

GROWTH CHARACTERISTICS OF MEXICAN OREGANO (*LIPPIA BERLANDIERI* SCHAUER) UNDER SALT STRESS

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ABSTRACT—This study evaluated growth patterns of Mexican oregano (*Lippia berlandieri*) under salt stress and tested the hypothesis that this species is salt tolerant. Plants were grown in Hoagland solution with no NaCl added (control) and with increasing NaCl concentrations added to produce osmotic potentials of -0.30 , -0.45 , -0.60 , -0.90 , and -1.20 MPa. Relative growth rate decreased linearly and leaf weight decreased 9-fold as osmotic potential decreased from control to -1.20 MPa. We therefore concluded that, compared to other non-halophytes, Mexican oregano is only moderately tolerant of salinity. Root growth was less affected by salinity than leaf and stem growth. Relative growth rate of Mexican oregano varied from 0.01 to 0.08 g/g/d. These low values suggest a slow recovery process for plants in native stands subject to continuous harvesting.

RESUMEN—Este estudio evaluó los patrones de crecimiento del orégano mexicano (*Lippia berlandieri*) bajo condiciones de estrés salino y probó la hipótesis de que esta especie es tolerante a la salinidad. Las plantas fueron desarrolladas en solución Hoagland libre de NaCl (testigo) y con diferentes concentraciones de NaCl para producir potenciales osmóticos de -0.30 , -0.45 , -0.60 , -0.90 , y -1.20 MPa. La tasa relativa de crecimiento disminuyó linealmente y el peso de las hojas disminuyó 9 veces al disminuir el potencial osmótico de las soluciones desde la testigo hasta el tratamiento de -1.20 MPa. Con esta base concluimos que, en comparación con otras plantas no halófitas, el orégano mexicano es sólo moderadamente tolerante a la salinidad. El crecimiento de la raíz fue menos afectado por la salinidad que el crecimiento de hojas y tallos. La tasa relativa de crecimiento del orégano mexicano varió de 0.01 a 0.08 g/g/d. Estos valores son bajos y sugieren una lenta recuperación de las plantas de las poblaciones naturales que están sujetas a continua recolección.

The term “oregano” refers to a flavor rather than to a particular plant species. According to Bernath (1996), there are at least 61 species of 17 genera belonging to 6 botanical families that are known and used as oregano in the world. Despite the taxonomic disparities, these species share the spicy, pungent flavor and aroma, determined by their essential oils, that is appreciated in flavoring foods (Yousif et al., 2000). In addition, oregano has been used for centuries as a medicinal plant because of its antibacterial properties (Escamilla-Sanchez et al., 1991; Bernath, 1996; Olivier, 1996).

For international commercial purposes, oregano is divided into 2 main groups, European or Mediterranean oregano and Mexican oregano (Bernath, 1996; Olivier, 1996). European oregano includes plant species, such as

Origanum vulgare, that are mostly in the family Lamiaceae (Bernath, 1996). Mexican oregano includes species that mainly belong to the family Verbenaceae, such as *Lippia berlandieri* and *L. graveolens* (Bernath, 1996). These are native shrubs from semi-desert areas of central and northern Mexico that are mainly harvested for exportation and satisfy about half of the oregano consumption in the United States, which increased 9-fold from the mid 1960s to the mid 1990s (Olivier, 1996).

Mexican oregano is usually harvested from native stands, often during the flowering season (Escamilla-Sanchez et al., 1991; Alaniz-Gutierrez et al., 2000). This results in serious risk for the reproduction of native populations and genetic erosion. Researchers have conducted studies on seed germination and in vitro re-

production of Mexican oregano in attempts to domesticate this species and protect native stands (Escamilla-Sanchez et al., 1991; Armendariz-Erives, 2000). Researchers also have emphasized the drought tolerance of this species based on its patterns of natural distribution (Hernandez-Reyna and Arias-Moreno, 1991; Alaniz-Gutierrez et al., 2000). However, the basic growth characteristics and responses to stress of Mexican oregano are largely unknown. A much larger volume of genetic, agronomic, and botanical information is available for the European oregano species (see studies compiled by Padulosi, 1996).

Mexican oregano is known to tolerate drought stress, growing in areas that receive <300 mm of annual rainfall (Hernandez-Reyna and Arias-Moreno, 1991; Alaniz-Gutierrez et al., 2000). It is not clear, however, if this species can tolerate saline conditions. Salinity stress is a major cause of plant growth impairment in the world and a common phenomenon in North American deserts. The objective of this study was to determine the relative growth rate and biomass allocation of Mexican oregano (*L. berlandieri*) as affected by salinity in culture solution. Because of its drought tolerance, we hypothesized that Mexican oregano could also withstand some degree of salinity stress. If so, the range of potential cultivation areas for this species might include vast areas of saline soils in North America.

METHODS—This study was conducted in the greenhouse of Unidad Regional Universitaria de Zonas Aridas, Universidad Autonoma Chapingo in Bermejillo, Durango, Mexico (25°53'17"N, 103°37'20"W). This area is part of the Chihuahuan Desert and has an average annual precipitation of 265 mm, which occurs mainly during the summer. Native populations of Mexican oregano are widely distributed throughout the study area (Alaniz-Gutierrez et al., 2000).

Oregano seeds were obtained from cultivated experimental plots located in the area of Viesca, Coahuila, Mexico. Seeds were germinated in plastic planters (2.5 cm × 2.5 cm × 5 cm) that contained sphagnum as the rooting medium. Several seeds were placed in each planter, irrigated uniformly with potable water, and thinned to 1 seedling per planter. Seedling emergence began 10 to 15 d after sowing.

Seedlings were maintained in planters for 45 d, reaching a height of about 10 cm. At this time, plants were removed from the planters, and the rooting material was washed carefully with water to

obtain the intact rooted plant. Plants were transferred to cubic plastic containers (18 cm wide, 18 cm deep, 20 cm high) containing complete Hoagland solution. Every container had a plastic lid with 1 perforation near each corner, through which, 1 plant was suspended with the roots immersed in the solution. Therefore, every container supported 4 plants for the duration of the experiment (30 d). The solution was aerated with an aquarium pump to provide oxygen for the roots.

Plants were subjected to 6 salinity treatments, which were achieved by adding 0 (control), 4.2, 6.3, 8.4, 12.6, and 16.8 g NaCl/L to the solution culture. Additions of NaCl were gradual to avoid osmotic shock to plants and resulted in osmotic potentials of -0.30, -0.45, -0.60, -0.90, and -1.20 MPa (as in Pessaraki and Tucker, 1985). The culture solution was renewed every 4 d, and pH was adjusted to 6.0. The solution containers with plants were arranged in a randomized block design to account for variation in temperature within the greenhouse. The 6 treatments were replicated 3 times.

Before osmotic treatments were imposed, the initial plant weight was estimated by cutting, drying, and weighing the shoots of 10 plants representative of the size of the plants at the beginning of the experiment. At the end of the experiment, plants were separated into leaves, roots, and stems and dried at 45°C until reaching a constant weight (approximately 2 d). Because each container supported 4 plants that grew uniformly, the weights of the 4 plants per container were averaged. The initial (estimated) and the final shoot dry weights were used to calculate relative growth rate (RGR) according to the formula $RGR = [\ln(\text{final weight}) - \ln(\text{initial weight})]/d$ growth (Glenn et al., 1998). Root:shoot ratio (RSR) was obtained by dividing the root dry weight by the dry weight of the aerial parts (leaves and stems). The data were analyzed by analysis of variance and regression. Treatment mean separation was performed by the Tukey test at 0.05 probability level.

RESULTS AND DISCUSSION—Root weight was not significantly ($P > 0.05$) affected by the changes in salinity, but leaf and stem weights decreased gradually as salinity stress increased (Table 1). Leaf weight and stem weight were 9-fold and 7.5-fold higher, respectively, in the control than in the -1.20 MPa treatment. Overall, leaves were slightly more sensitive than stems and much more sensitive than roots to salinity treatments. This also was observed by Pessaraki and Tucker (1985) and Munns and Termaat (1986).

The decrease in leaf and shoot biomass production as osmotic potential decreased from

TABLE 1—Production (g) of root, stem, and leaf per plant of Mexican oregano (*Lippia berlandieri*) as affected by salinity treatments in culture solution. Means within a column followed by the same letter are not significantly ($P > 0.05$) different (Tukey's test).

| Osmotic potential (MPa) | Root (g) | Stem (g) | Leaf (g) |
|-------------------------|----------|----------|----------|
| Control | 0.17 a | 0.45 a | 0.91 a |
| -0.30 | 0.22 a | 0.39 a | 0.85 ab |
| -0.45 | 0.12 a | 0.25 ab | 0.41 abc |
| -0.60 | 0.12 a | 0.17 ab | 0.32 abc |
| -0.90 | 0.09 a | 0.08 b | 0.22 bc |
| -1.20 | 0.06 a | 0.06 b | 0.10 c |

control to -1.20 MPa was striking (9-fold). Under the same treatments in culture solution, Pessarakli and Tucker (1985) found that cotton (*Gossypium hirsutum*) shoot production decreased only about 3-fold. Furthermore, Wan et al. (1993a) reported that the green tissue production of snakeweed (*Gutierrezia sarothrae*) also decreased 3-fold when soil water potential decreased from -0.02 MPa to -1.10 MPa. Cotton is considered a non-halophyte with high salinity tolerance (Maas, 1986; Brugnoli and Lauteri, 1991), whereas snakeweed is a desert shrub with high drought tolerance (Wan et al., 1993a, 1993b).

Our results showed that Mexican oregano is more sensitive to salinity than cotton and seems to be less drought-tolerant than snake-weed. However, it is possible that the drought tolerance of Mexican oregano is higher than it seemed from our results. Although no specific symptoms of toxicity were observed, the deleterious effects of increasing salinity might have been related to ion-specific effects. Under iso-osmotic levels, NaCl solutions have been more harmful to germination and seedling growth of pea (*Pisum sativum*) (Singh et al., 1990), *Atriplex prostrata*, and *A. patula* (Katembe et al., 1998) than polyethylene glycol solutions. Direct ionic-specific effects include excessive accumulations of Na^+ or Cl^- in the cytoplasm, which can induce changes in protein activity (Katembe et al., 1998). Another well-documented ion-specific effect is the disruption of normal mineral nutrition by competitive ionic interactions, such as Ca^{2+} or K^+ deficiencies

TABLE 2—Proportion of plant parts, as percentage of the total plant weight, of Mexican oregano (*Lippia berlandieri*) affected by salinity treatments in culture solution. Coefficient of determination (R^2) and P value are for linear regressions.

| Osmotic potential (MPa) | Root (%) | Stem (%) | Leaf (%) |
|-------------------------|----------|----------|----------|
| Control | 11 | 29 | 59 |
| -0.30 | 15 | 27 | 58 |
| -0.45 | 15 | 32 | 52 |
| -0.60 | 20 | 28 | 52 |
| -0.90 | 23 | 21 | 56 |
| -1.20 | 27 | 27 | 46 |
| R^2 | 0.96 | 0.24 | 0.59 |
| P | 0.0007 | 0.32 | 0.07 |

caused by an excess of Na^+ (Grattan and Grieve, 1992).

Pessarakli et al. (1989) and Pessarakli and Tucker (1988) subjected sweet corn (*Zea mays*) and eggplant (*Solanum melongena*), respectively, to salinity treatments similar to ours. Dry-matter production of sweet corn decreased 16-fold as osmotic potential decreased from control to -0.9 MPa, and dry-matter production of eggplant decreased 11-fold as osmotic potential decreased from control to -0.6 MPa. Clearly, these crops are more sensitive than Mexican oregano to salt stress. Corn and eggplant are classified (Maas, 1986) as moderately sensitive to salinity. Therefore, Mexican oregano could be classified as moderately salt tolerant, an intermediate category between tolerant (cotton) and moderately sensitive (corn). Other plants considered moderately salt tolerant are barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and olive (*Olea europaea*) (Maas, 1986).

The increase in salinity produced responses in Mexican oregano that varied with the type of plant tissue (Table 2). The proportion of leaves and stems with respect to the total plant biomass was not correlated with the osmotic potential of the solutions ($P > 0.05$). This indicates that shoots remained proportionally stable despite the changes in salinity. In contrast, the proportion of roots increased ($P = 0.0007$) as osmotic potentials decreased. It was clear that the importance of roots for plant survival increased as salt stress increased. This was confirmed by the increase in root:shoot ratio (RSR) ($P = 0.0001$) as osmotic potentials

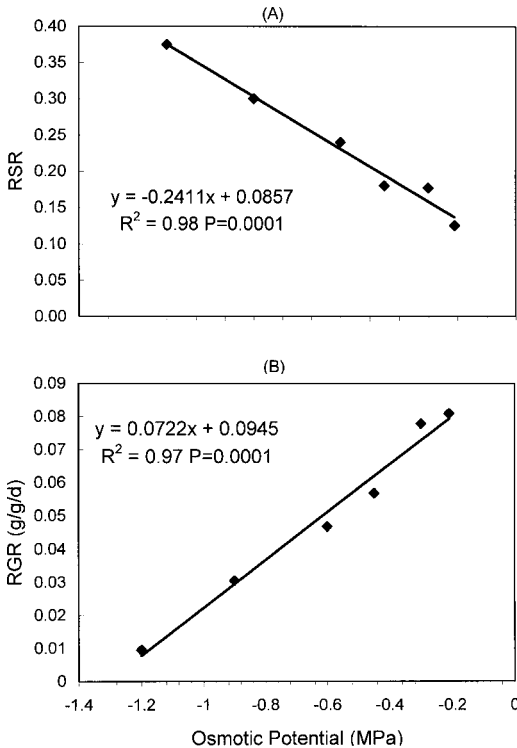


FIG. 1—A) Root:shoot ratio (RSR), and B) relative growth rate (RGR) of Mexican oregano (*Lippia bertandieri*) as affected by salinity treatments in culture solution.

decreased in culture solution (Fig. 1A). Increases in RSR also have been observed as a response to drought stress (Sisson, 1989; Wan et al., 1993a) and to nutrient deficiencies (Mata-González et al., 2002b) in the rooting medium. This supports the contention that root growth is favored over shoot growth when plants encounter sub-optimal soil resources. In contrast, when resources are plentiful in soil, plants do not require an extensive root system because the resource use efficiency of roots is greatly increased (Robinson, 1986).

Our results showed that 1 g of root was able to support 8 g of shoot in the control treatment. In contrast, 1 g of root could support only 3 g of shoot in the 2 highest salinity treatments (Table 1). Therefore, plants might only require about one-third of their root system to provide water and nutrients for a plant if these resources are adequately provided. In agreement with this, Robinson (1986) reported that only 20% of the root mass is necessary to satisfy

the N demand of a plant when N is readily available in the soil.

In contrast to RSR, RGR of Mexican oregano decreased linearly as osmotic potentials decreased (Fig. 1B), which is a typical response of non-halophytic plants subjected to salinity. The degree to which plant growth decreases with salinity is, however, species-specific. The RGR of halophytes, such as *Atriplex canescens*, decreases 50% when NaCl concentration in the root zone reaches 58 g/L (Glenn et al., 1994). In contrast, the RGR of Mexican oregano decreased 50% when NaCl concentration in solution was 8.4 g/L (-0.60 MPa) (Fig. 1B).

Relative growth rates of Mexican oregano varied from 0.01 to 0.08 g/g/d (Fig. 1B). These low RGRs are typical of perennial species adapted to grow in unproductive habitats (Grime and Hunt, 1975), such as *Artemisia tridentata*, whose RGR under adequate greenhouse conditions was 0.06 g/g/d (Schlesinger et al., 1989). The low RGR of Mexican oregano is perhaps a reflection of its adaptation to sites with low fertility. This species typically grows in southern exposures of small mountains (Hernandez-Reyna and Arias-Moreno, 1991), where soil water content and nutrient levels usually are lower compared to northern exposures (Mata-González et al., 2002a).

The low RGR of Mexican oregano indicates that regeneration of natural plant stands subjected to continuous harvesting could be slow. European oregano (*Origanum vulgare*) has an intrinsically higher RGR than its Mexican counterpart. Grime and Hunt (1975) reported that *O. vulgare* grew at a rate of 0.21 g/g/d under optimal greenhouse conditions, which is almost 3 times higher than the maximum rate determined for Mexican oregano. This implies that the risk of loss of diversity and genetic erosion, as well as the need for preservation of native stands of Mexican oregano, are higher than in European oregano. Genetic erosion is a problem faced by other species of oregano collected from native stands in Turkey, Morocco, Albania, and Greece (Padulosi, 1996).

In conclusion, our results only partially supported the hypothesis that Mexican oregano is salt tolerant. We can only assert that this species is a non-halophyte that is moderately tolerant to salinity. Therefore, cultivation efforts should avoid land affected by salinity. Roots were less affected than shoots under salt stress,

confirming a general pattern of plant growth under stress. Mexican oregano has low intrinsic growth rates, which makes plant regeneration a slow process and warrants studies tending to protect native populations. Our results, however, can be considered preliminary because of the highly artificial conditions, in terms of substrate composition, of our experimental setting.

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