GERMINATION AND EMERGENCE OF *Stachytarpheta cayennensis* AND *Ipomoea asarifolia*[1]

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RESUMO

Germinação e emergência de *Ipomoea asarifolia* e *Stachytarpheta cayennensis*

O entendimento de como a germinação e emergência de plantas daninhas respondem a fatores ambientais é de grande importância para a determinação da capacidade de adaptação e potencial de infestação, podendo ainda auxiliar no desenvolvimento de práticas de controle eficazes. A germinação de *Ipomoea asarifolia* (Desr.) Roem. & Schultz e *Stachytarpheta cayennensis* (Rich) Vahl. diminuíram linearmente com o decréscimo do potencial osmótico do meio de germinação, sendo que o aumento do estresse osmótico atrasou com maior intensidade a germinação de *Ipomoea* do que a de *Stachytarpheta*. A germinação de *Ipomoea* foi insensível à luz; já a de *Stachytarpheta* mostrou ter um comportamento fotoblástico positivo. O nitrato teve um efeito negativo na germinação de *Ipomoea* sob condições de luz ou escuro, mas estimulou a germinação de sementes de *Stachytarpheta* no escuro. A emergência de *Ipomoea* não foi significativamente afetada pela profundidade de semeadura. No entanto, em *Stachytarpheta,* a emergência das plântulas foi restrita a sementes plantadas na superfície do solo. A emergência de plântulas de *Ipomoea* de profundidades superiores a 6 cm ocasionou decréscimos significativos no percentual de biomassa alocada para as raízes, enquanto o percentual de biomassa alocado para as folhas decresceu para plântulas emergentes de profundidades superiores a 2cm.

Palavras chave: Nitrato, luz, estresse osmótico, profundidade de semeadura, gervão.

ABSTRACT

Understanding how weed seed germination and emergence respond to environmental factors is critical to determining their adaptive capabilities and potential for infestation, and could also aid in the development of effective control practices. Germination of *Ipomoea asarifolia* (Desr.) Roem. & Schultz and *Stachytarpheta cayennensis* (Rich) Vahl. decreased linearly with decreasing osmotic potentials. Also, increasing osmotic stress delayed germination of *Ipomoea* more than that of *Stachytarpheta*. *Ipomoea* germination was insensitive to light, while *Stachytarpheta* showed a positive photoblastic behavior. Nitrate had a negative effect on germination of *Ipomoea* seed under both light and dark conditions but stimulated dark germination of *Stachytarpheta*. *Ipomoea* emergence was not significantly affected by planting depth. However, for *Stachytarpheta* emergence was restricted to seeds planted at the soil surface. Emergence of *Ipomoea* seedlings from greater than 6cm significantly decreased the amount of biomass allocated to roots, while biomass allocated to leaves was decreased for

seedlings that emerged from depths greater than 2cm. These germination and emergence responses are discussed in relation to their ecological

implications and to weed control strategies.

Key words: Nitrate, light, osmotic stress, depth of planting.

INTRODUCTION

Knowledge of the seed germination requirements of weed species can be relevant to both agronomic and ecological perspectives. For example, much of the loss of seeds from the soil (decline in soil seed bank) seems to be attributable to germination (Schafer & Chilcote, 1970); therefore decline in the soil seed bank would be greatest under conditions favoring germination of specific species. The significance of seed germination to weed control programs can be stressed by the fact that, unlike dormant seeds that are often unaffected by most methods of weed control, germinating seeds are usually vulnerable. Thus, knowledge of germination patterns and the influence of environmental factors on seed germination is essential for development of preventive weed control programs.

Ipomoea asarifolia, a member of the Convolvulaceae, and *Stachytarpheta cayennensis, in* the Verbenaceae, are important perennial weedy species from pastures and crop areas in Brazil (Dias Filho, 1990; Dias Filho, 1994; Dias Filho *et al.* 1995; Lorenzi, 1991). Although native to tropical America, *S. cayennensis* also occurs as a weed in Australia (Kleinschmidt & Johnson, 1987), Hawaii (Haselwood & Motter, 1983) and India (Nair *et al.,* 1982). In addition, many *Ipomoea* species are also considered as troublesome weeds in warm temperate zones (Elmore *et al.,* 1990).

No biological or ecological information is available on the environmental factors influencing seed germination and emergence of these species. Such information would enable the understanding of these species adaptive capabilities and their potential for infestation, and could also aid in the development of effective control practices. Because in tropical pasture and agricultural areas

soil osmotic potential and nitrate levels can vary due to temporal changes in soil water availability, and the light environment can be extremely variable due to vegetation cover, it is important to know the germination response of tropical weed seeds to these factors. Also, in these environments soil cultivation and biotic agents may often change the position of weed seeds in the soil profile; hence, it is important to know the emergence response of weed seedlings to different planting depths. Therefore, the objectives of this research were to assess the effects of light, nitrate and osmotic stress on seed germination of *Ipomoea* and *Stachytarpheta,* and to determine the effect of planting depth on seedling emergence and biomass allocation of the two species.

MATERIALS AND METHODS

Seeds of *Ipomoea asarifolia* and *Stachytarpheta cayennensis* were collected from pasture areas in Brazilian Amazonia. After collection, seeds were cleaned and stored in low humidity at room temperature until initiation of the experiments 6-10 months later.

Osmotic stress

The effect of osmotic stress on germination was assessed in water solutions with osmotic potentials of -0.2, -0.4, -0.6, -0.8 and -1.0 MPa, prepared by dissolving appropriate amounts of polyethylene glycol (PEG 8000) in deionized water (Smith *et al.,* 1992). Deionized water alone was used for the 0 MPa standard in the experiment. Seeds were incubated in 9-cm glass petri dishes with two sheets of filter paper (Whatman No. 1. Whatman International Ltd. Maidstone, England) in a growth chamber (Percival, Model PT-80, Boone, IA) set at 12-h photoperiod and 31/24°C temperature regime.

Each petri dish had 20 seeds and received 7mI of the appropriate solution. Solutions and filter papers were renewed every other day to minimize water potential change through time (Emmerich & Hardegree, 1990). All petri dishes were sealed with a strip of Parafilm (Laboratory film. American National Can, Greenwich, CT). Prior to incubation, *Ipomoea* seeds were scarified with concentrated sulfuric acid for 20 min to overcome the hard seededness known to occur in this species. Preliminary trials indicated that this period was ideal to break seed dormancy in this species (M. B. Dias Filho, unpublished data). By previously breaking seed dormancy, any delay in germination during the experimental period could be attributed to treatment effect and not to natural dormancy. Germination counts were made every two days for eight days for *Ipomoea* or 18 days for *Stachytarpheta*. These periods have previously been determined as around 20 % above the ideal period for maximum germination for these species (M. B. Dias-Filho, unpublished data).

Light and nitrate

The effects of nitrate and light were determined in a growth chamber with temperature set at 31 °C (day) and 24 °C (night), and a photoperiod of 12 h. Fluorescent lighting provided a mean photosynthetic photon flux density (PPFD) of 200 µcool m^{-2} s^{-1}. Two sets of 9-cm glass petri dishes lined with two filter papers received 7 ml of either 0, 5 or 10 mM of KNO_3. Deionized water alone was used for the 0 mM standard. One set of petri dishes was wrapped in two layers of aluminum foil to exclude light. All petri dishes were sealed with a strip of Parafilm. The experiment was concluded after seven days for *Ipomoea* and 14 days for *Stachytarpheta* and germination was determined.

Planting depth

Seeds from each species were planted in 15-cm-diam plastic pots on the soil surface and at depths of 1, 2, 4, 6, 8, and 10 cm. Pots were filled with 250 g (air dry weight) of a potting soil mixture (peat:vermiculite:perlite, 1:1:1) and placed in a greenhouse with temperatures ranging from 33 ± 2.1°C during the day to 24 ± 2.2°C at night. In addition to natural light, 400 W lamps extended day length to 12 h and maintained PPFD, measured at the soil surface, at about 900 µmol $m^{-2} s^{-1}$. The soil was initially irrigated to saturation, then watered daily from the top. Ten or five seeds per pot were planted, respectively for *Stachytarpheta* and *Ipomoea.* Prior to planting *Ipomoea* seeds were scarified with concentrated sulfuric acid for 20 min. Emergence counts were recorded daily for 12 *(Ipomoea)* or 42 *(Stachytarpheta)* days. Preliminary trials indicated that these periods were at least twice as long as the ones required for maximum germination of these species in the greenhouse. Seedlings were considered to have emerged after they had broken the soil surface. At the end of the experimental period, the leaf, stem and root dry weights of all emerged seedlings were recorded. These data were used for the calculations of biomass allocation patterns: root, stem and leaf mass ratios (respectively, leaf, stem or root mass per unit of mass of whole plant, RMR, SMR and LMR).

Statistical analysis

For the germination experiments, each treatment was replicated five times in a completely randomized block design (osmotic stress) or a completely randomized design (light/nitrate). For the planting depth experiment, treatments were replicated four times in a completely randomized block design (each pot represented one replication of a given depth). The germination experiments were repeated once with similar results, the data of both experiments were pooled for presentation. Each data set was subjected to Levene's test for homogeneity of variance and to residual plots to check for homoscedasticity. Normal probability plots were constructed to examine the assumption of normality. When necessary, germination and emergence data were transformed by arsin (Raíz de y); no transformation was required for the biomass allocation data. Transformed data were retransformed for presentation. Data from the

osmotic stress trial were subjected to regression analysis. Data from the other experiments were subjected to analysis of variance: within each species and experiment, treatment means were separated by single degree of freedom contrasts or by the Bonferroni's method of multiple comparison (P= 0.05). Statistical analyses were performed by STATISTICA for Windows release 4.5 (STATISTICA for Windows, 1994) or SYSTAT Version 5.03 (Wilkinson, 1990).

RESULTS AND DISCUSSION

Osmotic stress

Both *Ipomoea* and *Stachytarphela* showed a similar response to increasing osmotic stress. Germination decreased linearly with decreasing osmotic potentials. Regression analysis performed on the accumulative percent germination throughout all experimental period confirmed the linear nature of the germination response in *Ipomea* (Germ= 74.4 + 72.9MPa; $F_{1.28}$= 231.05, P<0.0001: R^2=0.89) and *Stachytarpheta* (Germ = 72.43 + 65.8MPa; $F_1 1_8$=164.3, *P*< 0.0001: R2= 0.85). For *Ipomoea,* no germination was observed when osmotic stress exceeded -0.8 MPa, whereas *Slachrtarphela* seeds were able to germinate at -1.0 MPa (Figure 1). Related *Ipomoea* species have been shown to possess similar responses to increasing osmotic stress (Crowley & Buchanan, 1980; Hoveland & Buchanan, 1973).

Osmotic stress also affected germination timing of both species. Unstressed *Ipomoea* seed (0 MPa) germinated faster, showing a maximum value at day two (Figure 1). Under osmotic stress, germination of *Ipomoea* was delayed, being maximum at da^y four, for the -0.2 MPa treatment; at day six for the -0.4 MPa treatment, and at day eight for the -0.6 and -0.8 MPa treatments (Figure 1). For *Stachytarpheta,* germination of unstressed seeds was maximum at day 14, decreasing sharply at day 18 (Figure 1). Seeds incubated at -0.2 and -0.4 MPa showed a plateau of maximum germination from day 10 to the end of the experiment, while germination of seeds from the -

0.6 to -1.0 MPa treatments was maximum at day 14, decreasing slightly thereafter (Figure 1).

Stachytarpheta showed a remarkable capacity to germinate under low osmotic potentials. This characteristic, together with its known physiological response to water stress (Dias Filho *et al.,* 1995), may in part help to explain the occurrence of this and related species in overgrazed areas in pastures (Dias Filho, 1990; Haselwood & Motter. 1983; Kleinschmidt & Johnson, 1987). In these exposed areas, moisture content of the soil surface is subjected to a greater variability, and is normally lower than in adjacent areas protected by vegetation. Colonization of such areas is expected to be primarily by species capable to germinate and develop over a wide range of osmotic pressures.

Light and nitrate

Germination response to light indicated that *Ipomoea* seed was insensitive to light environment, while *Stachvtarphela* seed showed a positive photoblastic behavior (Figure 2). This suggests that management practices that help prevent seeds from receiving light, such as soil cultivation and mulching, could drastically reduce the occurrence of *Slachylarphela,* while no such effect would be observed for *Ipomoea. Slachylarphela* seeds that did not germinate in the dark were able to germinate normally once exposed to light (data not shown). This response suggests that the short term period (14 d) that the seed was deprived from light, under ideal moisture and temperature regimes, did not stimulate dormancy. Dormancy is known to be induced in this species in the field after long periods of incubation in the dark (M. B. Dias Filho, unpublished data).

Posl hoc contrasts showed that nitrate had a negative effect on germination of *Ipomoea* seed under both light ($F_{1.24}$= 5.38; *P*= 0.03) and dark ($F_{1.24}$= 8.71; P= 0.007) conditions (Figure 2). For *Stachvtarpheta,* no effect of nitrate could be detected on the final seed germination in the light.
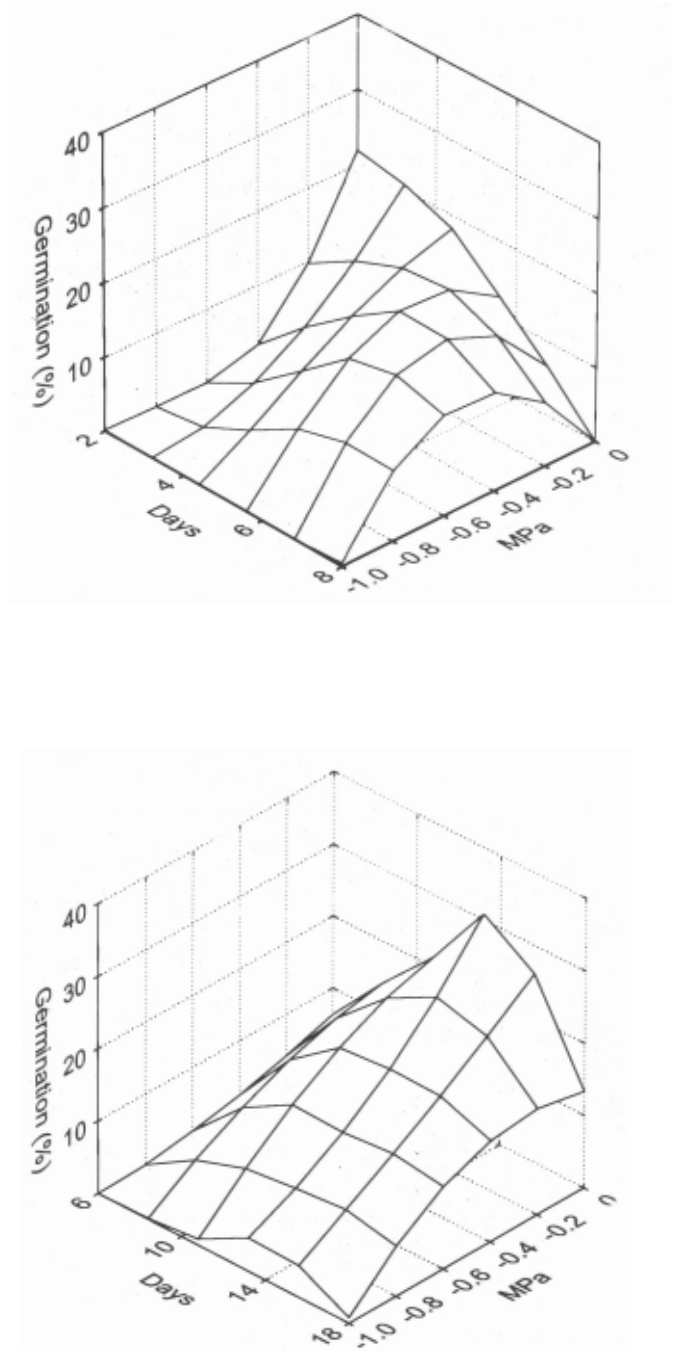
FIGURE 1. Effect of osmotic stress on the proportion of *Ipomoea* and *Stachytarpheta* seed germinating at different days after planting. Surfaces fitted by a bicubic spline smoothing procedure.
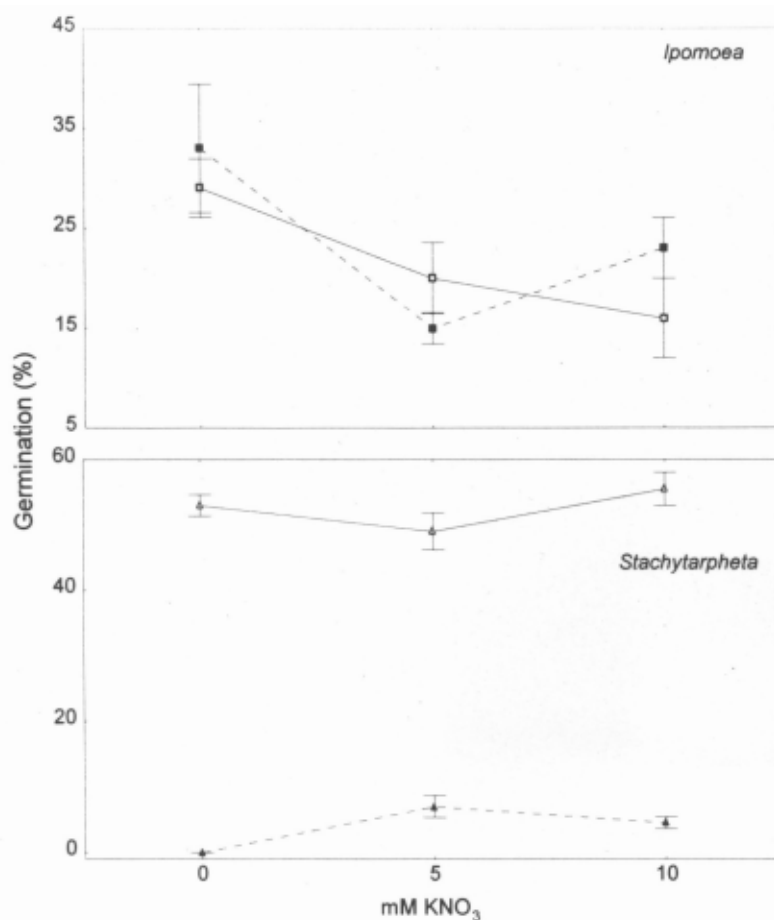
FIGURE 2. Effect of light and nitrate levels on percent germination of *Ipomoea* and *Stachytarpheta,* at seven *(Ipomoea)* or 14 *(Stachytarpheta)* days after planting. Each value is the mean of 20 seeds each, with five replications. Vertical bars are $\pm$ 1 standard error of the mean. Light = open symbols and solid lines: dark = closed symbols and dashed lines.

However, dark germination of this species was weakly, but significantly ($F_{1,24}$= 6.37; P= 0.02) stimulated by nitrate (Figure 2). Germination stimulation by nitrate in the dark has been suggested to act Iike a "gap detection mechanism", an ecologically important characteristic that may enable buried seeds to sense the presence of nearby plants (Pons, 1989). The observed response of Stachytarpheta to nitrate in the dark may, to some extent, suggest the presence of such mechanism. This characteristic could be of a great adaptive significance for a small seeded species (seed mass = 1.22 $\pm$ 0.04 mg), with a relatively small seedling size and growth rate (M. B. Dias Filho, unpublished data) like *Stachytarpheta,* by decreasing the risks of an early competition for external resources with other plants.

Planting depth

No significant *(P>* 0.05) effect of planting depth could be detected on seedling emergence of *Ipomoea* (Figure 3). However, a tendency for lower emergence was observed for seed placed on the soil surface (Figure 3), probably due to poor seed-soil contact and desiccation.
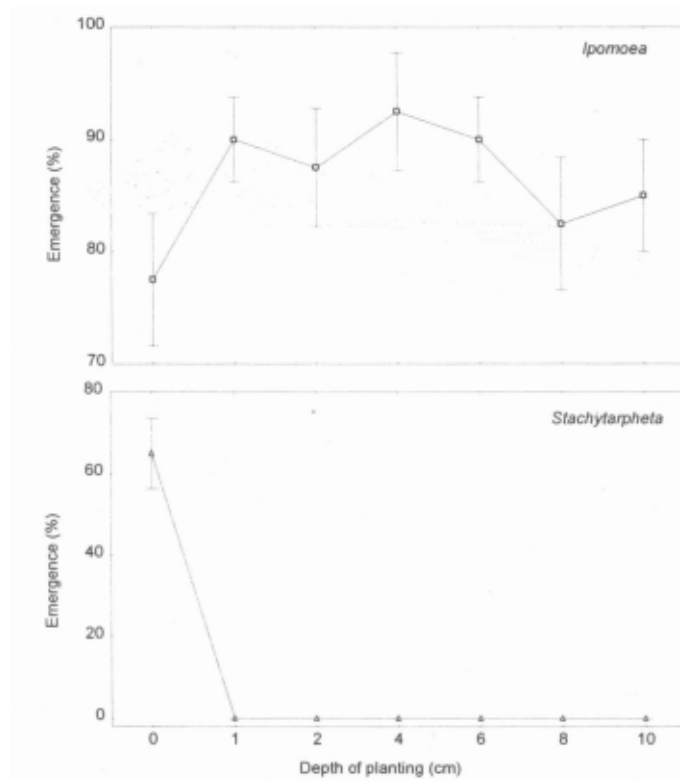
FIGURE 3. Effect of planting depth on percent emergence of *Ipomoea* and *Stachytarpheta, at* 12 *(Ipomoea)* or 42 *(Stachytarpheta)* days after planting. Each value is the mean of five *(Ipomoea)* or 10 *(Stachytarpheta)* seeds each, with four replications. Vertical bars are ± 1 standard error of the mean.

Results from the osmotic stress (Figure 1) and light (Figure 2) trials corroborate the observed emergence response of *Ipomoea* to planting depth. Many *Ipomoea* species have also been reported to have the ability to emerge from relatively great depths (for a review see Elmore et al. 1990), a characteristic that, for the present species, must be related to the lack of light requirement for germination and to the relatively large seed mass (64.31 ± 1.49 mg). The observed emergence response of *Ipomoea* shows that this species, which has epigeal germination, has a great degree of plasticity with respect to hypocotyl extension when buried at different depths. Because in agricultural environments, conditions such as seed position in the soil profile may be constantly changing due to practices like cultivation and to biological agents, this characteristic may be considered of great adaptive significance for this

species. Unlike *Ipomoea, emergence* in *Stachytarpheta* was restricted to seeds planted at the soil surface (Figure 3). This result agrees with the positive photoblastic nature of the seed of this species shown in the light experiment (Figure 2) and correlates with the natural occurrence of this species mainly on disturbed open areas like overgrazed pastures and road sides.

An important characteristic often overlooked in planting depth trials is the biomass allocation pattern of seedlings that emerge from different seeding depths. In the present study, the amount of biomass allocated to roots (RMR) by *Ipomoea* was significantly (Bonferroni's method, $P< 0.006$) higher for seedlings that emerged from depths up to 6 cm, decreasing at greater depths (Figure 4). Biomass allocated to stems (SMR) and leaves (LMR) had a tendency to be similar for depths up to 2cm, respectively, increasing (SMR)

or decreasing (LMR) at greater depths (Figure 4). These patterns suggest that emergence from greater depths compromises the ability of *Ipomoea* seedling to develop rapidly roots and leaves, as during the early phase of seedling development, greater amounts of stored seed reserves had to be allocated to hypocotyl elongation to enable the cotyledons to reach the soil surface. As a fast and efficient capture of above and below ground resources can be key for the survival and competitive ability of establishing seedlings (e.g., Harper, 1977; Fenner, 1987) it could be speculated that emerging from increasing depths may compromise the survival ability of *Ipomoea* seedlings by reducing the relative amount of biomass invested to leaves and roots, thus making these plants less capable to compete for external resources and, as a consequence, to endure and recover from stresses such as drought, mowing or herbivory.
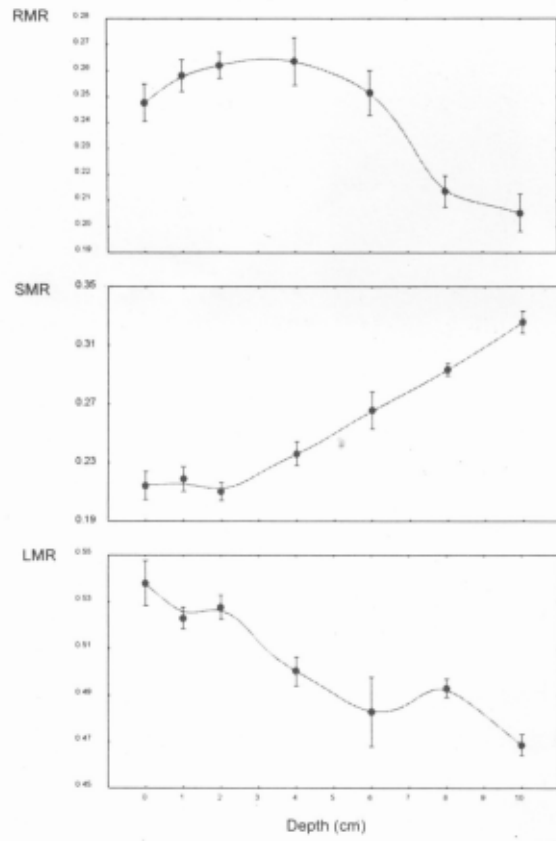

FIGURE 4. Biomass allocation to roots (RMR), stems (SMR) and leaves (LMR) of *Ipomoea* seedlings emerging from various depths. Vertical bars are ± 1 standard error of the mean (n= 5). Curves fitted by distance-weighted least squares.

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