in Virginia and an upland population from Kansas, respectively. Seeds were scarified by filing a narrow section of the seed coat. Seed mass was measured prior to scarification so that the relationship between seed mass and rate and duration of hypocotyl and first internode elongation could be examined. Seeds were planted at a 1 cm depth in flats divided into 36 compartments (each compartment approximately $3 \times 6 \times 8 \text{ cm}^3$), kept on heating pads (30°C) and exposed to intermittent misting resulting in continuously moist but well-aerated soil. Planting was conducted in a stratified random design with one seed from each of the three populations being planted in one of three contiguous compartments, but in random order. There was no opportunity for below ground competition and seedlings were harvested before any competitive effects due to shading could occur. Germination date was recorded and daily measurements of hypocotyl length were made until the first internode was > 1 mm, then daily measurements of the first internode were taken thereafter. First internode elongation was recorded until the expansion of the second leaf. Rates of hypocotyl and first internode elongation were determined by regressing the $\log(x + 1)$ transformed measurements against day. An initial measurement of 0.0 was assigned to all individuals on day 0, 24 h before the emergence of the hypocotyl above the soil surface. Prior to log transformation, the height of the hypocotyl was subtracted from all subsequent internode measurements. Thus, the log transformation on hypocotyl and first internode length was conducted on data with the same starting point of 0.0. Measurements of cotyledon length and width were taken for one

cotyledon on each seedling and cotyledon area on the nearly rectangular cotyledons was estimated by multiplying length \times width.

2.2.4. Juvenile characters

Thirty seedlings with three to six true leaves (approximately 4 weeks after germination) from each of the flood-zone var. *macrosperma* and neighboring upland populations were transferred from well-watered and drained conditions into each one of three watering treatments: (1) lightly watered, (2) well-watered and (3) water-saturated (total number of plants = 30 seedlings \times 2 populations \times 3 watering treatments = 180 plants). These treatments were the same as described for seedling characters experiment 1 (Section 2.2.1.) except that plants were grown in 8 cm clay pots. This experiment was conducted simultaneously with seedling characters experiment 1. All six treatments (two populations \times three watering treatments) were replicated across six blocks with five replicates per block. Total shoot length and number of leaves were measured for each plant at the initiation and termination of the experiment. Shoot and total root biomass were quantified. Separate root mass measurements were also made for the top and bottom halves of the pot, and for tap roots and lateral roots. All plants were harvested prior to initiation of flowering.

2.2.5. Seedling competition

Seedlings of each of the varieties (populations) in the one to three leaf stage were matched by level of development, i.e. number of leaves, and planted 2.5 cm apart in 8 cm clay pots ($n = 50$ replicate pots). Soil was the same as above and plants were fertilized once weekly as in previous experiments. To induce water stress, pots were watered once daily, except on cloudy days when they were not watered. The experiment was initiated in early May and terminated 38 days later while plants were still in exponential growth and prior to becoming pot bound. Shoots were harvested, and the number of leaves recorded.

2.3. Statistical analyses

The General Linear Models procedure from the SAS statistical package was used to conduct two-way fixed factor ANOVA to determine the role of population origin (either upland or var. *macrosperma*) and water treatment (lightly watered, well-watered and water-saturated) on early seedling (seedling characters experiment 1, Section 2.2.1.) and later seedling (juvenile characters, Section 2.2.4.) performance and allocation patterns (SAS Institute Inc., 1985). These analyses were restricted to seedlings planted shallow and did not include the deep flood treatment because of limited survivorship of seedlings planted deep and/or exposed to deep continuous flooding. If either the main effects or interaction were significant, then the General Linear Models procedure was used to conduct one-way ANOVA with each population and watering regime considered as a separate treatment. Tukey's tests were then conducted to contrast the performance of the different population and water treatment combinations. Proportions were square root arcsine transformed and biomass was log transformed prior to analysis in order to better meet the assumptions of ANOVA. Plants were grown in non-random placement within

blocks, e.g. all flood-treated plants in a block grew in the same tub. To avoid pseudoreplication, analyses were conducted on the means of each treatment for each block. Tolerance to flooding and dry conditions was examined using two methods. First, using ANOVA the absolute growth of var. *macrosperma* was contrasted with the upland form for the different treatments. Second, relative growth was determined for var. *macrosperma* vs. the upland form by dividing total biomass (roots and shoots) for each plant in the lightly watered and water-saturated treatments by its nearest conspecific in the same block which was grown under well-watered conditions. For example, relative tolerance to dry conditions of the upland population vs. var. *macrosperma* was determined by contrasting the ratios: (upland lightly watered)/(upland well-watered) vs. (var. *macrosperma* lightly watered)/(var. *macrosperma* well-watered). Mean relative performance was tested using one-tailed *t*-tests with the prior expectation that var. *macrosperma* would perform better under flooded conditions and worse under dry conditions than upland *C. fasciculata*. One-way ANOVA was used to determine the effect of population origin on traits measured in seedling characters experiment 3 (Section 2.2.3.). Differences among the populations for germination, percent emergence and survivorship under the different watering regimes were tested with χ^2 analyses with the null expectation being equal frequencies among the populations. Data are presented on an untransformed scale and 95% confidence intervals or two standard errors are presented on back-transformed scales.

For seedling characters experiment 3 (Section 2.2.3.), Pearson correlation analysis was performed between seed mass (log transformed), cotyledon area (log transformed), germination date (square root transformed) and rate and duration (square root transformed) of hypocotyl and first internode elongation.

3. Results

3.1. Seedling survivorship

Based on seedling experiments 1 and 2 (Section 2.2.1 and Section 2.2.2.), both upland and flood-zone forms of *C. fasciculata* have similar emergence rates above the soil when planted at shallow depths under dry, well-watered and water-saturated conditions (Table 1). However, var. *macrosperma* has an approximate 50% advantage when the soil is intermittently flooded. This advantage is maintained only for shallow seed burial under deep flooded conditions. Variety *macrosperma* has a significant advantage when the germinating seedlings are buried at a greater depth, but only under well-watered and drained conditions.

A much larger proportion of var. *macrosperma* plants were able to emerge above the water surface under both intermittent and deep-flooding conditions (Table 2). In addition var. *macrosperma* emerged beyond the water surface in the flooded treatment a week earlier (emergent date = 13.1 days \pm 2.3 vs. 20.3 days \pm 1.6 for var. *macrosperma* vs. the upland population, respectively, $P < 0.001$, Mann Whitney *U*-Test) and consequently spent fewer days submerged than flood intolerant *C. fasciculata* (number of days submerged = 8.6 \pm 0.6 vs. 16.0 \pm 1.5 for var. *macrosperma* and the upland site,

Table 1

Percentage of upland Virginia site *Chamaecrista fasciculata* and flood-zone *C. fasciculata* var. *macrosperma* seedlings which emerged beyond the soil surface under lightly watered, well-watered (but drained), water-saturated, and intermittent and high flooding treatments

Treatment	Emergence from soil (%)		Significance
	<i>C. fasciculata</i>		
	Upland	var. <i>macrosperma</i>	
<i>Shallow seed burial</i>			
Lightly watered	87.0	100.0	NS
Well-watered	93.0	100.0	NS
Water-saturated	80.0	86.7	NS
Intermittent flooded	51.0	77.0	$P < 0.001$
Deep flooded	33.3	46.7	NS
<i>Deep seed burial</i>			
Well-watered	60.0	100.0	$P < 0.001$
Water-saturated	0.0	3.3	NS
Deep flooded	0.0	0.0	NS

Seeds were planted at two depths: shallow (1 cm) and deep (5 cm). Sample size = 30 seeds per treatment, except the intermittent flooding treatment which had a sample size of 99 seeds. Significance levels determined by χ^2 analysis with 1 d.f.

respectively, $P < 0.001$, Mann Whitney *U*-Test). Subsequent survivorship was also higher for var. *macrosperma* with intermittent flooding (Table 2) and is possibly higher under high flooding, but decreased sample size prevents any strong inference.

3.2. Seed and seedling traits

Results from seedling characters experiment 3 (Section 2.2.3.) indicate that there are significant population effects for all seed and seedling traits measured (ANOVA tables not shown, lowest *F* value = 9.6, $P < 0.0001$, Table 3). The rapid emergence of var. *macrosperma* is due to a two-fold higher rate of elongation for both hypocotyl and first internode (Table 3). Similar duration and increased rates of elongation in var. *macrosperma* results in much longer stem lengths. Hypocotyl length was not environmentally

Table 2

Fate of upland Virginia site of *Chamaecrista fasciculata* and flood-zone *C. fasciculata* var. *macrosperma* seedlings after emerging beyond the soil surface under intermittent and constant flooding

Flooding treatment	Emergence beyond water surface			Survivorship		
	Upland	<i>macrosperma</i>	<i>P</i> level	Upland	<i>macrosperma</i>	<i>P</i> level
Intermittent	57% (51)	97% (77)	< 0.001	71% (51)	97% (77)	< 0.001
Deep	0% (10)	35% (14)	< 0.05	0% (10)	23% (14)	NS

All seeds planted at 1 cm burial depth. Significance levels determined by χ^2 analysis with 1 d.f. Sample sizes given in parentheses.

Table 3

Seed and seedling traits for two populations of *Chamaecrista fasciculata* and one population of *C. fasciculata* var. *macrosperma* grown under moist and well-drained conditions

Trait	<i>macrosperma</i>	Upland (Virginia)	Upland (Kansas)
Seed mass (mg)	38.8A (1.2)	11.7B (0.4)	9.0C (0.2)
Percent germination	90.0A	73.0B	62.0C
Days to germination	7.9A (1.3)	19.8B (2.6)	16.3C (1.6)
Cotyledon area (cm ²)	2.28A (0.10)	0.95B (0.08)	0.74C (0.06)
Duration of hypocotyl Growth (days)	5.2A (0.2)	4.7B (0.3)	6.1C (0.2)
Duration of first internode growth (days)	7.8A (0.3)	7.5A (0.3)	8.6B (0.4)
Rate of hypocotyl elongation (mm day ⁻¹)	4.6A (0.2)	1.8B (0.1)	1.9B (0.2)
Rate of first internode elongation (mm day ⁻¹)	9.3A (0.2)	4.8B (0.2)	3.8C (0.2)

Means followed by different letters are significantly different (for percent germination: χ^2 , 1 d.f., $P < 0.05$, Bonferroni correction for multiple comparison; for all other traits Tukey's test, $P < 0.05$). Two SE are given in parentheses.

sensitive because var. *macrosperma* maintained the same approximate three- to four-fold length advantage under all treatments in seedling characters experiments 1 and 2 (hypocotyl length = 20.4, 20.5, 13.7, 23.4 for var. *macrosperma* and 5.3, 5.7, 4.5, 8.2 for the upland population under lightly watered, well-watered, deep flooded and intermittent flooded conditions, respectively, average 95% CI = ± 3.7). Under well-watered and drained conditions, var. *macrosperma* germinated earlier (Table 3) and had a greater germination rate than either of the two upland *C. fasciculata* populations (Table 3).

Seed mass is inversely correlated with duration of first internode ($r = -0.523$, $P < 0.001$) and positively correlated with internode elongation rate ($r = 0.479$, $P < 0.001$) only for var. *macrosperma*. In all populations cotyledon size is positively correlated to seed mass ($r = 0.363$, $P < 0.001$; $r = 0.263$, $P < 0.05$; $r = 0.404$, $P < 0.001$), increased elongation rates of the hypocotyl (0.338, $P < 0.01$; $r = 0.431$, $P < 0.001$; $r = 0.603$, $P < 0.001$) and increased elongation rates of the first internode ($r = 0.312$, $P < 0.01$; $r = 0.438$, $P < 0.001$; $r = 0.456$, $P < 0.01$) for var. *macrosperma* and the Virginia and Kansas upland populations, respectively. Duration and rate of elongation were negatively correlated to each other for both the hypocotyl ($r = -0.617$, $P < 0.001$; $r = -0.388$, $P < 0.001$; $r = -0.735$, $P < 0.001$) and generally for first internode ($r = -0.910$, $P < 0.001$; NS; $r = -0.534$, $P < 0.001$) for var. *macrosperma* and the Virginia and Kansas upland populations, respectively. However, caution must be used in interpreting these latter results since a negative correlation between rate and duration may be a statistical artifact of having duration in the denominator of rate.

3.3. Performance under flooding and dry conditions

The results of the three experiments, seedling characters experiment 1 (Section 2.2.1.), juvenile characters (Section 2.2.4.) and seedling competition (Section 2.2.5.), are summarized here. Because of the low survivorship under greater burial depth and deep-flooding, biomass data for young seedlings are presented only for seedlings planted at shallow burial depth and exposed to lightly watered, well-watered and water-saturated conditions. There were significant differences between the populations and among the water treatments and significant population \times water treatment interactions for shoot biomass, root biomass, total biomass, shoot elongation and leaf production for both early seedling and later juvenile stages (ANOVA tables not shown, minimum F value = 4.9, $P < 0.01$).

Table 4

Response by upland *Chamaecrista fasciculata* and flood-zone *C. fasciculata* var. *macrosperma* to different soil moisture levels

Population	Watering treatment	Biomass (mg)		
		Root	Shoot	Total
Upland (young seedlings)	Light	69.4A (10.3)	176.5A (22.9)	245.9A (26.6)
	Well	170.1B (22.5)	366.7B (55.4)	536.9B (76.1)
	Saturated	129.0A (41.0)	298.7AB (83.1)	427.7A (122.3)
<i>Macrosperma</i> (young seedlings)	Light	86.1A (13.2)	252.1AB (29.2)	344.1A (33.1)
	Well	292.5C (24.1)	558.1BC (51.7)	850.5BC (68.9)
	Saturated	347.5C (69.7)	717.5C (153.8)	1065.0C (213.2)
Upland (juvenile seedlings)	Light	145.3B (37.5)	464.9A (121.6)	610.3A (157.7)
	Well	375.5B (118.8)	1312.9B (304.1)	1688.4B (392.9)
	Saturated	60.5A (16.1)	309.3A (67.3)	369.8A (81.6)
<i>Macrosperma</i> (juvenile seedlings)	Light	141.1B (26.0)	427.8A (103.1)	568.9A (127.3)
	Well	428.9C (82.8)	1047.3B (154.3)	1476.2B (227.9)
	Saturated	345.6C (59.3)	1127.3B (204.7)	1472.8B (257.1)

Root, shoot and total biomass are shown for three treatments: lightly watered, well-watered and water-saturated soils for young and then older juvenile seedlings. Young seedlings were followed from the germination stage whereas juvenile seedlings were exposed to the treatments at the seedling stage. $\pm 95\%$ confidence intervals are given in parentheses. Means followed by different letters are significantly different from one another within a column (i.e. root or shoot or total) and life stage (young seedlings or juvenile seedlings) (Tukey's test, $P < 0.05$).

Based on differences in total biomass accumulation, the water-saturated treatment had a negative effect only on the upland population (Table 4). Variety *macrosperma* had greater growth of roots and shoots under water-saturated conditions during both the early seedling and later juvenile stages compared with individuals from the nearby upland population (Table 4). The greatest difference in elongation and leaf production between the two populations was observed under water-saturated conditions, with var. *macrosperma* adding six times more shoot length and four times more leaves (Table 5). Relative to the controls (well-watered and drained conditions) there was a trend for flooding to enhance growth in terms of total biomass in var. *macrosperma* (Fig. 1). The upland population's response to flooding was most severe in the later juvenile stage with total biomass decreasing to 40% of plants that were well-watered (Fig. 1). Young seedlings of var. *macrosperma* also outperformed seedlings from the upland site under well-watered conditions. There was no difference between the two populations in biomass accumulation expressed at the later juvenile stages under well-watered conditions (Tables 4 and 5).

The lightly watered treatment had a significant stress effect for both forms of *C. fasciculata* (Table 4). No significant differences were observed between var. *macrosperma* and the neighboring upland population for either root or shoot biomass under dry conditions for both life stages (Table 4). In terms of growth relative to the well-watered environment, var. *macrosperma* had the greatest negative response to dry conditions but this was close to statistically significant only for the later juvenile plants (Fig. 1). Upland and var. *macrosperma* populations responded similarly to dry conditions in terms of number of leaves added over the course of the experiment, but var. *macrosperma* added significantly more shoot length (Table 5). Differences in vegetative growth under dry conditions were more extreme under a competitive environment (Table 6). When grown in the same pot under dry conditions, var. *macrosperma* produced twice as much shoot biomass and 20% more leaves than plants from the upland site.

3.4. Allocation to vegetative structures

Results from seedling characters experiment 1 and the juvenile characters experiment demonstrate significant differences among the populations and water treatments for

Table 5

Shoot elongation (cm) and number of leaves added by flood-zone *Chamaecrista fasciculata* var. *macrosperma* and a nearby upland population of *Chamaecrista fasciculata* under lightly watered, well-watered and water-saturated conditions

Treatment	Shoot growth (cm)		Number of leaves added	
	Upland	<i>macrosperma</i>	Upland	<i>macrosperma</i>
Lightly watered	10.2A (3.8)	18.6B (4.2)	9.2A (3.6)	8.3A (1.4)
Well-watered	46.8C (11.2)	43.8C (7.4)	28.6B (6.8)	18.1B (3.6)
Water-saturated	8.6A (2.4)	48.2C (8.2)	5.1A (1.6)	20.4B (3.4)

Data are from older seedlings. Two SE are given in parentheses. Means followed by different letters are significantly different (Tukey, $P < 0.05$). Separate contrasts were made for each trait.

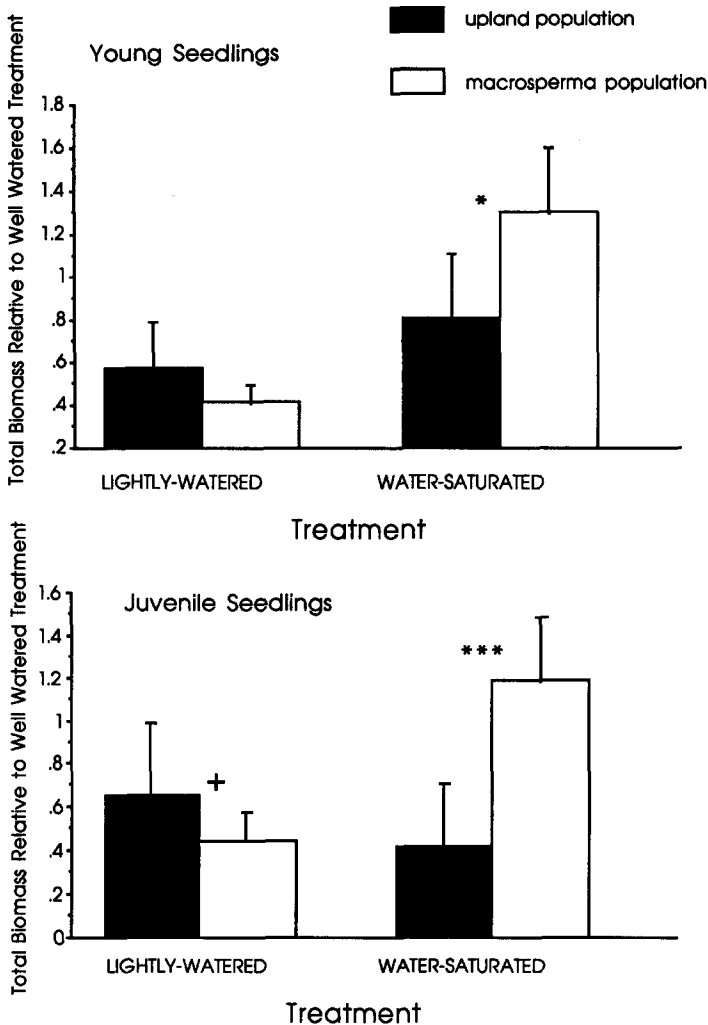


Fig. 1. Comparison of Virginian upland population and nearby flood-zone population var. *macrosperma* of *Chamaecrista fasciculata* in response to lightly watered and soil water-saturated conditions for young and juvenile seedlings. Response is measured in terms of total biomass relative to the biomass of the well-watered (but drained) treatment for each species. +, $P < 0.10$, *, $P < 0.05$, ***, $P < 0.001$, 1-tailed t -test, var. *macrosperma* is relatively less tolerant of dry conditions and more tolerant of flood conditions, respectively, than the upland population. Bars are 95% confidence intervals.

root-to-shoot ratios in older seedlings and root placement for both younger and older seedlings (ANOVA tables not shown, minimum F value = 7.3, $P < 0.005$). Population had no effect on root-to-shoot ratios in younger seedlings ($F = 0.06$, $P < 0.805$) or on proportion of the root biomass allocated to tap root in older seedlings ($F = 2.6$, $P < 0.11$), although there were water treatment effects on both traits ($F = 4.94$, $P < 0.01$, $F = 7.1$, $P < 0.005$, respectively). Unlike the performance traits above, the interaction

Table 6

Above ground biomass and leaf production of *Chamaecrista fasciculata* and *C. fasciculata* var. *macrosperma* when grown together in the same pot under competitive conditions

	Upland	<i>macrosperma</i>	Significance
Shoot biomass (mg)	83.9 ± (11.0)	167.3 ± (17.8)	$P < 0.001$
Number of leaves	5.1 ± (0.2)	6.0 ± (0.4)	$P < 0.001$

Significance levels determined by paired *t*-tests on transformed data, 48 d.f., and ±95% confidence intervals are given in parentheses.

between population and water treatment tended not to be significant on patterns of allocation, with only root-to-shoot ratio in older seedlings marginally affected by the interaction between the two main effects ($F = 3.3$, $P < 0.06$). Young seedlings of var. *macrosperma* had a lower root-to-shoot ratio under dry conditions compared with well-watered conditions (Tukey's test, $P < 0.05$). Older upland seedlings had a lower ratio under water-saturated conditions compared with well-watered var. *macrosperma* (Tukey's test, $P < 0.05$). The seedlings of both var. *macrosperma* and the neighboring upland population responded similarly to environment in terms of placement of the roots. As conditions became wetter more of the root biomass of young seedlings was placed shallower in the soil. This trend was also observed among the older juvenile plants except that under water saturated conditions var. *macrosperma* had a significantly greater proportion of root mass in the top half of the soil compared with the neighboring upland population (Tukey's test, $P < 0.05$). Wetter soil conditions also resulted in less of the root biomass allocated to tap root with the exception of the upland plants under water saturated conditions (Tukey's test, $P < 0.05$).

4. Discussion

Growth of neighboring populations of *C. fasciculata* is environment specific. Variety *macrosperma* is tolerant of water-saturated and intermittently flooded soils. In contrast, individuals from a nearby upland population of *C. fasciculata* exhibited lower seedling survivorship and reduced growth under the flooded conditions. Thus, var. *macrosperma* is a flood-tolerant ecotype of *C. fasciculata*. Many of the same morphological traits which generally distinguish flood-tolerant species from non-flood-tolerant species are responsible for the ecotypic differentiation for flood tolerance documented here. Under well-watered and dry environments, var. *macrosperma* has similar vegetative production as the non-flood-tolerant forms of *C. fasciculata*.

Shoot elongation is greater in flood-tolerant var. *macrosperma* than in flood-intolerant upland populations. Photosynthetic carbon gain can be significantly reduced following submergence (Ridge, 1987). Many flood-tolerant species respond to flooding with shoot elongation, e.g. *Rumex* (Blom, 1990; Laan and Blom, 1990), *Polygonum* species (Mitchell, 1976) and populations of *Agrostis stolonifera* (Aston and Bradshaw, 1966). In addition, greater duration of shoot elongation differentiates deep-water varieties of rice from other varieties (Keith et al., 1986). However, higher rates of shoot

elongation in var. *macrosperma* suggest that a selective premium has been placed on decreased time of shoot submergence, perhaps reflecting a limited tolerance of both forms of *C. fasciculata* to extended periods of shoot submergence. In further contrast to many flood-tolerant species such as *Rumex* species (Voeselek and Blom, 1989), which demonstrate a plastic response of shoot elongation to inundation and show increased shoot elongation only at specific life-stages (Van der Sman et al., 1993), the amount of elongation by var. *macrosperma* is constant across environments and is maintained throughout its life. Fixed shoot elongation suggests that competition for canopy light may also be a determinant of var. *macrosperma*'s architecture.

The major morphological feature which distinguishes var. *macrosperma* from other forms of *C. fasciculata* is the massive size of its seed. In all three populations of *C. fasciculata* a positive correlation between cotyledon size and rate of shoot elongation was observed. The higher concentration of seed energy reserves as carbohydrates rather than lipids in legume seeds may facilitate germination under low partial pressures of oxygen (Crawford, 1989). This suggests that the evolution of rapid shoot elongation at the hypocotyl and first internode stage may be associated with increased seed mass and cotyledon size which provides greater carbohydrate reserves in var. *macrosperma*. The large seeds of var. *macrosperma* may therefore be analogous to food reserve organs, such as rhizomes and tubers, found in many flood-tolerant species (Crawford, 1992, 1993). There was no difference in survivorship among the seedlings under deep flooding and deep burial, suggesting that increased seed mass has not evolved to allow var. *macrosperma* to germinate at greater burial depth.

Rapid generation of new laterals is observed in *Rumex* species following flooding (Laan et al., 1989). In flood-tolerant species these laterals have a higher root-porosity due to increased levels of aerenchyma (Armstrong, 1979; Laan et al., 1989) and may function in place of the old root system (Jackson and Drew, 1984). The increased proportion of root biomass close to the soil surface for older seedlings of var. *macrosperma* in water-saturated soils indicates that var. *macrosperma* also produces new lateral roots in response to flooding. In contrast, root biomass and root-to-shoot ratios decline from well-watered to water-saturated conditions for the neighboring upland population. The low root biomass of flood-intolerant plants may arise from either root die-back and/or lower root initiation during flooding. The new lateral roots produced by var. *macrosperma* under flooded conditions do not have aerenchyma (C.B. Fenster, personal observation, 1994) which may indicate that the new laterals do not have increased porosity. While individuals from the flood-zone and upland site differ as old seedlings, young seedlings from both populations place their roots near the soil surface in response to water-saturated soil. These results suggest that the ability of older var. *macrosperma* seedlings to synthesize new laterals near the soil surface following flooding is a retention of juvenile root allocation patterns from its presumed flood-intolerant ancestor.

The increased propensity for shallow lateral root growth with flooding in var. *macrosperma* suggests that it would be less tolerant to dry conditions. However, a shallow rooting trait could enable the plant to benefit from water in the sub-surface night-time condensation zone on dry sites. Furthermore, under dry conditions, the roots of var. *macrosperma* and its upland conspecific are placed in similar positions in the soil

column. Trade-offs between performance under flood and dry conditions have been observed among three heath species and three *Polygonum* species and may account for their local distribution (Bannister, 1964; Carter and Grace, 1990, respectively). Intraspecific studies on *Spartina patens* demonstrate a marsh ecotype to have lower survival and tiller production compared with a nearby dune ecotype under drought conditions (Silander, 1979, 1985). Reciprocal transplantation of the progeny of trees of the *Betula pendula/pubescens* complex across a cline from dry heath to waterlogged bog demonstrated little difference in growth and survival, but there was poor regrowth following a heath fire in bog genotypes compared with their heath conspecifics (Davy and Gill, 1984). In contrast, when exposed to dry conditions, flood-tolerant var. *macrosperma* may suffer in terms of *relative* growth, but it still performed as well as flood-intolerant *C. fasciculata* in terms of young seedling survivorship and *absolute* vegetative growth. When a competitive regime was superimposed upon dry conditions, var. *macrosperma* actually out-performed a neighboring upland form of *C. fasciculata* in terms of vegetative growth. This observed lack of trade-off may reflect several processes. First, if var. *macrosperma* avoids hypoxia induced by flooding by increased shallow rooting then it may not have evolved any differences in root porosity which would be expected to lessen tolerance to dry aerated soils, since highly porous roots would be expected to desiccate easily. Tolerance to dry conditions may be evidence in itself of little difference in root porosities between the two forms. In total, the lack of intolerance to dry conditions by var. *macrosperma* may reflect a selective premium placed on superior vegetative performance across different environmental conditions and may be an outcome of greater allocation to vegetative growth.

Although flood intolerance clearly limits *C. fasciculata* from invading var. *macrosperma* habitat, it is not obvious from vegetative performance what limits var. *macrosperma* from successfully invading the terrestrial habitat of *C. fasciculata*. Large seed mass may limit seed dispersal but seed dispersal by explosive pod dehiscence is very limited in *C. fasciculata* (Fenster, 1991). In addition, the increased height of var. *macrosperma* would probably offset the greater individual seed mass in terms of its ability to disperse seeds. On long-term time scales differences in dispersal distances should not limit colonization or migration events between sites separated by hundreds of meters. Instead, the spatial segregation of *C. fasciculata* and var. *macrosperma* may be associated with another attribute associated with increased seed mass and superior vegetative performance. *Chamaecrista fasciculata* is strictly annual (except in the southern portion of its range) and reproductive success must be strongly correlated with flower and seed production. Increased seed mass of var. *macrosperma* is likely associated with increased seedling survivorship under flooding as discussed above. However, trade-offs among yield components are common and are often a serious constraint to crop improvement (Adams, 1967). Variety *macrosperma*'s increased seed mass is associated with fewer ovules and fewer seed per fruit. When grown in the greenhouse, fruit of var. *macrosperma* contain on average $8 \text{ ovules} \pm 0.2$ (2 SE) while fruit from the close upland population contain $10 \text{ ovules} \pm 0.4$. Thus under non-competitive conditions and well aerated soils the potential reproductive success of *C. fasciculata* may be greater than var. *macrosperma*. Reciprocal transplant studies confirm this (T.E. Finney and C.B. Fenster, unpublished data, 1996). Only var. *macrosperma* can tolerate

the marsh but var. *macrosperma* has only slightly lower survivorship and equivalent vegetative biomass as the upland form in the upland site. However, upland plants produce two- to five-fold more seed and seed biomass than var. *macrosperma* in the upland site (T.E. Finney and C.B. Fenster, unpublished data, 1996). If seed mass and increased allocation to vegetative structures in var. *macrosperma* does constrain seed number, then this potential trade-off of flood tolerance with reproductive allocation may account for the distributional limits of the different forms of *C. fasciculata*. The examination of the genetic correlations underlying seed mass, seed number and reproductive allocation are presently under investigation.

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