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The importance of flood events on the establishment of seedlings and cuttings of saltcedar (*Tamarix ramosissima*)

EVANGELINA NATALE[2,3,✉] & HERMINDA REINOSO[1]

[1] *Laboratorio de Morfología Vegetal, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto. Ruta 36, km 601, (5800) Río Cuarto, Córdoba, Argentina.* [2] *Fundación Conservación y Desarrollo (ConyDes). Sobremonte 1653, (5800) Río Cuarto, Córdoba, Argentina.* [3] *Laboratorio de Plantas Vasculares, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto. Ruta 36, km 601, (5800) Río Cuarto, Córdoba, Argentina.*

ABSTRACT. The expansion of invasive plants is considered one of the greatest threats to biodiversity. The environments more threatened by the presence of exotic invasive species are the riparian areas due to their small surface and high diversity. The advance of *Tamarix* in these environments may be linked to transient conditions of higher water availability, which would allow initial colonization. The objective of the present work was to determine which hydric conditions promote germination and establishment of *Tamarix* cuttings by measuring in the laboratory the effects of different periods of flooding. It was observed that the germination was high under irrigation at field capacity and was decreased after 10 days of flood, while the establishment through cuttings required at least 15 days duration of a flood event. These results will contribute to the identification of critical environmental conditions that facilitate the establishment of the genus in new areas and guide actions for its early control.

[Keywords: biological invasion, stress, salinity, plants]

RESUMEN. Importancia de eventos de inundación sobre el establecimiento de plántulas y estacas de tamariscos *(Tamarix ramosissima)*. La expansión de especies vegetales invasoras es considerada una de las mayores amenazas a la biodiversidad. Los ambientes más amenazados por la presencia de especies exóticas invasoras son las áreas riparias debido a su escasa superficie y su alta diversidad. El avance de *Tamarix* sobre estos ambientes podría estar relacionado a situaciones transitorias de mayor disponibilidad hídrica, que permitirían la colonización inicial. El objetivo de este trabajo fue detectar las condiciones hídricas que promueven la germinación y establecimiento de estacas de tamarisco, a través de la determinación, en laboratorio, de la importancia de diferentes periodos de inundación. Se observó que la germinación fue elevada con riego a capacidad de campo y se vio disminuida luego de un periodo de inundación de 10 días; mientras que el establecimiento por estacas necesitó al menos de 15 días de inundación. Estos resultados contribuirán a la identificación de aquellas condiciones ambientales críticas que facilitan el establecimiento del género en nuevas áreas y orientarán acciones para su control temprano.

[Palabras clave:invasión biológica, estrés, salinidad, plantas]

INTRODUCTION

The riparian environments host some of the most complex ecosystems. In particular, riparian forests are the most diverse floristic and structural systems, and despite representing only a small percentage of the landscape harbor a critical biodiversity. For these reasons, conservation of the riparian areas should be a priority in the strategies of management of watersheds (Reynolds et al. 2014). The riparian landscapes in arid and semi-arid regions of Argentina are frequently invaded by species of the *Tamarix* genus (Natale et al. 2012), a genus native to Asia, north of Africa and southeast of Europe. Some species are invasive in USA, México and Australia after their introduction in the country with ornamental purposes, as windbreaks or to control erosion (Glenn & Nagler 2005). The genus *Tamarix* produces seeds twice a year. It is estimated that an adult plant releases over half million seeds per year that are spread by the wind and water over new environments at distances that can reach 20 km in one year. Furthermore, portions of branches or stems have the ability to develop adventitious roots and sprout given enough moisture (Baker 1974). This situation raises great concern due to the history of *Tamarix* as a very invasive genus (Matthews 2005).

Studies about the invasive capability on *Tamarix* have focused on its tolerance to a range of stress conditions, including high and low temperatures, drought, flooding and high salt concentration in the soil (Di Tomaso 1998; Shafroth et al. 2005). Species from the *Tamarix* genus have been traditionally considered tolerant to drought conditions

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✉ enatale@exa.unrc.edu.ar

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(Glenn & Nagler 2005). However, results obtained by Natale et al. (2010) showed that this tolerance does not manifest when it comes to the initial establishment phase for both seedlings and cuttings. It was shown that *T. ramosissima* is significantly sensitive to water deficit, exhibiting null levels of germination at osmotic potentials over -0.4 MPa, which are comparable to the potentials found in soils in semi-arid regions of Argentina. Similarly, the species barely resists salinity levels under 13.4 g/L NaCl (0.2 M) during the establishment of seedlings and cuttings. This level is remarkably lower than the values reported for adult individuals of the same species (Glenn et al. 1998; De Loach et al. 2000; Glenn & Nagler 2005).

These data acquires further importance considering that germination and cuttings establishment are the most vulnerable phases during the invasion process, since they depend on external factors. They constitute the most important phases to overcome for the colonization of a new environment to succeed (Zalba et al. 2008). All these results raise the question of how is it possible to find *Tamarix* populations in high salt or extreme drought environments. The explanation to this phenomenon could be related to the opening of opportunity windows and the duration of such windows in time. The occurrence of transient conditions of higher water availability and lower salinity that would allow initial colonization by the invasive species. Therefore, the objective of the present work was to determine which hydric conditions promote germination and establishment of cuttings of *Tamarix* by measuring in the laboratory the effects of different periods of flooding on vegetative and sexual propagation. Based on the planed experiments, we aim to obtain relevant information for the identification of environmental conditions that favor the establishment of the genus in new areas, contributing to direct early control and eradication actions essential for the effective management of invasive species.

MATERIALS AND METHODS

Seeds and cuttings collections

Seeds and cuttings were collected from 100 plants randomly selected from a population of *T. ramosissima* located near the town of Dalmasio Velez Sarsfield (32°36'47" S, 63°39'18" W), in the south of Córdoba province, Argentina. Materials were collected on March 2010. Plants were selected providing that they were at least 10 m apart. Seeds were stored in paper bags and seeded three days after collection. Based on previous experiences from Natale et al. (2010), cuttings of 10 cm long and between 0.3 and 0.5 cm of diameter were collected and the damaged tissue covered with lanolin.

Seed trial

Seeds were placed on trays (800 cc) with a mix of sand and soil (1:1) from the area of study. Approximately, 2180 seeds per tray were employed in seedlings survival trials. The trials were conducted in a growing chamber (16 h light at 28° C/8 h darkness at 20° C, 80% RH). Six different treatments were applied to simulate different periods flooding: 0 (field capacity), 10, 20, 30, 40 and 60 days. The trays were watered periodically to maintain the flooding conditions corresponding each different treatment. Each trial consisted in six treatment replicates. The water employed in the trials was from the local water network of the Río Cuarto City (33°07'56'' S, 64°20'48.39'' W). The local water contains a total of 309 mg/L of solids in solution consisting of Na+ (22.2 mg/L) and Ca^{2+} (44.8 mg/L) cations, Cl^- (8.6 mg/L) and SO_4^{2-} (13.4 mg/L) anions, and no reported carbonates. For each treatment, the germination proportion was registered after 10 days of trial and the survival proportion was determined 30 days after stress release (end of flooding event).

Cutting trial

Cuttings were placed on plant pots (300 cc) with a mix of sand and soil (1:1) from the area of study. Two hundred cuttings were planted in individual containers and kept under natural photoperiod conditions. After ten days, cuttings showing higher vigor were selected for further treatment.

The trials with cuttings were conducted under natural photoperiod. The flooding lasted 0 (Control), 15, 20, 30, 40 and 90 days; at 0 days the moisture of the substrate was 50% of its water holding capacity. Thirty days after stress release, the proportion of sprouting and total biomass were determined. For the trials with cuttings, 6 replicates were performed, each consisting of four cuttings in an individual container. Each cutting represented 25% of the replicate. To be considered a cuttings sprouted should have both roots as shoots.

Data analysis

The obtained proportions were transformed (arcsine) to fulfill normality and homoscedastic assumptions (Shapiro-Wilk) and residual dispersion versus predicted value graphics respectively, which are necessary for the parametric analysis of variance. Only the sprouting proportion in the cuttings establishment trials fulfilled the normality assumptions and was, therefore, analysed trough Analysis of Variance (ANOVA) followed by Fisher Least Significant Difference test (LSD). The variables biomass, proportion of germination and proportion of seed survival after 30 days of stress release were analysed by means of the non-parametric Kruskal-Wallis test and means comparison tests. All the analyses were performed employing the Infostat 2008 software.

RESULTS

Trial with Seeds

Significant differences were found for the proportion of germination, with the treatment of irrigation to field capacity showing the highest values (*P*=0.043) (Table 1); whereas 10 days of flood were enough to produce 80%

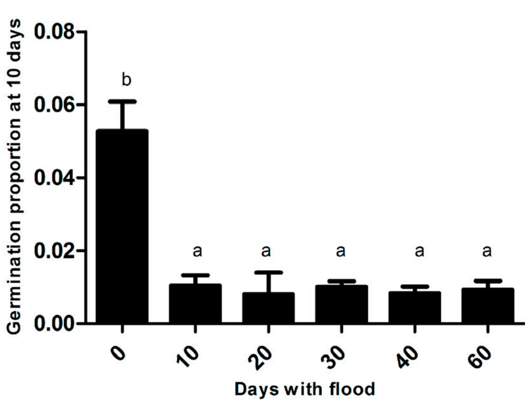

Figure 1. Average proportion of germination of *T. ramosissima* exposed to different time periods of flooding. Different letters indicate significant differences between treatments (*P*<0.01) and bars represent the standard error.

Figura 1. Valores promedio de proporción de germinación de *T. ramosissima* expuestos a diferentes períodos de inundación. Letras diferentes indican diferencias significativas entre los tratamientos (*P*<0.01) y las barras representan el error estándar.

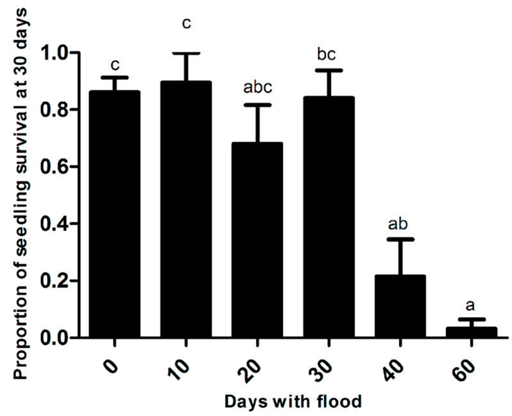

Figure 2. Average proportion of survival of *T. ramosissima* seedlings exposed to different time periods of flooding. Different letters indicate significant differences between treatments (p<0.01) and bars represent the standard error.

Figura 2. Valor promedio de proporción de supervivencia de plántulas de *T. ramosissima* expuestas a diferentes períodos de inundación. Letras diferentes indican diferencias significativas entre los tratamientos (p<0.01) y las barras representan el error estándar.

decrease in germination (Figure 1). Concerning the variable proportion of seedlings survival, significant differences were found among treatments (*P*=0.006) (Table 1). Irrigation up to field capacity and 10 days of flooding were the treatments with better response, followed by treatments consisting of 20 and 30 days of flooding that showed over 60% survival, although the difference was not significant. Finally the survival dropped under 25% at 40 days of flooding and was almost 0 at 60 days of flooding (Figure 2). From these results it can be deduced that the plantlet survival varies between 75 and 100% when the flooding events last up to 30 days but after this period the survival decreases to 50% and becomes 0 after 60 days of flood.

Trial with cuttings

In these trials significant differences were obtained between treatments (*P*=0.023) (Table 1). No sprouting was observed in the control treatment (Figure 3), and the average survival for the other treatments ranged between 30 and 60%. Significant differences were detected for the variable biomass production (*P*=0.03) (Table 1) between the control treatment, showing null biomass production and 20 and 40 days of flooding, which produced the highest amount of biomass. In addition, 20 days of treatment showed a significant difference with the treatment consisting of

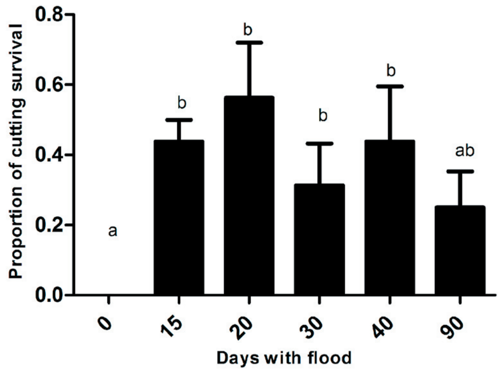

Figure 3. Average percentage of cutting survival for *T. rammosissima* exposed to different time periods of flooding. Different letters indicate significant differences between treatments (P<0.01) and bars represent the standard error.

Figura 3. Valores promedio de proporción de supervivencia de esquejes de *T. ramosissima* expuestos a diferentes periodos de inundación. Letras diferentes indican diferencias significativas entre los tratamientos (P<0.01) y las barras representan el error estándar.

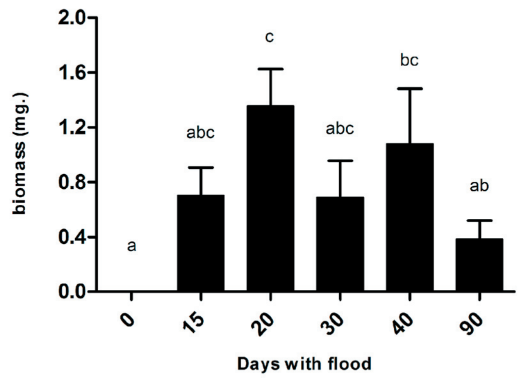

Figure 4. Avarage total biomass production of *T. ramosissima* cuttings exposed to different time periods of flooding. Different letters indicate significant differences between treatments (P<0.01) and bars represent the standard error.

Figura 4. Valores promedio de producción de biomasa total de esquejes de*T. ramosissima* expuestos a diferentes periodos de inundación. Letras diferentes indican diferencias significativas entre los tratamientos (P<0.01) y las barras representan el error estándar.

Table 1. Average values and standard deviation of the percentage of cutting survival, total biomass, percentage of germination at day 10 and percentage of seed survival after 30 days of stress release, obtained in trials with *T. ramosissima.*
Tabla 1. Valores promedio y desvío estándar del porcentaje de supervivencia de esquejes, biomasa total, porcentaje de germinación al día 10 y porcentaje de supervivencia de semillas después de 30 días del relajamiento del estrés, obtenidos en ensayos con *T. ramosissima.*

Treatment	Field conditions 0 days		Flood 15 day		20 days		30 days		40 days		90 days		P	Analysis
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Percentage of cutting survival	0.00	0.00	0.44	0.13	0.56	0.31	0.31	0.24	0.44	0.31	0.25	0.20	0.023	Anova
Biomass (mg)	0.00	0.00	0.70	0.41	1.36	0.54	0.69	0.54	1.08	0.80	0.39	0.27	0.030	Kruskal-Wallis
	0 days		15 day		20 days		30 days		40 days		60 days			
Germination percentage at 10 days	0.23	0.04	0.10	0.03	0.07	0.06	0.10	0.02	0.09	0.02	0.10	0.03	0.043	Kruskal-Wallis
Percentage of seed survival at 30 days	86.10	10.31	89.47	21.05	68.04	27.26	84.10	19.51	21.58	25.84	3.23	6.45	0.006	Kruskal-Wallis

over 90 days of flooding in which the cuttings biomass was reduced approximately 30% (Figure 4).

Discussion

The results suggest that both reproductive strategies show different behaviour under flood events. On the one hand, to trigger massive germination, it would be necessary to maintain irrigation to field capacity and avoid adverse flooding events that have a negative impact on germination. It is likely that the low germination response observed later is related to embryo death caused by excess of water that decreases oxygen intake in the tissues (Pardos 2004). On the other hand, establishment of *Tamarix* cuttings requires a period of 15 to 40 days of flooding events, that might be explained by the need of a period of time to wash-off the inhibitors, which might be preventing the emission of root primordia by the stem. It is known that some species have stems that are difficult to root, and only succeed after they become free of certain phenolic compounds such as lignin, flavonoids and anthocyanins among others (Hartmann and Kester 1988). This washing effect seems to be necessary for only a given period of time after which the extraordinary water supply becomes detrimental as seen after 90 days of flooding events where sprouting events decrease drastically. In most species long periods of flooding induce hypoxia. It is known that the lack of oxygen favours the accumulation of toxic compounds in the roots that inhibit nutrient and mineral absorption through cell membranes causing cell death, biomass loss and ultimately plant death (Drew 1997). Proton transport into the cells membrane is affected under oxygen-deficiency stress, the initial production of lactic acid after the onset of the stress period, as well as the inhibition of H^+-ATPases due to ATP deficiency, rapidly leads to a decrease in cytoplasmic pH. This cytoplasmic acidosis can inhibit enzyme activities and glycolysis in the cytosol, therefore produce a extreme energy deficit in the plant (Kulichikhin et al. 2008).

Since all experimental conditions represent simplified environments (Natale et al. 2010), results from this type of experimental analysis must be cautiously considered when trying to predict the behaviour of plants in the field. Nevertheless, the results obtained in this study allow us to highlight some features of the germination and establishment processes of *T. ramosissima* which may help to understand local variations in its invasive capacity. Our results support field observational studies conducted by Reynolds et al. (2014) on the invasion mechanisms employed by exotic species in riverside landscapes. The author showed that the rainfall constitutes an important element for the establishment of *Tamarix* in the flooded plains, where no high salt concentration was registered; this, was probably caused by the occurrence of flooding events that washed off frequently the surface of that particular area. The authors also affirm that any increment in the salt concentration present in the soil must have occurred gradually after the trees were established. According to Pardos (2004), sporadic soil flooding might favour the germination and enable the establishment of new populations by clearing the vegetation and laying down new sediments.

The data published by Natale et al. (2014) also shows a similar situation. In this work the authors found that the salinity levels of flooding areas where the *Tamarix* patches were found (0.69 dS/m) were substantially lower that the levels found in the patches of xerophytic native forest (13 dS/m). These field results together with the data obtained in the laboratory would indicate that the species of the *Tamarix* genus have an opportunistic strategy, where dispersion and colonization processes relay on flooding events that last enough for its establishment. In addition, a particular characteristic of the genus could also contribute to this scenario; *Tamarix* is capable of releasing an important amount of propagules at different times of the year (Baker 1974) increasing the probability of occurrence of the appropriate environmental conditions. Thereby the postulates of the resource-enrichment hypothesis (Davis et al. 2000), which proposes that in order for an invasion to succeed the adequate number of propagules must be released during a time of high resource availability, would be fulfilled (Gilbert & Lechowicz 2005). This hypothesis should, however, be corroborated in the future through experimental field trial.

Finally, the present results give rise to some recommendations for the generation of control strategies of this genus in riparian areas. According to the results obtained in the trial with seeds, one short flooding event occurring during seed production time, could favor massive germination and therefore seedlings establishment. While during long lasting flooding events, The advance of the patch would occur through aerial portions of the plant that remain on the surface of the substrate.

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