

Evidence for the Existence of Distinct Alligatorweed Biotypes

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ABSTRACT

The growth of a broad-stemmed (BSA) and a narrow-stemmed (NSA) form of alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] was compared in a 5-week study under a variety of simulated-floating, terrestrial, and emersed-rooted conditions. Neither dry weights nor stem lengths of BSA and NSA were significantly different when grown under simulated-floating conditions of full-strength Hoagland's nutrient, nutrient + 1% NaCl, nutrient + 1 ppm Cd, or in well water without nutrients. Very poor growth of both BSA and NSA occurred in these treatments, apparently as the result of nutrient inavailability and/or deficiency. BSA produced significantly greater dry weights than NSA when grown rooted-emersed in sand or topsoil or terrestrially in topsoil, but no differences were observed when the plants were grown terrestrially in sand. Specific stem lengths ($\text{cm}\cdot\text{g}^{-1}$) of NSA were significantly greater than those of BSA in all treatments. No changes occurred in the morphology of either BSA or NSA during the 8-week study or during a 1-year observational period in which the plants were grown hydroponically in a greenhouse. The results of this study and numerous field observations indicate that BSA and NSA represent distinct biotypes of alligatorweed and are not environmentally-induced variations of genetically identical plants.

INTRODUCTION

Alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] an aquatic, mat-forming weed of the family Amaranthaceae, was introduced into the United States from South America around 1897 and since has spread rapidly across the southern United States. This plant was a major aquatic weed problem until after the release of biological control agents during the mid-1960's and early 1970's (21). For many years alligatorweed has been a difficult weed to control in rice fields in Louisiana (8), and interference by this weed with recreation, fishing, irrigation, agricultural drainage, and flood control programs has been well documented (6). The amphibious nature of this plant has also allowed it to invade cultivated fields as well as the aquatic habitat (25). Alligatorweed is common in soybean fields near infested lakes and streams in the lower Mississippi River Valley.

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Although weed control programs have eliminated alligatorweed as a serious problem in many places, it continues to persist in a few areas. For instance, in the Carolinas, introduced natural enemies initially seemed to have eliminated the problem; but alligatorweed has reappeared as a pest (Gary Buckingham, USDA, Gainesville, Florida, personal communication). Consequently, alligatorweed control continues to be an important topic for many scientists in both state and federal agencies.

Although much attention has been paid to the ecological relationships between alligatorweed and the biological agents that were introduced for its control, little consideration has been given to the fact that alligatorweed populations have considerable morphological variation from one area to another. This morphological variability generally has been considered to be environmentally induced, without consideration that some of the observed differences may have been genetic. Perhaps this is the result of general acceptance that alligatorweed was introduced at Mobile and New Orleans and, from there, spread to the entire southern United States, including Texas and California (25).

Environmentally-induced variation in the morphology of aquatic plants is well known. Hutchinson (11) and Sculthorpe (19) discussed at length phenomena such as heterophylly, air roots, development of aerenchyma and lacunae, the reduction of cuticle, stomata, and vascular tissues, and the elongation of internodes in response to aquatic conditions. The production of new ramets and the lengthening or swelling of waterhyacinth petioles have been demonstrated clearly to be environmentally induced (1). Similar environmentally-induced morphological variation has been demonstrated for *Alternanthera sessilis* (L.) R. Br. ex DC., a close relative of alligatorweed (5).

In alligatorweed, many of the observed morphological variations have been attributed properly to the effects of the environment. Alligatorweed quickly develops a tough, fleshy, root-like rhizome, when subjected to a non-flooded existence. Terrestrial stems of alligatorweed are also smaller in diameter, more lignified, and have smaller internodal cavities and shorter internodes than aquatic stems. Plants grown in highly saline water develop thickened leaves, which readily revert to normal when the plants are returned to fresh water (7). Although these morphological variations are directly the result of prevailing environmental conditions, others appear to be innate. Separate populations of alligatorweed growing in the field under apparently identical conditions are often distinctly different in appearance. One population may have very slender stems and

short, rounded leaves, whereas another may have broad stems and long, narrow leaves. Two such "varieties" of alligatorweed have been reported by Quimby *et al.* (16). Heterophylly, clearly a genetic trait in alligatorweed, was reported by zur Burg *et al.* (26) and by Gangstad *et al.* (7). The degree of glabrosity of stems and leaves may also vary considerably. These morphologically distinct plants, when grown under identical conditions for extended periods of time, remain morphologically distinct. This strongly suggests that the populations represent genetically distinct biotypes. If so the populations should have physiological differences which may influence the biological and chemical control of alligatorweed.

The objective of this study was to compare the growth of two morphologically distinct forms of alligatorweed when cultured under several environmental conditions.

METHODS AND MATERIALS

Plants of a narrow-stemmed biotype of alligatorweed (NSA) were collected at Gainesville, Florida, during the spring of 1979. A broad-stemmed biotype of alligatorweed (BSA) was obtained from a greenhouse culture at the Southern Weed Science Laboratory, USDA, SEA-AR, Stoneville, Mississippi. The greenhouse culture of BSA originated from plants originally collected at Blue Lake, near Itta Bena, Mississippi, and subsequently grown hydroponically as described by Quimby and Kay (15). Plants of the NSA biotype had relatively slender stems with short internodes, compared to the broader stems and longer internodes of the BSA. The pubescence on both stems and leaves of NSA was considerably finer and more reduced than that on BSA. The leaves of NSA were small and rather blunt-tipped, whereas those of BSA were large, long, and acutely pointed. Both biotypes were grown in outdoor cages in plastic pans containing full-strength Hoagland's no. 1 nutrient (10) with Sequestrene 330 providing iron at 1 ppm until sufficient material was available for experimentation. Two-node stem cuttings (10 cm) of BSA and three- to 4-node (10 cm) stem cuttings of NSA were emersed in tap water under fluorescent lighting in the laboratory. After 1 week, plants of each type were selected for uniformity of shoot and root development, and initial fresh weights of the stem cuttings were recorded.

The study was conducted as a completely random design having eight treatments, with three replications per treatment. Each experimental unit contained three stems each of BSA and NSA and constituted one replication of each biotype for a given treatment. The stems of BSA and NSA were placed in the opposite ends of a plastic pan measuring approximately 25 x 35 x 12 cm with an approximate volume of 10 liters. Simulated-floating conditions were provided by supporting the stem cuttings in an upright, emergent position with a piece of 1.25 cm mesh hardware cloth that was placed over the pans. All simulated-floating treatments were made up in well water. Treatments having solid substrates contained an 8-cm layer of either sand or a commercially-prepared topsoil. Emersed conditions were simulated by flooding the substrate to a depth of four cm, and terrestrial conditions were simulated by

cutting holes in the bottom of the pans to provide drainage. Treatments are summarized in Table 1. The study was conducted in an outdoor screened enclosure for a period of 5 weeks from 11 July to 15 August 1979. All treatments were watered as needed with well water.

TABLE 1. SUMMARY OF EXPERIMENTAL CONDITIONS USED FOR THE GROWTH COMPARISON STUDIES OF NSA AND BSA.

Hydroponic culture	Treatments	Solid substrate
Nutrient only		Florida Sand, emersed
Nutrient + 1% NaCl		Topsoil, emersed
Nutrient + 1 ppm Cd		Florida Sand, terrestrial
Well water only		Topsoil, terrestrial

At harvest, plants were separated into leaves, stems, roots, and original stem cuttings. The total length of all new stems was measured. Tissues were dried at 65 C for 48 h and weighed. Specific stem lengths ($\text{cm}\cdot\text{g}^{-1}$) were calculated. All three plants of one biotype from each experimental unit were treated as a single unit for growth measurements. The diameters of an additional 25 BSA and 25 NSA stems were measured with calipers at the mid-points of the internode between the third and fourth fully-expanded leaf pairs. Additional plants of both biotypes were maintained in hydroponic culture in a greenhouse for one year for observation for possible long-term morphological changes.

Growth data were subjected to an analysis of variance and means were compared with Duncan's Multiple Range Test. A two-sample t-test was used for comparison of stem diameters.

RESULTS

In all simulated-floating treatments the growth of both BSA and NSA was so poor that pre-planned comparisons to assess biotype-substrate interactions were not possible. Consequently, the responses of the two biotypes under simulated-floating conditions will be discussed separately from the responses under emersed and terrestrial conditions.

Simulated-Floating Treatments

The very poor growth of both BSA and NSA in all simulated-floating treatments was accompanied by severe chlorosis. Table 2 shows that the dry weights of all plants grown in full-strength nutrient were not significantly different than those grown in well water without nutrients. Although these observations seem to support Sculthorpe's contention that rooting in soil is necessary for growth of alligatorweed (19), Weldon (23) has shown that large mats of alligatorweed may remain totally free-floating for many seasons without any apparent adverse effect on the survival or growth of the plants. Good growth of free-floating alligatorweed has also been reported previously by zur Burg *et al.* (26). Numerous field observations by the present authors and experimentation with alligatorweed growing in nutrient solutions totally devoid of any soil substrate confirm Weldon's observation that rooting in soil is unnecessary for the growth of alligatorweed.

TABLE 2. GROWTH MEASUREMENTS ON PLANTS GROWN IN SIMULATED-FLOATING TREATMENTS.^a

Treatment	Biotype	Dry weight of new growth (g)				Total stem length	Specific stem length
		Leaves	Stems	Roots	Total		
						(cm)	(cm.g ⁻¹)
Nutrient Only	BSA	0.34ab	1.04ab	0.09a	1.47ab	152ab	146c
	NSA	0.09b	0.34ab	0.02a	0.45ab	91ab	276b
Nutrient + 1% NaCl	BSA	0.56a	1.22a	0.27a	2.04a	181a	148c
	NSA	0.24ab	0.53ab	0.08a	0.86ab	142ab	269b
Nutrient + 1 ppm Cd	BSA	0.33ab	0.80ab	0.09a	1.22ab	111ab	143c
	NSA	0.08b	0.19b	0.02a	0.29b	67ab	350a
Well Water	BSA	0.36ab	0.94ab	0.19a	1.49ab	78ab	87d
	NSA	0.12b	0.26ab	0.05a	0.44ab	46b	178c

^aValues in a column followed by the same letter are not statistically different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of three replications.

The problem of poor growth in the simulated-floating treatments appeared to be related to nutrient availability. The chlorosis and concomitant poor leaf, stem, and root development of both biotypes were similar to the effects of severe iron deficiency observed previously in alligatorweed (12). Since iron chelates, such as the Sequestrene 330 used in this study, have limited photostability, the observed chlorosis and depressed growth were very likely the result of photodegradation of the iron chelate and the consequent inavailability of iron to the plants. The pH of the water or interactions among the nutrient solutions and minerals in the water also may have rendered one or more nutrients unavailable. No analyses of either nutrient solutions or plant tissues were made, however. The plants of both biotypes that were grown hydroponically for the subsequent 1-year observation period were all healthy. This supports the hypothesis that nutrient inavailability may have been responsible for the poor growth of the simulated-floating treatments.

In all simulated-floating treatments, the dry weights of individual plant tissues, total new growth, and the stem lengths of BSA exceeded those of NSA within a given treatment (Table 2). Variation was extremely great, however, and the apparent differences were not statistically significant. Morphologically, however, the differences were striking. In all simulated-floating treatments, the specific stem lengths of NSA were significantly greater than those of BSA in the same treatments. Specific stem length (cm.g⁻¹)

directly reflects stem diameters, in this case the smaller stem diameters of the NSA biotype in comparison to BSA.

Rooted-Emersed and Terrestrial Treatments

The dry weights of leaves, new stems, and total new growth of BSA were significantly greater than those of NSA when grown rooted in sand or topsoil under emersed conditions or in topsoil under terrestrial conditions (Table 3). The growth of NSA appeared to slightly exceed that of BSA when grown under terrestrial conditions in sand, but differences were not statistically significant. There were no significant differences between the root dry weights of BSA and NSA within any treatment. Stem lengths of NSA consistently exceeded those of BSA within a treatment, and differences were significant in all treatments except sand-emersed. Specific stem lengths of NSA were significantly greater than those of BSA regardless of treatment, clearly reflecting the narrow-stem morphology of the NSA biotype.

Both biotypes grew best in topsoil under terrestrial conditions (Table 3). This may reflect both greater nutrient availability in top soil and better root function under non-flooded conditions. Reddish-brown deposits (ferric oxide) in the substrate adjacent to the roots and encrusting the roots in all rooted-emersed treatments suggested that oxygen had been transported through the stems to the root system and that, therefore, the roots had not been subjected to a totally anoxic environment. Both biotypes grew better in topsoil

TABLE 3. GROWTH MEASUREMENTS ON PLANTS GROWN ROOTED-EMERSED OR TERRESTRIALLY.^a

Treatment	Biotype	Dry weight of new growth (g)				Total stem length	Specific stem length
		Leaves	Stems	Roots	Total		
						(cm)	(cm.g ⁻¹)
Sand-Emersed	BSA	0.89c	1.87c	1.15ab	3.91c	157d	85c
	NSA	0.41d	0.84d	0.89b	2.14d	172d	211a
Topsoil-Emersed	BSA	1.79b	4.73b	0.67b	7.02b	411c	88c
	NSA	1.01c	2.08c	0.55b	3.63cd	515b	248a
Sand-Terrestrial	BSA	0.93c	2.08c	0.61b	3.62cd	140d	71c
	NSA	0.93c	2.13c	1.21ab	4.72c	317c	145b
Topsoil-Terrestrial	BSA	3.51a	6.82a	1.65a	11.98a	542b	78c
	NSA	1.88b	4.26b	1.26ab	7.40b	632a	148b

^aValues in a column followed by the same letter are not statistically different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of three replications.

than in sand under both emersed and terrestrial conditions. This most likely reflected a higher nutrient content of the topsoil than of the sand.

DISCUSSION

Differences in growth and morphology observed in this study appear to be genetic and are most clearly reflected in the specific stem lengths. In all cases specific stem lengths of BSA were significantly less than those of NSA within the same treatment (Tables 1 and 2). An obvious conclusion is that differences observed in specific stem lengths reflect differences in stem diameters. Under all experimental conditions BSA stems were visibly greater in diameter than those of NSA, lending support to our hypothesis that BSA and NSA are two distinct biotypes of alligatorweed and are not simply environmentally-induced variations of genetically identical plants. Mean diameters of the additional 25 stems of each biotype were 4.8 ± 0.6 mm and 2.6 ± 0.3 mm, respectively, for BSA and NSA, and a "t" test indicated that these differences were highly significant. Observations of the growth of BSA and NSA over the subsequent year produced no evidence suggesting any convergent changes in morphology. Thus, the differences in growth and morphology observed in this study were apparently genetic and BSA and NSA have been correctly designated as true, distinct biotypes of alligatorweed. Proof of genetic differences by means of chromosome counts were not attempted because of a previous report that the 2N chromosome number for alligatorweed was 100 (4). Field observations of the authors (unpublished) and previous reports (7, 16, 26) suggest that several other alligatorweed biotypes may exist elsewhere in Florida and the southern United States.

The existence of morphologically and physiologically distinct alligatorweed biotypes may eventually prove to be the basis for many of the difficulties experienced in both chemical and biological control of alligatorweed infestations. Vogt *et al.* (22) noted that small stem diameters restricted the pupation of the alligatorweed flea beetle (*Agasicles hygrophila* Selman and Vogt), even though prepupae could enlarge the stem cavity somewhat in the more slender stems and pupate. Coulson (3) noted that stems of rooted alligatorweed or plants growing without adequate nutrients were often too lignified and/or too small to allow successful pupation of the flea beetles. The stems of the NSA biotype in the present study appeared to be too small and, possibly, too lignified (i.e., hard) for adequate flea beetle pupation. The field population from which our initial NSA plants were obtained was rooted and flooded throughout the summer of 1979. A few adult flea beetles were present on this field population throughout the entire growing season but never in sufficient numbers to affect the alligatorweed growth. Little larval activity was observed, and there was no evidence of pupation or emergence of new adults at anytime. The possibility exists that pollution or pesticide usage (13, 14) may have interfered with the development of the flea beetle population, even though this NSA field population was not adjacent to a heavily-travelled roadway. Differences in responses of alligatorweed to herbicides eventually may be shown to be the result of genetic

differences in susceptibility. Although no studies of biotypic differences in resistance to herbicides have been published on alligatorweed, one study has suggested biotypic differences in resistance of waterhyacinths to 2,4-D (9), and several studies have indicated herbicide resistance in biotypes of a number of terrestrial plants (2, 17, 18, 20, 24).

The origin of alligatorweed biotypes in the United States must be speculative. Alligatorweed may have entered the United States on numerous occasions at several locations other than Mobile and New Orleans. In South America, physically-isolated populations of alligatorweed may have evolved into genetically distinct biotypes. Thus, the presence of different alligatorweed biotypes in the United States could be the result of the introduction of previously-existing biotypes from different localities in South America. Another possibility is that the distinct morphological characteristics of different biotypes may be physical manifestations of genetic changes that have occurred in response to repeated herbicide usage. Monocultural uniformity of alligatorweed biotypes observed in the field would be expected, since alligatorweed is known to propagate only by vegetative means in the United States.

Finally, it should be noted that broad-stemmed and narrow-stemmed biotypes (not necessarily limited to the BSA and NSA used in this study) appear equally well adapted to terrestrial, rooted-emersed, and free-floating existence. Transferring such biotypes from one environment to another does not alter their basic biotypic distinctiveness. This is highly suggestive of genetic diversity and strongly supports our contention that distinct biotypes of alligatorweed are extant in the southern United States.

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