

Absorption of nutrient exudates from terrapin eggs by roots of *Ammophila breviligulata* (Gramineae)

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Ammophila breviligulata Fern. is a dominant grass in the nutrient-poor soil of sand dunes along the east coast of North America. More than half its range overlaps that of *Malaclemys terrapin* Schoepff., a brackish water turtle nesting in sand dunes. Roots of *A. breviligulata* often proliferate around buried terrapin nests. Gamma spectrometry was used to determine whether nutrients are translocated from terrapin eggs into *A. breviligulata*. Eggs, collected in the field within 48 h of being laid, were injected with ⁷⁵Se, ¹³⁷Cs, ⁵⁴Mn, and ⁵⁹Fe. Labelled eggs were covered with dune soil in pots containing *A. breviligulata*. Plants were then monitored over 15 weeks for the appearance of isotopes. Within 45 days, all isotopes except ⁵⁹Fe were found in aerial shoots, more than 30 cm from buried eggs. Iron was absorbed but remained in the roots. The other three isotopes steadily accumulated in shoots for 90 days, after which they began to be translocated to underground tissues. Thus, *A. breviligulata* absorbs nutrients exuding from buried terrapin eggs, utilizing this novel nutrient supply in mineral-deficient soils of barrier dunes.

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L'*Ammophila breviligulata* Fern. est une variété de graminée dominante sur le pauvre sol nourricier que constituent les dunes de sable de la côte Est de l'Amérique du Nord. Plus de la moitié du domaine sur lequel elle s'étend coïncide avec le territoire de pont de la *Malaclemys terrapin* Schoepff., tortue d'eau saumâtre. Les racines de l'*A. breviligulata* prolifèrent à proximité des nids de tortue enfouis dans le sable. Les auteurs ont utilisé la spectrométrie gamma pour déterminer s'il y avait déplacement des éléments nutritifs des oeufs de tortue dans l'*A. breviligulata*. On a injecté dans ces oeufs, ramassés sur le terrain dans le 48 h suivant la ponte, une solution composée de ⁴⁵Se, de ¹³⁷Cs, de ⁵⁴Mn et de ⁵⁹Fe. Une fois marqués, les oeufs ont été enfouis sous le sable, dans des pots contenant des *A. breviligulata*. On a ensuite suivi les plantes sur une période de 15 semaines, pour y détecter l'apparition d'isotopes. Dans l'espace de 45 jours, ils ont tous été retrouvés, à l'exception du ⁵⁹Fe, dans les tiges aériennes, à plus de 30 cm de distance des oeufs. Le fer avait été absorbé, mais demeurait dans les racines. Les trois autres isotopes ont continué de s'accumuler régulièrement pendant 90 jours, après quoi ils ont commencé à se déplacer dans les parties souterraines de la plante. Il s'avère donc que l'*A. breviligulata* absorbe les éléments nutritifs contenus dans les oeufs de tortue, exploitant ainsi une source nourricière providentielle venant pallier à la pauvreté des dunes en minéraux.

Introduction

Research on roots continues to broaden our knowledge of their capabilities, as originally characterized by laboratory studies. Excavations show that individual root systems are usually composed of axes whose structure and geotropic responses differ widely (Kutschera 1960). Recent analyses (Vermeer and McCully 1982; McCully and Canny 1985) document surface features of field-grown roots that may greatly contribute to plant nutrition by regulating rhizosphere interactions, including microbial growth and nutrient transport (Curl and Truelove 1986). Field conditions appear to prompt the expression of heretofore unrecognized root structure and capabilities, increasing the likelihood of discovering new traits in plants growing in specialized habitats.

The present study was inspired by a plant–animal interaction found in barrier sand dunes along the eastern coast of North America (Lazell and Auger 1981). Roots of the dominant grass, *Ammophila breviligulata*, concentrate around eggs buried in dunes by the brackish water turtle, *Malaclemys terrapin* (Diamondback Terrapin). Affected turtles die either during

development or as they try to emerge through a confining mass of roots (Fig. 1). Based on massive root proliferation around eggs as compared with the much smaller root volume found in normal dune sand, Lazell and Auger (1981) speculated that *A. breviligulata* draws nutrients from terrapin eggs. However, no evidence was provided either for root penetration into eggs or for nutrient transfer from eggs into *A. breviligulata*. Subsequent study of dozens of root-infested eggs showed that rather than penetrating terrapin eggs, roots usually remain outside the shell (Stegmann 1985).

We test the hypothesis that *A. breviligulata* roots can absorb nutrients exuding from terrapin eggs. The substances must migrate out of an egg membrane – shell complex before being absorbed by roots.

Limited availability of terrapin eggs dictated that we use a nondestructive means of sampling *A. breviligulata* for uptake of nutrients from eggs. Gamma spectrometry was used because it allows continuous quantification of minerals reaching a target organ. The same plant part can be repeatedly monitored for several isotopes simultaneously, since tissue need not be solubilized for sampling. In addition, gamma spectrometry allowed us to use the small volumes of mineral source solution needed to fit into terrapin eggs.

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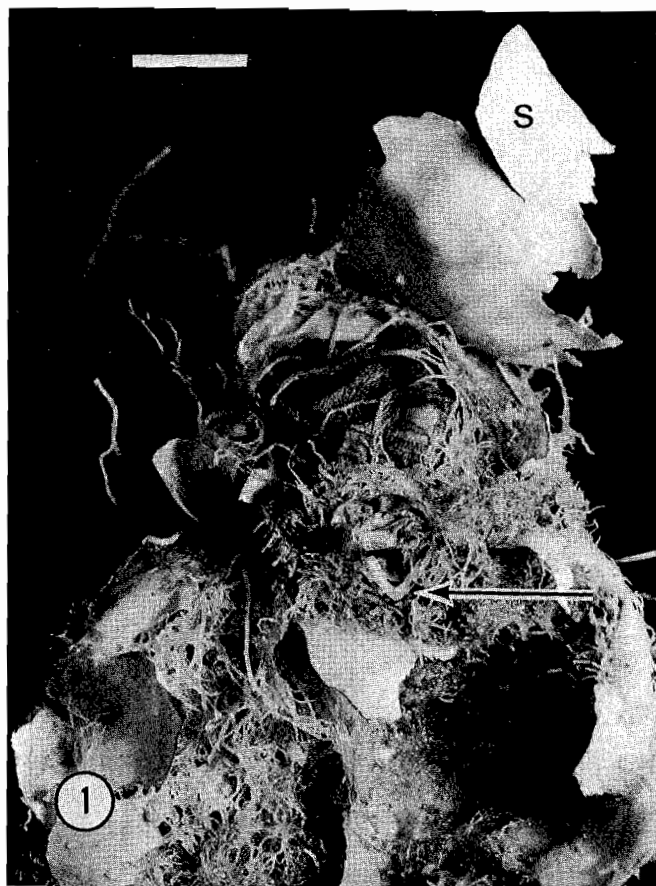


FIG. 1. Portion of terrapin nest encased in *A. breviligulata* roots. The lower jaw (arrow) persists in this individual that became entangled during hatching. S, egg shell fragment. Bar = 1 cm.

Materials and methods

Plants of *A. breviligulata* were collected in June 1984 from coastal sand dunes in Gloucester, MA, about 30 miles (48 km) north of Boston. Specimens of similar size were chosen at the start of the experiment. Individuals were maintained in 16-cm pots, filled with native sand, and illuminated 16 h per day with a mixture of fluorescent and incandescent light at 3700 lx ($25 \pm 2^\circ\text{C}$). Before isotope uptake experiments, the endogenous pool of nutrients was depleted by watering plants with deionized H_2O for 1 week.

Plants were treated in three ways. Three individuals were presented with isotopes injected into terrapin eggs, as described below. Two others were supplied with the same isotopes, delivered through the watering solution, to confirm that *A. breviligulata* would take up those nutrients if they were available in the soil solution. Finally, three more individuals were supplied only with deionized H_2O . These specimens were to check for cross-contamination from isotopes delivered to other plants in nearby pots.

Four gamma-emitting radionuclides, ^{137}Cs , ^{75}Se , ^{54}Mn , and ^{59}Fe , were used to assess whether mineral nutrients can be translocated from turtle eggs to roots. ^{137}Cs resembles K in its uptake and transport within plants (Witherspoon 1964) and therefore serves as a K analog. ^{75}Se acts as an analog of S (Bollard 1983), while ^{54}Mn and ^{59}Fe are themselves micronutrients (Clarkson and Hanson 1980).

To present plants with egg-enclosed tracers, 48-h old terrapin eggs were collected from the field (Stegmann 1985) and injected with 1 μCi (1 Ci = 37 GBq) of each of the four isotopes. Cesium was administered as $^{137}\text{CsCl}$, selenium as $\text{H}_2^{75}\text{SeO}_3$, manganese as $^{54}\text{MnCl}_2$, and iron as $^{59}\text{FeCl}_2$. The hole created by the hypodermic needle was sealed with molten TackiwaxTM and that seal was secured in turn by an epoxy adhesive. To guard further against leakage over the 3.5 month

presentation period, eggs were oriented with sealed holes facing upward. These precautions assured that isotopes detected in *A. breviligulata* had reached plants by diffusing across egg shells and dune soil to reach the roots.

One labelled egg, containing four isotopes, was presented to each of three plants in separate 16-cm pots. For the two plants watered directly with tracers, 1 μCi of each isotope was added to 150 mL of deionized H_2O , which was then used to water each plant.

Pots with tracer-fed plants were placed in trays along with control plants watered only with deionized H_2O . Trays prevented leakage of isotopes into the growth room, and the control plants were used to check for artifacts due to isotope migration between plants of different pots. All plants were watered every 3 days with 150 mL of deionized H_2O and were maintained indoors under artificial light for 15 weeks.

Gamma spectrometry

Gamma emission was monitored using a scintillation counter based on a NaI crystal detector, and supported by a photomultiplier, a 1024 channel pulse height analyzer, and associated equipment. Emission from all four isotopes was quantified simultaneously, since the pulse height analyzer plots the number of gamma photons at characteristic energy levels over the range of gamma radiation. The emission peak for ^{137}Cs is 0.66 MeV, those for ^{75}Se 0.26, ^{54}Mn 0.83, and ^{59}Fe 1.10 MeV. The scintillation crystal was surrounded by a shield of 5 cm thick lead bricks, with an aperture across from the crystal. Counts were first taken with the plant placed in front of the aperture. A background count was then obtained with the plant removed from the aperture. The pulse height analyzer subtracted background from plant-generated counts.

Counts were made on the three eggs injected with the four isotopes before those eggs were buried in pots containing *A. breviligulata*. Plants were then monitored for radioactivity over 15 weeks. During that time, counts were made on aerial shoots approximately 35 cm above soil level, using an aperture 10 cm wide. After the 15-week growth period, plants were uprooted and separated into roots, below-ground shoots, and aerial shoots. Each was dried and measured for radioactivity by two scintillation crystals simultaneously. This method produced a 10-fold increase in resolving power. Thus, two types of results were collected: data indicating uptake of the four isotopes over time were plotted (Fig. 2) after accounting for decay constants (half-life of ^{137}Cs , 30 years; of ^{75}Se , 127 days of ^{54}Mn , 291 days; and of ^{59}Fe , 45 days) and the final distribution of isotopes within plants by the end of the growth period was determined (Fig. 3).

Isotope uptake over time (Fig. 2) and their final distribution in *A. breviligulata* (Fig. 3) are expressed as percentages, but of different totals. The former are calculated over the total counts supplied to eggs at the onset of the experiment, while the latter are computed over the total counts recovered in plant, soil, and egg at the end of the 15-week growth period. Differences in final isotope distribution were statistically analyzed with a *t*-test, after homogeneity of variances had been established with a Bartlett's test (Sokal and Rohlf 1981). Means were compared by Tukey's significant-difference method with significance being assumed at $P < 0.05$.

Results

Although plants were similar in size at the start of the experiment, individuals supplied with turtle eggs became noticeably larger by the end of the 15-week growth period. Control plants, devoid of this potential nutrient source, averaged only 57% of the dry weight achieved by plants supplied with an egg. In addition, roots of egg-supplied plants had proliferated around the egg. Branching frequency of roots had increased, forming a mass of fine laterals near the egg. Similar proliferation occurs around terrapin eggs in nature (Fig. 1; Stegmann 1985).

Of the four isotopes enclosed within eggs and presented to *A. breviligulata*, all but ^{59}Fe were detected in shoots (Fig. 2), more than 30 cm distant from the isotope source. Even after

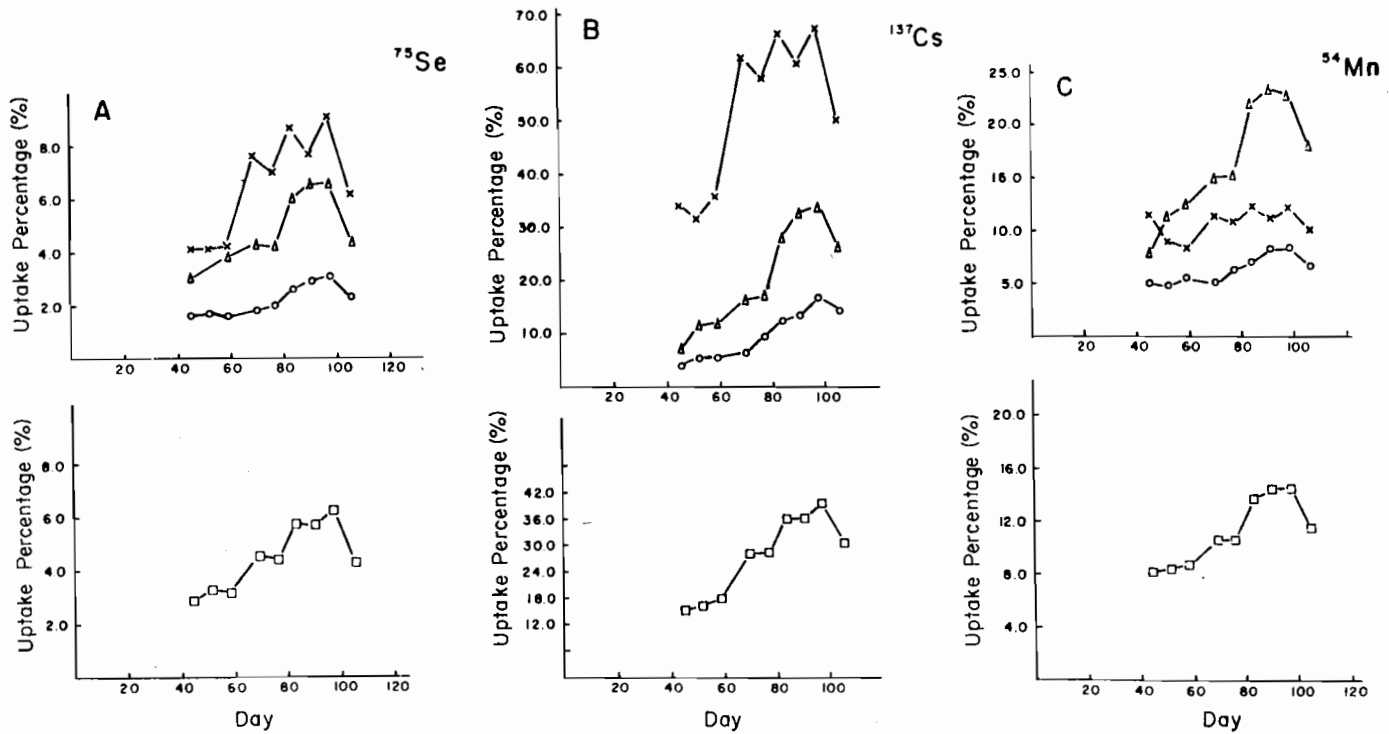


FIG. 2. Isotope uptake (percent) into aerial shoots of *A. breviligulata*, calculated from ratio of counts at day of measurement over counts initially injected into terrapin egg. Top graph of each pair shows uptake by individual plants (\times , Δ , and \circ). Bottom figure indicates average uptake.

15 weeks, ^{59}Fe was only found in root tissue. The other, more mobile isotopes differed in time required to appear in aerial shoots, rate of incorporation, and final concentration within tissue (Figs. 2 and 3).

Replicate plants within a treatment showed different rates of uptake, and isotope concentration in shoots seemed to fluctuate with time. Nevertheless, trends are apparent in the uptake of egg-encased isotopes. Selenium, cesium, and manganese all accumulated in aerial shoots between days 45 and 98 (Fig. 2). Surprisingly, aerial shoots showed a decrease in content of each isotope thereafter. The greatest rate of isotope uptake seemed to occur between weeks 9 and 14.

While uptake rate is indicated in Fig. 2, the relative amount of each isotope incorporated by the end of the 15-week growth period as well as the distribution of each within the plant body are presented in Fig. 3. After 15 weeks, 65% of the ^{137}Cs detected in pots was located in tissue of *A. breviligulata* (Fig. 3). Almost half (48%) the cesium incorporated by plants was located in aerial leaves, while the remainder was detected in underground roots and shoots. The portion of cesium (65%) within plant tissue was significantly ($P < 0.05$) more than the percentage of ^{54}Mn (18%) and of ^{75}Se (11%) taken up by plants. Iron was also detected but only 3% of this nutrient appeared in plant tissue, and that was restricted to the root system. This pattern of differential uptake, dominated by cesium incorporation, was seen in each plant, as well as being expressed in the average (Fig. 2). No radioactivity was detected in those control plants that had not been intentionally exposed to isotopes, indicating that isotopes in tissues came from sources within each pot, rather than from contamination from other pots.

Isotope distribution within the plant body varied widely among the radionuclides tested (Fig. 3). Cesium and manganese accumulated primarily in the shoot system. While twice as

much cesium was found in aerial leaves than in underground shoots, manganese concentrated in the underground shoot system. In contrast to ^{137}Cs and ^{54}Mn , two-thirds of the ^{75}Se incorporated over 15 weeks was located in root tissue. The remaining portion was divided almost equally between subterranean and aerial shoots; less than 10% of the accumulated ^{75}Se was detected in aboveground leaves. As with differential uptake patterns in entire plants (Fig. 2), these isotope distributions were consistent among the three plants presented with nuclide-augmented terrapin eggs. The two control plants, initially exposed to isotopes in the watering solution rather than in eggs, showed a similar distribution of nuclides at the end of the 15-week growth period. Finally, the small amount of ^{59}Fe taken up by *A. breviligulata* occurred only in the root system in all five plants supplied with isotopes (three test + two controls).

Discussion

Our results indicate that *A. breviligulata*, growing in mineral-deficient soils of barrier sand dunes (Kachi and Hirose 1983), can utilize buried turtle eggs as a supply of nutrients. The richness of this source is suggested by massive root proliferation around eggs unearthed from *A. breviligulata* stands in the field (Fig. 1; Lazell and Auger 1981). Root density is often two or three orders of magnitude greater in the volume of sand surrounding eggs than in normal dune sand (Stegmann 1985). The root mass develops from greatly enhanced branching rather than by increased axis elongation. Although not of this magnitude, analogous proliferation of laterals has been experimentally induced by exposing barley roots to localized mineral supplies (Drew 1975).

We cannot be certain of the types of molecules exiting from the egg. Flexible shells of turtle eggs similar to those of

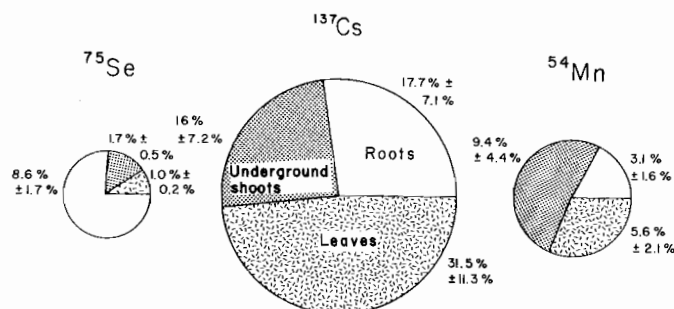


FIG. 3. Isotope incorporation and distribution within egg-exposed *A. breviligulata* plants expressed as percentage of total radioactivity recovered at the end of the 15-week growth period. Uptake into entire plant is reflected in circle radii. Values for total incorporation are as follows (mean \pm 95% confidence interval): ^{75}Se , 11.3 ± 5.5 ; ^{137}Cs , 65.2 ± 49.5 ; and ^{54}Mn , 18.1 ± 15.2 %. Isotope distribution within the plant is illustrated by size of the compartments within the circles. Percentages represent averages from three plants and add up to the percentage of initially supplied isotope incorporated by plants.

Malaclemys terrapin are permeable to gases, water, ions, and nitrogenous wastes (Tracy *et al.* 1978; Packard and Packard 1983; Packard *et al.* 1983). Our data (Fig. 2) reflect migration of inorganics, rather than larger molecules, across the shell since the isotopes were supplied as inorganic species. Metabolism of isotopes into larger molecules, such as selenium into amino acids (Bollard 1983), was prevented by killing the eggs in the injection process.

Over 40 days were required for leaf radioactivity to significantly exceed background levels (Fig. 2). Contributing to the delay must have been diffusion time out of flexible egg shells and through sand, root proliferation toward the nutrient source, mobility within the plant of absorbed nutrients, and initial pool size of S, K, and Mn, endogenous analogs to the isotopes used. Since plants were supplied with deionized H_2O throughout the experiment, progressive depletion of the nonradioactive nutrient pool may partially explain the steady rise of radioactivity in leaves. However, the abrupt loss of counts within aerial shoots after 100 days suggests a redirection of transport out of above-ground sectors. Vigor of aerial shoots decline rapidly, and it is likely that nutrients were shunted into subterranean shoots in preparation for overwintering. In Massachusetts field populations, aerial shoots senesce in November and die in winter. Underground plagiotropic rhizomes store nutrient reserves that support the growth of orthotropic tips the next spring (Maun 1985). Shunting of egg-driven nutrients from aerial shoots into underground shoots is apparent after day 100 (Fig. 2).

Of what importance are terrapin eggs to the ecology of *Ammophila*? Despite the implications of a carnivorous scenario among east coast sand dunes (Lazell and Auger 1981), it seems more reasonable to interpret the root-egg interaction as a response of *A. breviligulata* roots to fortuitous concentration of nutrients. Egg-borne nutrients clearly reach aerial shoots (Figs. 2 and 3). However, in populations of *A. breviligulata*, eggs seem too infrequent to constitute a selective force guiding evolution of the grass.

On the other hand, grass roots appear to have consistent impact on reproductive output of brackish water turtles, including *Malaclemys terrapin*. Members of this species occur along the Atlantic coast of America from Massachusetts to the Yucatan Peninsula (Obst 1986). *Ammophila breviligulata* is common in the northern portion of this range. At our study

site, *Malaclemys terrapin* prepares 25–30% of its nests among *A. breviligulata*. Roughly half such nests become infested with roots over the 70 to 90 day incubation period (Lazelle and Auger 1981), and among root-bound nests, half the eggs typically perish, compared with only 6% mortality in nests unencumbered by roots. Thus, one-quarter of the hatchlings developing in grassy areas are killed by *A. breviligulata* root growth. These figures are based on 8 years of field data collected in Massachusetts (P. Auger, unpublished data). They indicate the price imposed by *Ammophila* as it exploits buried clusters of terrapin eggs. Although the grass does not invade eggs, roots nonetheless form a mechanical barrier around eggs (Fig. 1). Terrapins are detrimentally affected, while *Ammophila* benefits, from the interaction.

Dune stabilization programs frequently use artificial plantings of *A. breviligulata*, *Uniola paniculata*, and similar grasses (Hawk and Sharp 1967; Nickerson and Thibodeau 1983) because the plants reduce erosion and dune movement. While grass planting may help preserve coastal dune morphology, such practices may reduce populations of brackish water turtles, prized by conservationists. The most successful nests of *M. terrapin* occur in grass-free areas, and over 70% of nests are buried there. Prime nesting areas are therefore converted to high-risk areas as a result of grass planting. Such management practices may further reduce habitats for *M. terrapin* and other turtles (Obst 1986).

The root-egg interaction reported here is an expression of the way in which roots may respond to localized nutrient sources. It provides additional evidence for heterogeneity of root systems in the field (Kutschera 1960; McCully and Canny 1985) and suggests ways in which roots adjust to improved local conditions.

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