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Abscisic Acid Induction of Aerial Leaf Development in *Myriophyllum* and *Proserpinaca* Species Cultured *In Vitro*¹

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ABSTRACT

The influence of abscisic acid (ABA) on induction of aerial leaf development in seven species of the aquatic heterophyllous angiosperm genus *Myriophyllum* (*M. humile*, *M. heterophyllum*, *M. pinnatum*, *M. aquaticum*, *M. spicatum*, *M. verticillatum*, *M. tenellum*) and two species of *Proserpinaca* (*P. palustris* and *P. intermedia*) was examined. Shoots of each species were cultured in half-strength Murashige and Skoog salts supplemented with 86.7 mM sucrose and synthetic ABA (0 to 100 μ M) at 25 C under a 16-hr photoperiod for 20 days. All species, except *M. tenellum*, developed pectinately divided (dissected) leaves on submerged shoots. When cultured in the presence of ABA in concentrations of 1.0 μ M and greater, submerged shoots of all species developed leaves with anatomical and morphological features characteristic of leaves produced on aerial shoots. These included: 1) development of stomata, 2) reductions in leaf and epidermal cell length, 3) promotion of laminar expansion with reticulate venation (*Proserpinaca* spp.), 4) redistribution in epidermal chloroplast abundance, and 5) increased cuticularization. It is suggested that endogenous ABA plays a common role in the regulation of aerial leaf development in many heterophyllous, amphibious species. Possible ABA/ethylene interactions controlling leaf development are discussed.

Key words: stomate differentiation, cuticle, watermilfoil, mermaidweed, Haloragaceae, growth regulators.

INTRODUCTION

Aquatic angiosperms exhibit some of the most variable growth and developmental patterns observed in higher

plants. These plants, especially amphibious species, display a combination of ancestral land characteristics as well as more recently acquired aquatic traits (Kane and Albert 1982). These characteristics include the necessary physiological, anatomical, and morphological plasticities which enable rapid growth and reproduction in a variety of terrestrial and aquatic emergent, floating, and submerged environments. This flexibility in adaptation can, in part, contribute to the noxious aquatic weed problems posed by certain species (Grace and Wetzel 1978; Anderson 1982).

This phenotypic plasticity is manifested in the phenomenon of heterophylly, the occurrence of leaves of different size, morphology, and/or anatomy on the same plant, usually spatially separated between submerged and aerial shoots. Heterophyllic development is well exemplified in the amphibious genera *Myriophyllum* (watermilfoil) and *Proserpinaca* (mermaidweed) of the Watermilfoil family (Haloragaceae). Submerged shoots of most species bear pectinately divided (dissected) astomatous leaves composed of filiform pinnae (aquatic leaves). In response to growth above the water surface or beaching following fragmentation, aerial shoots produce smaller cutinized stomatous pinnatifid (dissected) or lanceolate serrate leaves (aerial leaves) depending on the species. Resubmergence of shoots results in a switch to production of aquatic leaves. Consequently, the extreme degree of foliar plasticity displayed by most *Myriophyllum* and *Proserpinaca* species has long been a source of taxonomic difficulty (Aiken 1981; Ceska and Ceska 1985).

Numerous heterophyllic aquatics have been used to study the environmental and physiological factors influencing leaf morphogenesis. Effects of light intensity and quality (Schmidt and Millington 1968; Spence and Dale 1978; Bodkin et al. 1980), photoperiod (Bostrack and Millington 1962; Wallenstein and Albert 1963; Davis 1967; Cook 1969), temperature (Bostrack and Millington 1962; Wallenstein and Albert 1963; Manual 1973), carbon dioxide level (Bristow 1968; Bristow and Looi 1968), osmotic stress (Jones 1955; McCully and Dale 1961; Deschamp

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and Cooke 1983; 1984; Kane and Albert 1987a), edaphic factors (Aiken and Picard 1980), and exogenous treatments with plant growth regulators (Davis 1967; Wallenstein and Albert 1963) on heterophyllic development have been described. In spite of these studies, little is known of the endogenous mechanisms regulating heterophylly.

The role of abscisic acid (ABA) as a possible endogenous hormonal regulator of leaf development in heterophyllous aquatic angiosperms has received increased attention since Anderson (1978; 1982) first reported that submerged plants (winterbuds) of *Potamogeton nodosus* Poir. atypically develop stomatous floating-type leaves following exposure to ABA. Subsequent reports of ABA-induced development of aerial leaf characteristics including stomatal development in several other taxonomically distinct aquatics (Mohan Ram and Rao, 1982; Deschamp and Cooke; 1983; Liu 1984; Young and Horton 1985; 1987; Kane and Albert 1987a) provide indirect evidence that endogenous ABA may play a regulatory role in the control of aerial leaf development in other heterophyllic amphibious species.

Given the amphibious heterophyllic nature of most members of the Watermilfoil family in North America (Aiken 1981), we decided to examine the role of ABA as a possible regulator of aerial leaf development in seven *Myriophyllum* and two *Proserpinaca* species cultured *in vitro*.

MATERIALS AND METHODS

Plant Source and Species Identification. *Myriophyllum* and *Proserpinaca* specimens were obtained from the localities listed in Table 1. With the exception of *M. tenellum* and *M. aquaticum*, identifications of *Myriophyllum* species were made using inflorescence and fruit characteristics according to Aiken (1981). Identifications of *Proserpinaca* species were made according to Fassett (1957).

Stock Plant Culture. Preliminary results indicated that shoot segments taken from non-sterile *aerially-grown* stock

plants could be more effectively surface sterilized and established *in vitro* with minimal tissue damage than when stem segments from submerged stock plants were used. Consequently, aerial shoot development in each species was induced by rooting 2 cm long apical cuttings in peat pots containing a 3:1 (v/v) mixture of potting soil and perlite. Pots were kept moist in glass covered aquaria placed in environmental chambers maintained at 25 ± 2 C in a 16-hr photoperiod. Illumination was provided by cool-white fluorescent tubes (Sylvania F48T12/CW/VHO) at an average photosynthetic photon flux density (PPFD) of $350 \mu\text{mol s}^{-1} \text{m}^{-2}$. Measurements were made at plant level with a LI-1935B spherical quantum sensor (Li-Cor Inc., Lincoln, NE). All stock plant cultures and experiments to be described were maintained under this light and temperature regime.

Establishment of Aquatic Plant Stocks In Vitro. All species were established *in vitro* from the aforementioned aerial stock plants by first surface sterilizing defoliated stem segments (consisting of two to three nodes) in aqueous 1.05% (v/v) NaOCl containing 0.01% (v/v) Tween-20 for 12 min, followed by three 5-min rinses in sterile deionized water. Stem segments were then transferred into 500 ml aluminum foil capped Erlenmeyer flasks containing 250 ml sterile liquid basal medium. This basal medium (BM) consisted of half-strength Murashige and Skoog mineral salts (Murashige and Skoog 1962) supplemented with 0.28 mM myo-inositol, 0.6 μM thiamine-HCL, and 87.6 mM sucrose. The medium was adjusted to pH 5.7 with 0.1 N NaOH before autoclaving at 1.2 kg cm^{-2} for 20 min at 121 C.

Clonal stocks of the aquatic from of each species were increased by further propagating the lateral branches which developed from the initial stem explants after three to four weeks culture in liquid BM. Flasks were not shaken. Stocks were routinely subcultured at three week intervals. All transfers were performed in a laminar air flow transfer hood.

Experimental Design. The effects of exogenous ABA on induction of aerial-type leaf development were tested under non-inductive conditions (i.e., while shoots were submerged). Apical shoot cuttings (1.0 cm long), obtained from *in vitro* aquatic plant stocks, were transferred into 100 ml sterile liquid BM contained in 250 ml Erlenmeyer flasks that were loosely capped with aluminum foil. All treatments were performed in duplicate, with each treatment flask containing three shoots. Synthetic ABA (90% mixed isomers, Sigma Chemical Co., St. Louis, Mo.) was prepared as a concentrated aqueous stock solution and sterilized by Millipore filtration (pore size: $0.22 \mu\text{m}$) before being added to sterile liquid BM. For comparative purposes, aerial leaf development in each species (aerial controls) was induced by rooting shoot cuttings in 250 mL Erlenmeyer flasks containing 100 mL BM solidified with 1.5% (w/v) TC Agar™ (Hazelton Research Products, Inc., Lenexa, KS). Cuttings of all species developed numerous roots by day seven.

Experiments were 20 days in duration. Leaf and adaxial epidermal cell dimensions and stomatal density (stomata/mm²) were used to quantify treatment effects on leaf development. For leaf measurements, a single leaf was

TABLE 1. COLLECTION DATA FOR *MYRIOPHYLLUM* AND *PROSERPINACA* SPECIES USED IN THIS STUDY.

Species	Locality
<i>Myriophyllum humile</i> (Raf.) Morong.	Unnamed pond, intersection Shermantown and Pendar Rds. No. Kingstown, R.I.
<i>Myriophyllum spicatum</i> L.	Olney Pond, Lincoln, R.I.
<i>Myriophyllum heterophyllum</i> Michx.	Empoundment pond of Moscow Brook, Moscow, R.I.
<i>Myriophyllum pinnatum</i> (Walt.) BSP.	Rocky Brook Reservoir, Peacedale, R.I.
<i>Myriophyllum verticillatum</i> L.	Hiland Lake, Washentaw and Livingston Co., Minn.
<i>Myriophyllum aquaticum</i> (Vell.) Verde.	Piersol Lake, Shelby Co., Tenn.
<i>Myriophyllum tenellum</i> Bigel.	Larkin Pond, So. Kingstown, R.I.
<i>Proserpinaca palustris</i> L.	Small marsh intersection of Lafayette and Audubon Rds. No. Kingstown, R.I.
<i>Proserpinaca intermedia</i> Mack.	Ephemeral pond on access road to the Great Swamp Management Area, W. Kingston, R.I.

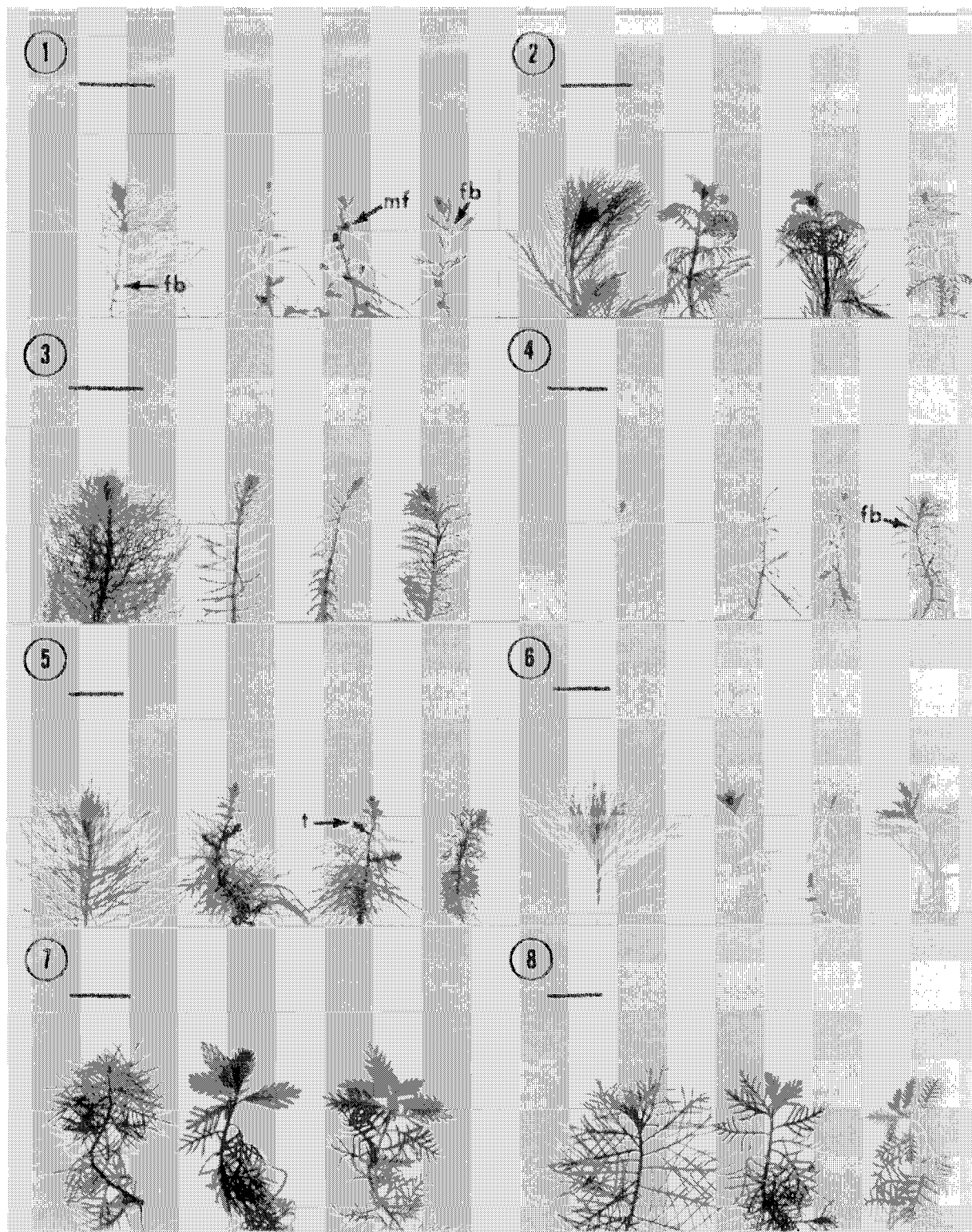


Figure 1-8. Effects of ABA on induction of aerial leaf morphologies on submerged shoots of *Myriophyllum* and *Proserpinaca* species cultured for 20 days. Fig. 1-6. Treatments left to right: submerged control; submerged plus 1.0 μM ABA; submerged plus 10 μM ABA; aerial control. Fig. 7, 8. Treatments left to right: submerged control; submerged plus 1.0 μM ; aerial control. Fig. 1. *Myriophyllum humile*; note presence of floral buds (fb) on submerged and aerial controls. Exposure to ABA enhanced fruit maturation (mf) on submerged shoots. Fig. 2. *Myriophyllum spicatum*. Fig. 3. *Myriophyllum heterophyllum*. Fig. 4. *Myriophyllum pinnatum* with flowering aerial shoots. Fig. 5. *Myriophyllum verticillatum*; note development of turions (t) in presence of 10 μM ABA. Fig. 6. *Myriophyllum aquaticum*. Fig. 7. *Proserpinaca palustris*. Fig. 8. *Proserpinaca intermedia* Scale bars = 10 mm.

removed from each replicate shoot ($n = 6$) at a node about 5.0 mm below the apex. In this position, the leaves were fully expanded, uniform in shape, and represented those leaves which were formed during the experimental period. Leaves were kept moist under glass coverslips on microscope slides. Using a dissecting microscope, total leaf lengths were determined to within 0.5 mm. The adaxial stomatal density for each replicated leaf was calculated by counting the number of stomata occurring in 15 successive microscopic fields of known area along the leaf midrib as recommended by Metcalfe and Chalk (1979). Counting was initiated at the point of insertion of the second subdivision (pinna) from the leaf base and progressed toward the distal end of the leaf. Dimensions of five adaxial epidermal cells in a median longitudinal row above the midrib were also measured with a microscope equipped with a calibrated ocular micrometer. Epidermal cells adjacent to stomatal complexes were not measured.

Scanning Electron Microscopy. Leaf samples to be examined by scanning electron microscopy (SEM) were fixed in formalin-acetic-alcohol (FAA) for 24 hr. Samples were then rinsed in two changes of 50% ethanol, further dehydrated through a graded ethanol series (70 to 100%), critical point dried, mounted on metal stubs, and then sputter coated with gold/palladium. Leaves were then examined with a Cambridge Stereoscan S-4 microscope at 20 kV.

RESULTS

Induction of aerial-type leaf morphologies. Submersed shoots of all *Proserpinaca* and *Myriophyllum* species except, *Myriophyllum tenellum*, produced yellow-green pectinate aquatic leaves bearing filiform pinnae when cultured *in vitro* in liquid BM. All aquatic leaves produced were surrounded by a highly chloroplastic epidermal layer. Leaf venation consisted of a single midrib vein branching into each pinna. *Myriophyllum* species (except *M. tenellum*) cultured on agar-solidified BM produced smaller and thicker dark green cutinized pinnatifid aerial leaves bearing numerous abaxial and adaxial stomata. These stomata were in a cutinized epidermal cell layer with chloroplasts confined to the guard cells. Vasculature in both aerial and aquatic leaves in all *Myriophyllum* species was the same. In contrast, both *Proserpinaca* species developed dark-green cutinized lanceolate serrate leaves with reticulate venation on aerial shoots in culture.

ABA-induced aerial leaf characteristics. Exposure to ABA induced the development of aerial-type leaf characteristics on submerged shoots in all species (Fig. 1-8). In all *Myriophyllum* species (except *M. tenellum*), exposure to ABA at concentrations of 0.1 μM and greater promoted development of relatively smaller and thicker dark-green cutinized pinnatifid leaves morphologically similar to those produced on aerial controls. However, these species exhibited differential sensitivities of cell division and elongation to 0.1 μM ABA. In *Myriophyllum humile*, *M. spicatum*, and *M. heterophyllum*, reductions in leaf length (Fig. 9) at 0.1 μM ABA could be attributed to reductions in cell division (i.e., reduced cell number) because no effect was observed on epidermal cell length. In *Myriophyllum verticillatum* and

M. aquaticum, reductions in leaf length correlated with reductions in epidermal cell length. Reductions in pinna length closely paralleled reductions in total leaf length in all species (data not shown). Mean epidermal cell width was not influenced by ABA treatment in any of the *Myriophyllum* species examined (data not shown). However, in the presence of ABA, the lateral epidermal cell walls of all *Myriophyllum* species except *M. spicatum* and *M. heterophyllum* were slightly more sinuous relative to the rectilinear epidermal cells of leaves on submerged control shoots (see Fig. 17-19).

Submersed shoots of *Proserpinaca palustris* and *P. intermedia* in the presence of 1.0 μM ABA (Fig. 7, 8) developed cutinized lance-serrate leaves with expanded lamina and reticulate venation that were identical to leaves produced on aerial plants. Abscisic acid treatment, however, did significantly reduce total leaf and epidermal cell length in both *Proserpinaca* species (Fig. 9).

ABA-Induced Stomatal Development. Exogenous ABA atypically induced differentiation of stomata on developing leaves of submerged shoots in *Myriophyllum humile*, *M. heterophyllum*, *M. spicatum*, and *M. verticillatum*, and further promoted stomatal development in *M. aquaticum*, *Proserpinaca palustris*, and *P. intermedia* (Fig. 10). typical effect of ABA treatment on induction of stomatal differentiation is shown for *M. spicatum* in Fig. 11-13.

In *Myriophyllum tenellum*, undivided scale-like leaves (Fig. 14) distinguish this species from all other *Myriophyllum* species in North America (Aiken 1981). Although not heterophyllic in terms of leaf shape, ABA exposure also induced development of stomatous leaves on submerged shoots of this species (Fig. 15). Aerial plants cultured on agar-solidified BM produced leaves with widely scattered stomata (Fig. 16). Due to the slow growth rate of *M. tenellum in vitro*, only the presence or absence of stomata was recorded after 45 days of growth.

Submersed shoots of *Myriophyllum aquaticum* produced leaves with widely scattered stomata in the absence of ABA (Fig. 10-17) under standard light conditions (350 $\mu\text{mol s}^{-1} \text{m}^{-2}$). However, plants cultured under lower light intensities (50 to 75 $\mu\text{mol s}^{-1} \text{m}^{-2}$) produced astomatous leaves. Stomata which did develop on submerged shoots of *M. aquaticum* in the absence or presence of ABA and on aerial shoots *in vitro* were occluded by the cuticular protuberances of their respective guard cells (Fig. 17-19). Comparison of scanning electron micrographs of the epidermis (Fig. 19) of leaves produced on aerial plants cultured *in vitro* with those of greenhouse-grown plants (Fig. 20) revealed the development of bulbous epidermal cells over-arching unoccluded sunken stomata and substantially more wax deposition in the latter.

Identical to leaves produced on aerial shoots, aerial-type leaves produced on submerged shoots in all species in the presence of 1.0 or 10 μM ABA developed epidermal layers in which chloroplasts were restricted to the guard cells of the stomata.

Species Specific Responses. Similar to field plants (Aiken 1981), *Myriophyllum humile* produced numerous flower buds on both submerged and aerial shoots in culture (Fig. 1). Although buds were observed to open only on aerial shoots, fruit development occurred on both submerged

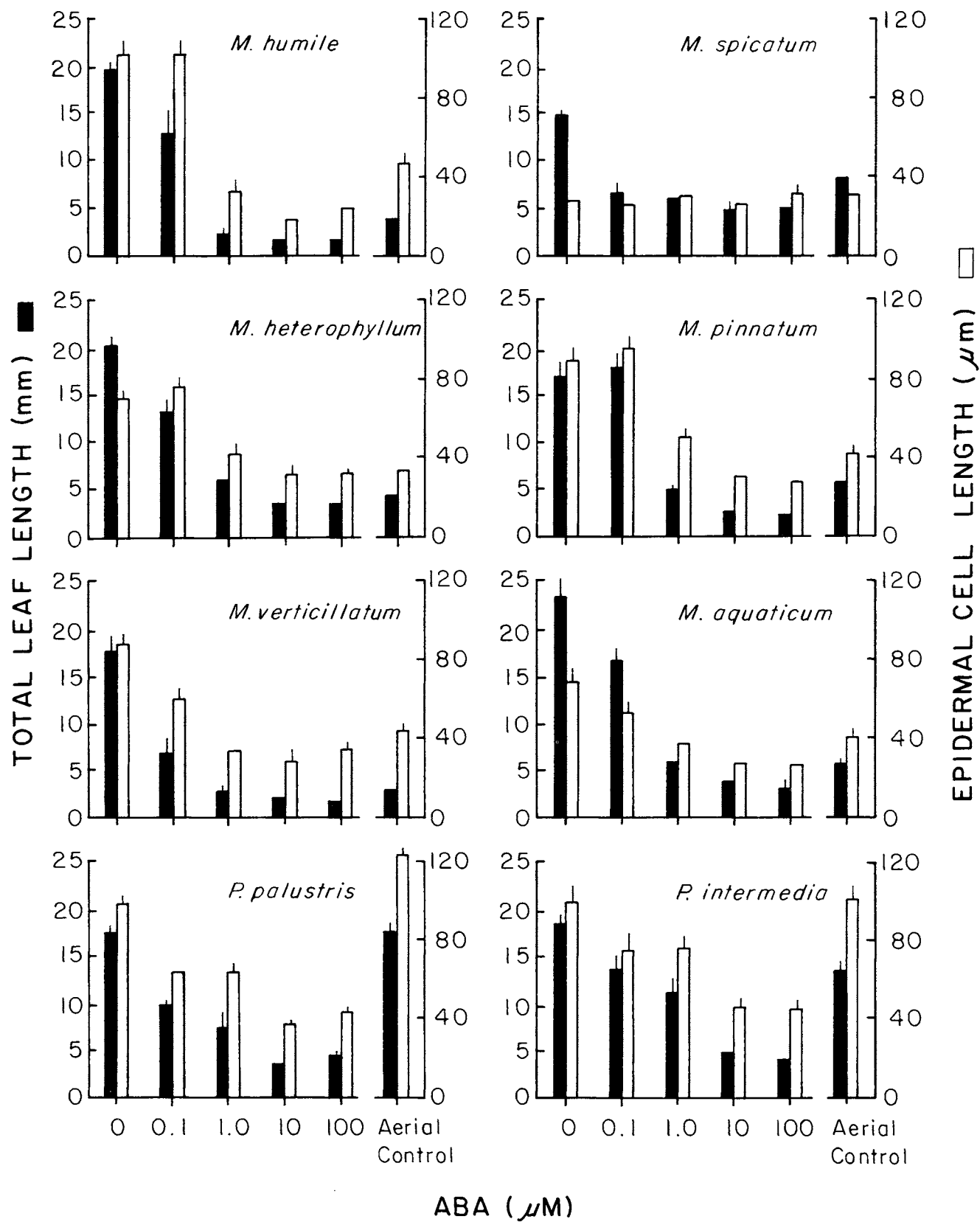


Figure 9. Effects of ABA on leaf and adaxial epidermal cell length in *Myriophyllum* and *Proserpinaca* species after 20 days. Leaf length values represent means \pm SE. Epidermal cell length values represent means \pm SE.

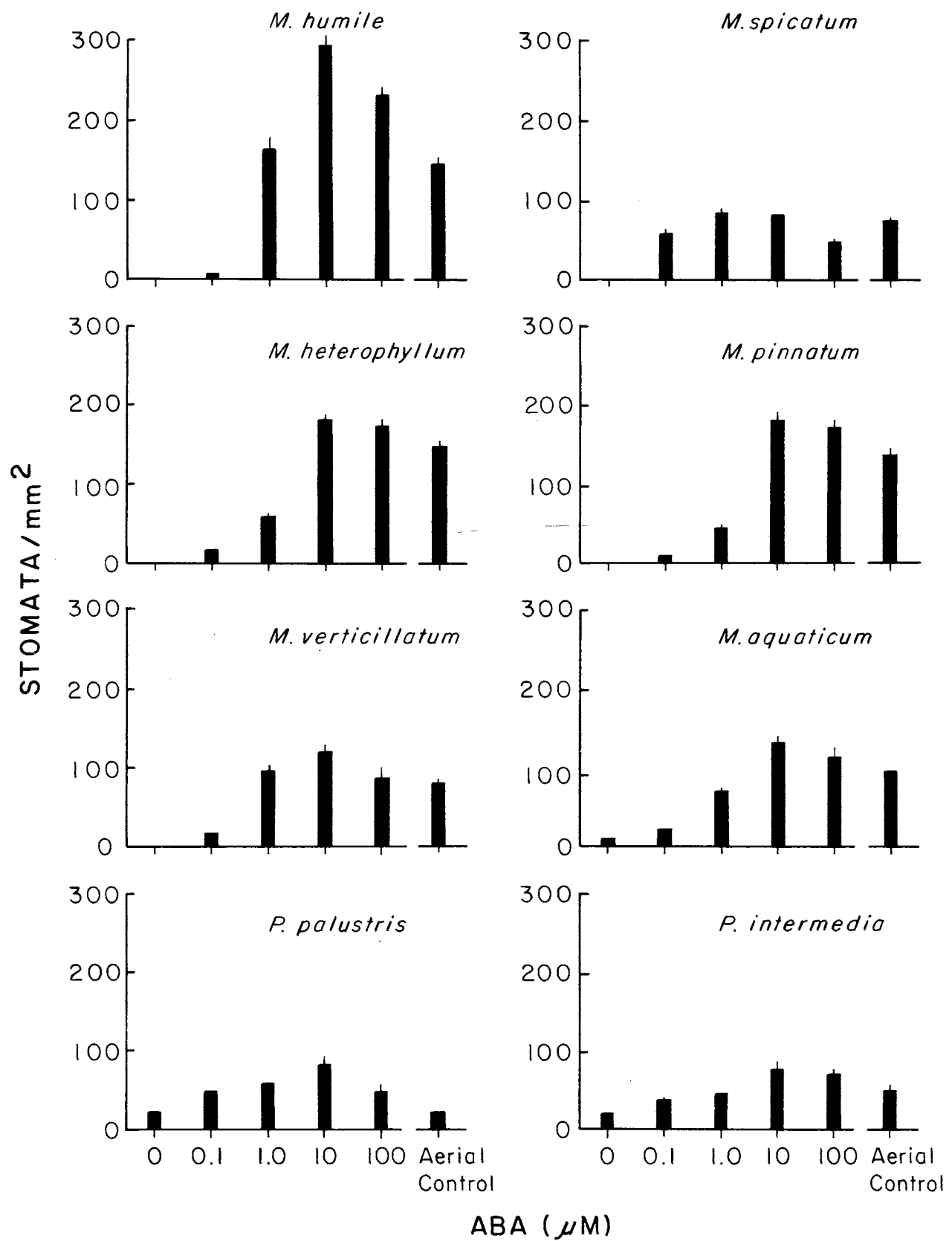


Figure 10. Effects on ABA on adaxial stomatal development in *Myriophyllum* and *Proserpinaca* species after 20 days. Stomatal density values represent means \pm SE.

and aerial plants. Flower bud development on submerged shoots was inhibited by ABA. Exposure to 0.1 or 1.0 μM ABA inhibited flower bud formation by 65.4% and 96.7%, respectively. Although plants exposed to 10 or 100 μM ABA did not flower, exogenous ABA stimulated the maturation of fruits present prior to treatment (Fig. 1). Aerial shoots of *Myriophyllum pinnatum*, *Proserpinaca palustris*, and *P. intermedia* also flowered in culture, however, flowering was never observed on submerged shoots.

Consistent with previous reports (Weber and Nooden 1976), submerged shoots of *Myriophyllum verticillatum* produced turions (dormant buds) in the presence of 10 or 100 μM ABA (Fig. 5).

DISCUSSION

In all species of *Myriophyllum* and *Proserpinaca* studied, representing approximately 60% of the known species occurring in North America (Aiken 1981), exogenous ABA induced the development of aerial leaf characteristics on submerged shoots. These anatomical and morphological characteristics included: 1) development of stomata, 2) reductions in leaf and epidermal cell lengths (except *Proserpinaca* spp.), 3) promotion of laminar expansion with reticulate venation (*Proserpinaca* spp.), 4) redistribution in epidermal chloroplast abundance, and 5) apparent increased cuticularization. Results of earlier studies indicate that the induction of aerial-type leaf development on submerged shoots, in response to ABA treatment, is not limited to heterophyllic amphibious monocotyledonous species (Anderson 1978) but also occurs in dicot (Deschamps and Cooke 1983) and aquatic fern (Liu 1984) species. The present study further demonstrates that the promotive effect of exogenous ABA on aerial leaf development is a response common among the heterophyllic members of the Haloragaceae.

In most amphibious species, aerial leaf development is generally initiated following emergence of the shoot apex above the water surface (Sculthorpe 1967). Apparently the change in water relations experienced by the emerging shoot apex plays an important role in aerial leaf form determination. In both *Myriophyllum heterophyllum* (Kane 1984) and *Callitriche heterophylla* Pursh (Deschamps and Cooke 1983), induction of aerial leaf development has been correlated with reductions in cell turgor pressure during water stress. In many terrestrial species, water stress, specifically loss of cell turgor (Pierce and Raschke 1981), modulates ABA content through regulation of biosynthesis and metabolism (Davies and Mansfield 1978; Zeevaart and Creelman 1988). Although only limited information is available, water stress also promotes the accumulation of ABA in at least some amphibious angiosperms (Milborrow and Robinson 1973; Weiler 1979). Goliber (1988; 1989) has recently reported correlated changes in endogenous free ABA content with dimorphic leaf development in the heterophyllic aquatic *Hippuris vulgaris* L., a species in which aerial-type leaf development is also induced by exogenous ABA (Kane and Albert 1987a). Consequently, given the responses to exogenous ABA exhibited by the *Myriophyllum* and *Proserpinaca* species examined in the present study, it is plausible that heterophyllic development in these species

is regulated, in part, by endogenous ABA through increased biosynthesis and subsequent accumulation at sites of action during emergence-induced water stress.

Other physiological factors besides changes in total ABA content may be important in the regulation of heterophyllic development. Analysis of data reported by Weiler (1979) shows that submerged shoots of *Myriophyllum aquaticum* (previously *M. brasiliense*) producing submerged leaves contain a relatively higher level of ABA than aerial shoots. As noted in the present study, submerged shoots of this species developed aerial-type leaves in response to exogenous ABA. It seems possible that developmental control may also involve the spatial separation of endogenous ABA from sites of action (receptors), possibly through chloroplastic compartmentation (Loveys 1977).

Furthermore, the abrupt switch to development of the elongate submerged dissected leaf form occurring in species of *Myriophyllum* and *Proserpinaca* following submergence may not be simply the result of a reduction in ABA synthesis (i.e., alleviation of emergence-induced water stress), but may also be indirectly regulated by ethylene-mediated changes in ABA turnover and by ethylene directly. Ethylene synthesis and accumulation occurs following submergence (Metraux and Kende 1983) and significantly promotes elongation growth in leaf tissues of many amphibious species (Cookson and Osborne 1979; Samarakoon and Horton 1983). Work by Zeevaart (1983) indicates that ethylene, or promoters of ethylene synthesis such as carbon dioxide (Kao and Yang 1982), enhance ABA degradation in certain terrestrial species. A similar ethylene effect on ABA metabolism in amphibious species would explain Bristow's (1968) report of the formation of submerged-type leaves on aerial shoots of *Myriophyllum aquaticum* and other amphibious species when grown in a carbon dioxide enriched atmosphere. It, therefore, may be necessary to examine the effects of any environmental factor on heterophyllic development based upon the relative effects on both endogenous ethylene and ABA content. This approach may clarify the physiological basis for the apparent similarities in effect of diverse environmental stimuli on heterophylly in different aquatic species (Deschamps and Cooke 1984; 1985).

Regulation of leaf development in *Proserpinaca* spp. appears to be more complex than in *Myriophyllum* since interactions between ABA and gibberellins (GAs) are also involved. Unlike the *Myriophyllum* species studied, aerial plants of *Proserpinaca* spp. display heterophyllous development in response to changes in photoperiod. Under long-day photoperiods (≥ 14 hr), aerial shoots produce lanceolate leaves with expanded lamina, while short-days (≤ 14 hr) induced development of small, thickened dissected leaves (Wallenstein and Albert 1963; Davis 1967). The photoperiodic effect on leaf development on aerial shoots may be mediated through quantitative and/or qualitative changes in endogenous GA content, since exogenous GA₃ alone will induce development of lanceolate serrate leaves under noninductive short days (Wallenstein and Albert 1963; Davis 1967; Kane and Albert 1982). However, when submerged, shoots develop large and elongate dissected aquatic leaves regardless of photoperiod. Under submerged conditions, GA treatment induces formation of

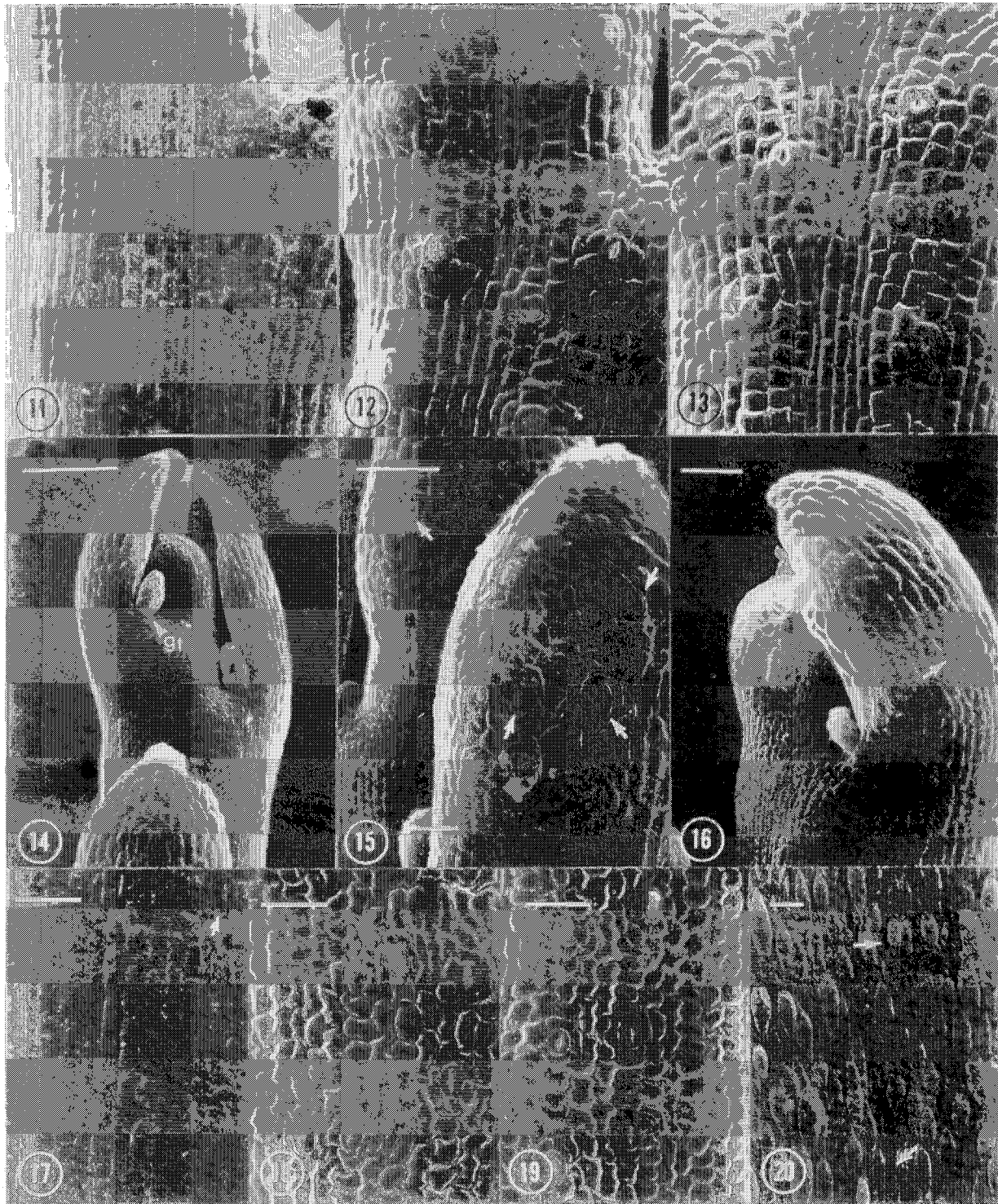


Figure 11-20. Scanning electron micrographs depicting ABA induction of aerial leaf characteristics on submerged shoots in *Myriophyllum* species. Fig. 11. Astomatous adaxial surface of a submerged leaf of *Myriophyllum spicatum*. Fig. 12. Stomatal differentiation on developing leaves on submerged shoot of *Myriophyllum spicatum* in the presence of 1.0 μ M ABA. Fig. 13. Adaxial stomatous leaf surface on aerial shoot of *Myriophyllum spicatum*. Fig. 14. Submerged shoot apex of *Myriophyllum tenellum* bearing astomatous scale-like leaves with glandular trichomes (gt). Fig. 15. Stomatal differentiation (arrows) on leaves produced on submerged shoots of *Myriophyllum tenellum* in the presence of 10 μ M ABA. Fig. 16. Scattered stomata (arrow) on aerial leaves of *Myriophyllum tenellum*. Fig. 17. Scattered occluded adaxial stomata (arrow) on submerged leaves of *Myriophyllum aquaticum*. Fig. 18. Occluded adaxial stomata and sinuous epidermal cells on leaves produced on submerged shoots of *Myriophyllum aquaticum* in the presence of 1.0 μ M ABA. Fig. 19. Adaxial surface of an aerial leaf of *Myriophyllum aquaticum* produced in culture. Fig. 20. Sunken stomata (arrows) and cuticularization of the adaxial surface of an aerial leaf of *Myriophyllum aquaticum* produced on greenhouse-grown shoots. Scale bars = 50 μ M.

very large leaves with extremely elongated filiform dissections (Kane, unpublished). The present finding that ABA treatment induced lanceolate serrate leaf formation on submerged shoots in both *Proserpinaca palustris* and *P. intermedia* suggests that a two hormone system involving both GA and ABA plays a role in the regulation of heterophyllic development in *Proserpinaca*. We have provided further evidence for the presence of this dual hormone system elsewhere (Kane and Albert 1987b).

Given the terrestrial origin of present day aquatic angiosperms, Arber (1920) has reasoned that heterophylly is a prerequisite for the ability of a terrestrial plant to inhabit an aquatic environment. Although Cook (1968) has similarly argued that foliar plasticity is important, he has stressed that it is the *mechanism* of plasticity that ultimately confers what evolutionary success these plants have in exploiting the submerged, water-air interface and terrestrial environments. It is becoming apparent that taxonomically diverse heterophyllic species, particularly those exhibiting leaf dimorphism upon submergence or emergence, have probably evolved similar mechanisms in which ABA plays a central role in regulating growth and development. Exemplified by the work of Anderson et al. (1986), further elucidation of the physiological mechanism(s) involved may provide the basis for development of more effective control practices for problematic aquatic species.

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Karyotypes of Hydrilla (Hydrocharitaceae) Populations in the United States

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ABSTRACT

Standardized chromosome lengths and arm ratios of hydrilla collected from eleven geographically distinct populations in the United States were not significantly different. The karyotype of these populations which consisted of 5 acrocentric, 2 submetacentric and 1 metacentric chromosomes that ranged in total length from 1.69-5.54 μm was similar to previously published karyotypes. The triploid number of 24 chromosomes was observed in populations where only diploids had previously been reported and endopolyploidy is suggested.

Key words: Chromosomes, genetics, diploid, triploid, endopolyploidy, monoecious, dioecious.

INTRODUCTION

Polymorphism and genetic variation of hydrilla [*Hydrilla verticillata* (L.f.) Royle] have been discussed in reviews by Pieterse (1981) and Cook and Lüönd (1982). According to Cook and Lüönd (1982):

"Some races of *H. verticillata* seem to be dioecious, others monoecious. In climatically 'tropical' regions the plants are usually monoecious with male and female flowers at separate whorls, often on separate branches, in temperate regions the plants appear to be dioecious. However, in culti-

vation plants may develop one sex for one or two seasons and then revert to the other sex a season later. This observation sheds some doubt on the truly dioecious nature of some races, however, European material has been observed in the wild and in cultivation since ca. 1830 and it has never yet developed male flowers."

In the subtropical climate of Florida, where hydrilla has been studied for 30 years, staminate flowers have never been observed, and it was believed that only female hydrilla existed in the United States. However, staminate flower production by hydrilla that had been collected from Washington, D.C. (Vandiver et al. 1982), and Delaware (Steward, 1983, personal communication) was reported under experimental conditions in Florida. Likewise, in the summer of 1983, profuse staminate flower production was observed in monoecious hydrilla populations in North Carolina lakes (Langeland and Schiller, 1983).

Prolific vegetative reproduction, aided by man's transportation of vegetative material, has allowed rapid spread of hydrilla. However, this means of dispersal is most effective over short distances under natural conditions. The occurrence of monoecious hydrilla in the United States suggests the potential for viable seed production which is a natural mechanism for long distance dispersal. Conant et al. (1984), have observed seed production and germination under experimental conditions by hydrilla collected from Delaware and North Carolina. Hydrilla was observed to produce viable seed in North Carolina lakes (Langeland and Smith, 1984) but the viability of seeds and seedlings was low (Langeland and Smith, 1988). This low viability may be a result of defective sex cell formation because Harlan et al. (1984) have reported that hydrilla populations in North Carolina are triploid ($2n = 3x = 24$).

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