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Germination Limits Survival of *Trithrinax campestris* (Palm Tree) in Argentina

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Between 1934 and 2010, the palm tree *T. campestris* population in Entre Ríos province (Argentina) decreased significantly, mainly due to the advance of the agricultural frontier. The aims of this work were to analyze this dramatic geographic decrease of *T. campestris* and to study the endogenous and exogenous factors that determine its low germination percentage and limited survival. Our results showed that factors limiting *T. campestris* population growth even under protected areas destined to field bovine production include the soil chemical properties (fields with *T. campestris* plants had lower pH, EC and sodium concentration and lower OM, concentrations of minerals and CEC values than those without palm plants) and the solute leakage during seed imbibition. When *T. campestris* seeds were incubated for germination at both 25° and 35°C for one year, none of this

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seeds germinated; however, seed viability was significantly high at the end of the imbibition period. The rainfall changes associated with the Global Climate Change occurred since the 1970s would also limit the survival of this palm in its native environment.

Keywords: Geographic distribution; global climate change; land use change; seed germination; solute leakage.

1. INTRODUCTION

The Mesopotamian Espinal, in Entre Ríos province, Argentina, comprises forests belonging to the Ñandubay district in the Espinal phytogeographic Province. The Espinal Province is an ecoregion located between 28º and 40º S, to the south of the Chaqueño Park, covering approximately 329,395 km^2 and forming an arch that surrounds the Pampas Grassland ecoregion. The rural landscape of the Mesopotamian Espinal in most areas of the Entre Ríos province has been modified due to the advance of the agricultural frontier [1,2].

The palm tree *Trithrinax campestris* (Burmeist.) Drude & Griseb. ('caranday') is originally from Uruguay and Argentina. It is very resistant to drought and one of the most cold-hardy palms in the world. This palm is naturally found in groups of several individuals or in large forests where it is strongly dominant. Its leaves, which are among the hardest in palm trees, stay on the palm for several seasons after dying, covering the trunk and making this species very recognizable. This palm shows hermaphrodite plants (a single specimen producing viable seeds), and its flowering is very short and not very ramified. Germination is difficult, erratic, and requires no less than six months from sowing. Fibers and fruits are used for weaving and to produce a palm wine respectively. However, plants are worldwide appreciated as a drought resistant ornamental small palm.

The Entre Ríos grain cropping increased continuously; at the same time fields from bovine production associated with the presence of *T. campestris* palms decreased (Mendoza, personal communication). Although we can speculate that it would be the result of a change in exogenous factors such as temperature and rainfalls or seed attributes which depleted seed germination, nowadays there is no report neither the environmental nor the endogenous seed factors involved.

Weather generators have been used extensively in climate change and variability studies to determine the potential impact on agricultural production [3]. WGEN [4] is one of the earliest stochastic weather generator models widely used in the Decision Support System for Agrotechnology Transfer (DSSAT) crop models [4,5]. WGEN simulates daily time series of precipitation amount.

The aims of this work were to analyze the dramatic geographic decrease of *T. campestris* in Entre Ríos province and to study the endogenous and exogenous variables that determine its low germination percentage and limited future survival.

2. MATERIALS AND METHODS

The study was conducted in the Villaguay Department, Entre Ríos province, Argentina, between 2010 and 2013. The protected forest plots selected were located at 31º 47' 59'' S and 59º 11' 38'' W. The *Trithrinax. campestris* palm tree geographic area distribution was achieved through references in the literature [6] and aerial photographs. The distribution of *T. campestris* plants from the limit imposed by the fence of the protected forest plot was determined with a GPS GARMIN eTrex Legend model (Kansas, USA).

The soil chemical properties of composed samples (30 cm depth) from protected forest plots (around 10,000 m^2 each) with and without *T. campestris* plants (ten protected forest plot each) were determined in triplicate. Electrical conductivity (EC), organic matter (OM) and pH were analyzed in a 1:5 (v/v) water extract. Nutrient concentration analysis included nitrogen (Kjeldahl method), phosphorus (colorimetrically), potassium, calcium, sodium and magnesium (atomic absorption). The cation-exchange capacity (CEC) was determined with 1 M ammonium acetate at pH = 7.

Four replications of 100 seeds were uniformly distributed on a single sheet of filter paper adequately wetted with 40 ml of distilled water (and weekly rewetted) in transparent polyethylene boxes and incubated for six months in two germination chamber at 25° or 35°C respectively. Two types of seeds were tested; (a) 'large seeds', which showed 1.19±0.031 g $seed^{-1}$ and a diameter of 13.0±1.13 cm $seed^{-1}$ and (b) 'small seeds', which showed 0.64±0.034 g $seed^{-1}$ and a diameter of 10.6±0.87 cm $seed^{-1}$.

To estimate the viability of seeds and solute leakage, the tetrazolium test and the electrical conductivity test [7] were performed.

The experimental design was a completed randomized block. Data were subjected to analysis of variance and means were separated by Tukey tests ($P < 0.05$).

Weather rainfall changes were tested using WGEN PAR program [4] to generate monthly parameters from daily values of rainfall, for which series of 30 years were taken starting from January 1934. The precipitation component of WGEN is a Markov chain-gamma model. A first-order Markov chain was used to generate the probability of occurrence of wet or dry days considering whether the previous day was wet or dry. When a wet day is generated, the two-parameter (α and β) gamma distribution is used to generate the precipitation amount, where α and β are shape and scale parameters, respectively. For $0 < \alpha > 1$, the density function of precipitation decreases as precipitation increases. The shape is appropriate for precipitation amounts since small values occur more frequently than larger ones.

Weather records such as daily maximum-minimum air temperature and precipitations between 1934 and 2010 were taken from the meteorological station of the National Institute of Agricultural Technology (INTA) Experimental Station Paraná, Entre Ríos province, Argentina (31º 85' S and 60º 53' W and altitude 116.5 m).

3. RESULTS

Between 1934 and 2010, the geographic area occupied by the *T. campestris* palm in Entre Ríos province (Argentine) decreased significantly (Fig. 1).

The chemical analysis showed that fields with *T. campestris* plants had lower pH, EC and sodium concentration than those without palm plants. On the other hand, OM, concentrations of minerals (nitrogen, calcium, magnesium, potassium) and CEC values were higher in fields with *T. campestris* plants than in those without its (Table 1).

The concentration of *T. campestris* palms was higher in the border of the protected forest plot (Fig. 2A) and decreased towards the center of the protected forest plot. The last plants were observed 82.00(±19.91) meters from the external limit of the protected forest plot (Fig. 2B).

When *T. campestris* seeds of two different size ('large' and 'small') were incubated for germination at both 25° and 35°C for one year, none of this seeds germinated. Seed viability was significantly higher in 'large' seeds than in 'small seeds' during the imbibition at both 25° and 35°C (Table 2).

Table 1. Soil chemical properties in fields with and without *Trithrinax campestris* palm trees in Entre Ríos province (Argentina). Means (n = 10) ± standard errors are indicated

Chemical properties	Field grown with *T. campestris* plants	Field grown without *T. campestris* plants
pH	$5.94{\pm}0.102^{b}$	$8.40{\pm}0.144^{a}$
Electric conductivity (ds m^{-1})	$0.05{\pm}0.095^{b}$	$0.56{\pm}1.064^{a}$
Organic matter (%)	$6.91{\pm}0.536^{a}$	$3.30{\pm}0.256^{b}$
Nitrogen (%)	$0.33{\pm}0.026^{a}$	$0.19{\pm}0.015^{b}$
Phosphorus (ppm)	$7.39{\pm}1.195^{a}$	$7.00{\pm}1.032^{a}$
Calcium (meq 100 g^{-1})	$22.36{\pm}0.896^{a}$	$14.00{\pm}0.56^{b}$
Magnesium (meq 100 g^{-1})	$4.98{\pm}0.29^{a}$	$3.00{\pm}0.175^{b}$
Potassium (meq 100 g^{-1})	$1.01{\pm}0.245^{a}$	$0,34{\pm}0.083^{b}$
Sodium (meq 100 g^{-1})	$0.58{\pm}0.102^{b}$	$1.64{\pm}0.288^{a}$
Cation exchange capacity (meq 100 g^{-1})	$30.17{\pm}0.782^{a}$	$19.00{\pm}0.492^{b}$

Different lower-case letters indicate significant differences ($P \leq 0.05$) between plots

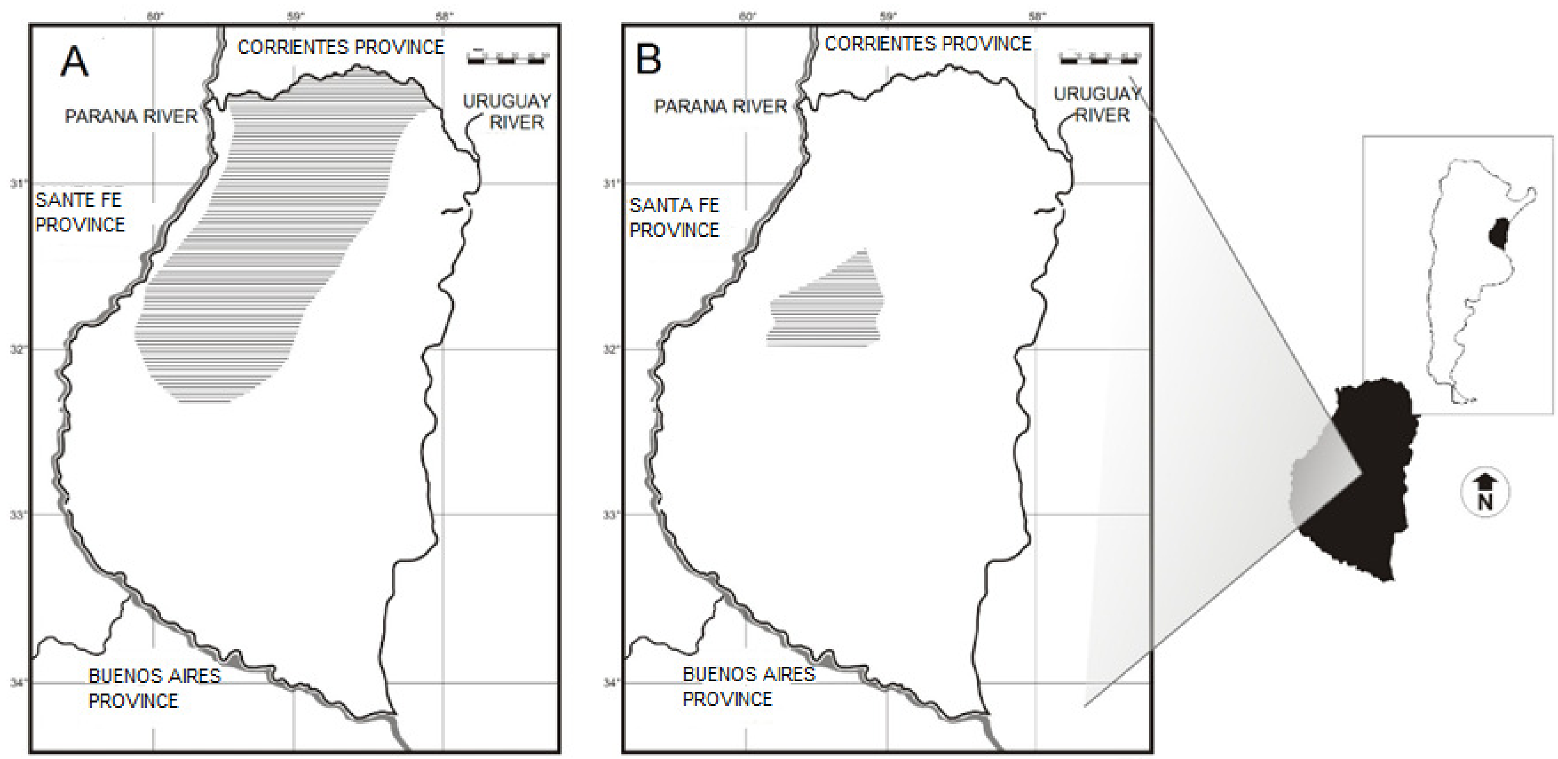

Fig. 1. Decrease in *Trithrinax campestris* distribution from 1930 [6] (A) to 2010 (B) in Entre Ríos province (Argentina)

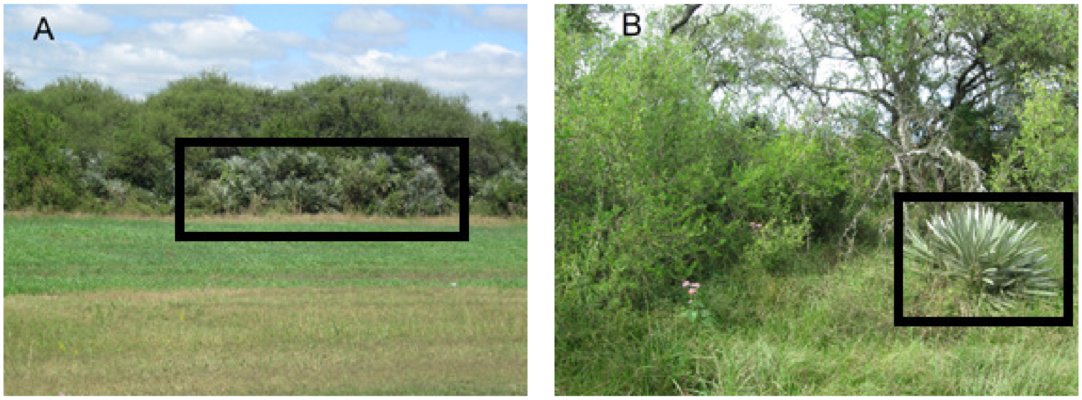

Fig. 2. *Trithrinax campestris* plants grown near the limit of the protected forest plot (A) or deeply inside it (B). The maximum distance from the last *T. campestris* found inside the protected forest plot and the external its limit was 82.00±19.91 meters (n = 10)

Table 2. Germination percentage (%) of *T. campestris* seeds after one year at 25 or 35°C and seed viability at the end of the experiment. Seed viability is the mean of four replications of 100 seeds each (n= 4) and the standard errors are indicated

Germination percentage (%)				Seed viability (%)			
'Large seeds'		'Small seeds'		'Large seeds'		'Small seeds'	
25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
0.00	0.00	0.00	0.00	$93.33{\pm}1.36^{aA}$	$91.25{\pm}1.11^{aA}$	$88.33{\pm}1.58^{aB}$	$83.37{\pm}2.12^{aB}$

Different lower-case letters indicate significant differences between each seed size ($P \leq 0.05$), while different capital letters indicate significant differences between temperature germination

Table 3. Changes in fresh weight and volume in both fruit and seeds during the last three months of maturation on *T. campestris* mother plants. Each value is the mean of four replications of 100 seeds each (n= 4) and standard errors are indicated

	October	November	December
Fresh weigh (g fruit^{-1})	0.866±0.013	1.109±0.016	3.266±0.089
Fresh weight (g seed^{-1})	0.452±0.008	0.866±0.013	1.334±0.034
Fruit volume (dm^3 fruit^{-1})	2.161±0.028	2.338±0.028	2.348±0.062
Seed volume (dm^3 seed^{-1})	0.535±0.013	0.985±0.018	1.168±0.034

During the first two days of imbibition at both 25° and 35°C, *T. campestris* seeds absorbed water but hardly increased their fresh weight (Figure 3A). Although 'large seeds' achieved higher fresh weight than 'small seeds', there was no change in the water absorption pattern (Fig. 3B).

Although inflorescences are initiated in April (data not shown) a significant increase in fresh weight and volume in both fruits and seeds was observed during the three last maturation months before harvest time (Table 3).

When fruits, immature seeds and matured seeds were soaked in water, solute losses were significant. Mature *T. campestris* seeds showed the highest solute losses (Fig. 4).

Table 4. Maximum and minimum temperatures (°C) for Entre Ríos province (Argentina) during the last eight decades. Standard errors are indicated

Years	Temperature (°C)	
	Maximum	Minimum
1934-1939	$23.50{\pm}1,38^{a}$	$12.161.12^{a}$
1940-1949	$24.07{\pm}1,48^{a}$	$12.39{\pm}1.18^{a}$
1950-1959	$23.75{\pm}1,48^{a}$	$12.23{\pm}1.24^{a}$
1960-1969	$23.97{\pm}1,47^{a}$	$13.02{\pm}1.23^{a}$
1970-1979	$23.35{\pm}1,43^{a}$	$12.95{\pm}1.25^{a}$
1980-1989	$23.52{\pm}1,51^{a}$	$13.09{\pm}1.31^{a}$
1990-1999	$23.88{\pm}1,41^{a}$	$13.64{\pm}1.24^{a}$
2000-2010	$24.09{\pm}1,42^{a}$	$12.97{\pm}1.29^{a}$

Different lower-case letters indicate significant differences ($P \leq 0.05$) in maximum or minimum temperature

The climatic series between 1934 and 2010 plotted in Table 4 showed that there were no significant temperature changes in Entre Ríos province during these years.

In addition, the same climatic series showed an increase in rainfall since 1970s (Table 5). The use of WGEN, a model to generate long-term daily weather variables for crop simulations showed significant β coefficients of the Gamma distribution fit for precipitations. The only trends in P (W/W) having values of r > 0.7 were in March (negative trend) and May, which presented the greatest positive slope. On the another hand, trends in P (W/D) having values of r > 0.7 were found in December, February, April, May, October and November (all positive trends), with the steepest slope in December. If a day is wet WGEN calculates the amount of precipitation using a gamma distribution expressing two, always positive parameters, α and β (shape and scale, respectively). February, March, May, September and October showed negative α trend values (r> 0.7), while only October presented a positive trend in β with r> 0.7 (Table 6).

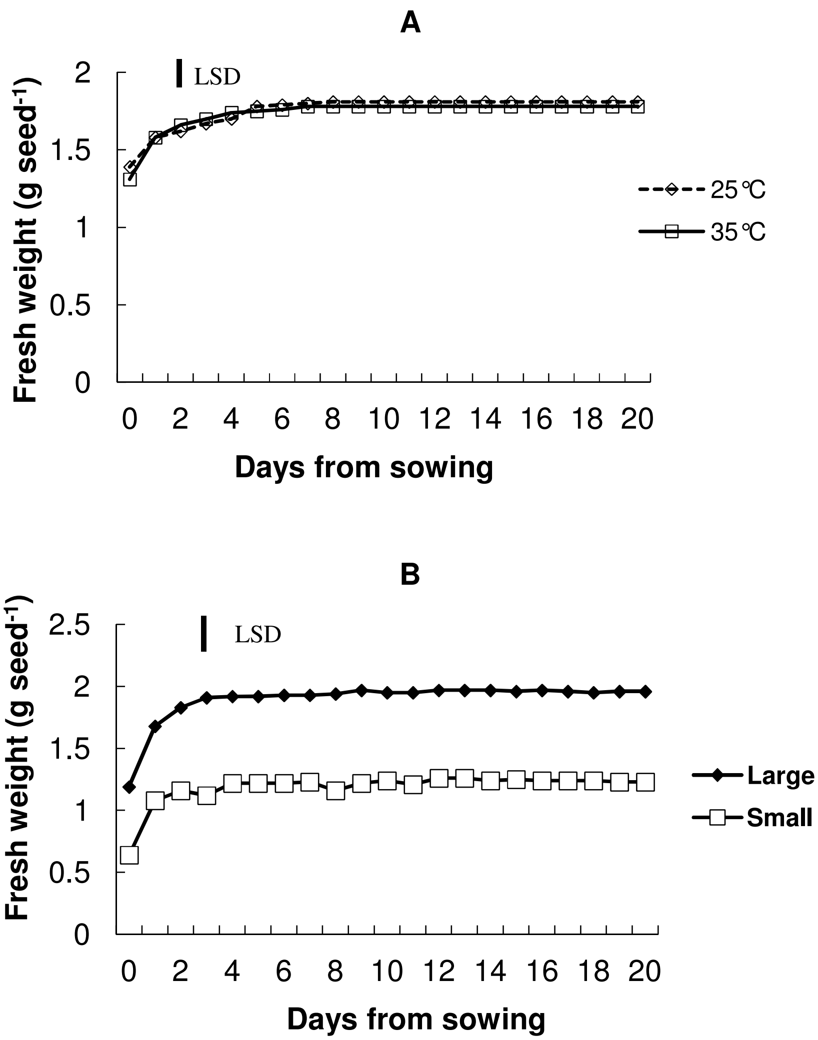

Fig. 3. Increase in fresh weight in *T. campestris* seeds germinated at 25°C and 35°C ('large seeds') (A) or with different seed size (B). Vertical lines indicate least significant differences (LSD)

Table 5. Changes in monthly and total rainfall (mm) for Entre Ríos province (Argentina) during the last eight decades

Decade	Month												Total annual precipitation
	J	F	M	A	M	J	J	A	S	O	N	D	
1930-1939	139.70	36.31	49.44	73.27	24.99	42.26	39.31	28.73	50.40	94.49	85.29	131.06	795.24
1940-1949	90.17	57.76	73.69	118.94	55.42	50.56	24.42	26.17	57.26	92.70	107.97	93.91	848.97
1950-1959	150.36	30.33	44.04	88.68	64.48	50.01	27.23	35.42	27.33	109.57	102.53	66.70	796.68
1960-1969	108.73	32.12	65.50	79.88	27.71	21.74	29.51	29.58	40.99	89.24	92.12	113.34	730.46
1970-1979	130.15	43.42	73.69	99.16	43.67	39.40	32.18	36.05	77.33	85.82	133.46	112.30	906.63
1980-1989	125.96	69.29	47.11	102.94	62.29	33.22	30.68	33.61	71.96	111.17	108.81	112.27	909.31
1990-1999	109.22	36.58	65.17	142.34	69.91	41.76	36.03	32.78	38.02	133.21	124.72	140.91	970.65
2000-2010	95.70	45.22	63.60	179.80	39.06	53.66	21.32	50.78	76.80	143.04	157.94	168.60	1095.52

Table 6. The slope of linear trend of monthly P (W/W), P (W/D), α and β parameter where the correlation coefficient was greater than 0.7; for 30 years starting from January 1934 for Entre Ríos province (Argentina). P (W/W) is the probability of a wet day on day i given a wet day on day i-1, and P (W/D) is the probability of a wet day on day i given a dry day on day i-1. α and β are the shape and scale parameters of the Gamma distribution, respectively

	P(W/W)		P(W/D)						α					β
	March	May	December	February	April	May	October	November	February	March	May	September	October	October
Slope	-0.0018	0.0035	0.0028	0.0016	0.0023	0.0006	0.0018	0.0017	-0.0102	-0.0066	-0.0047	-0.0041	-0.0060	0.2895

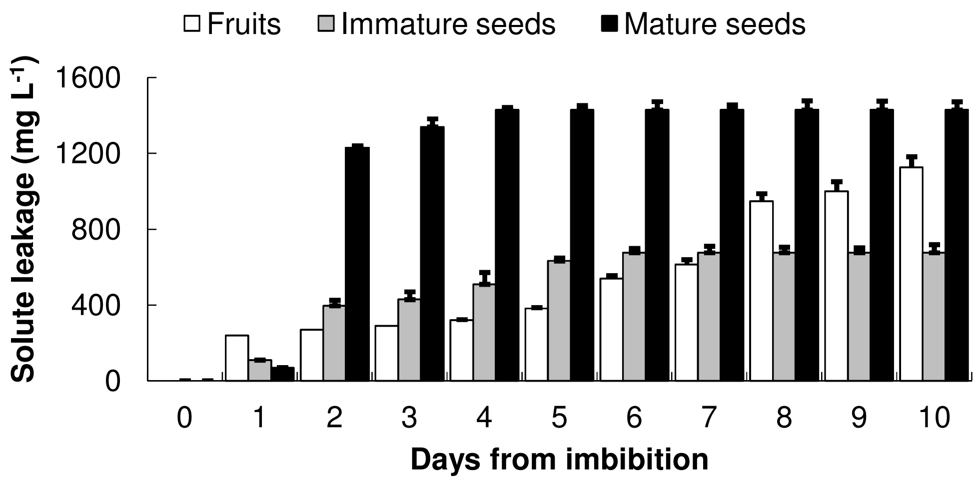

Fig. 4. Changes in solute leakage during the first ten days of the imbibition period of fruits, immature seeds and mature seeds of *T. campestris*. Vertical lines indicate standard errors

4. DISCUSSION

Thus, the only option currently available to produce seeds of native tree species is to use natural forest remnants as seed production fields, which do not allow controlling the environmental production factors. Additionally, these seed production fields have to be located as close as possible to the site under restoration to increase the chances of using locally adapted genetic materials [8,9]. Unfortunately, the *T. campestris* palm tree distribution in Entre Ríos province significantly decreased between 1930 and 2010 (Fig. 1) [6].

The *T. campestris* palm tree population decreased mainly due to the advance of the agricultural frontier [1,2,10]. However, the results of the present study showed that there are other reasons that limit the population growth even under protected forest plot destined to field bovine production. For example, we found that *T. campestris* plants need relatively acid soils with low EC and high OM and nitrogen content with high CEC (Table 1). In addition, *T. campestris* palms need open fields to grow. We found plenty of plants near the limit of the protected forest plot (Fig. 2A) but only isolated plants inside it (Fig. 2B). This is in agreement with the fact that the last *T. campestris* plants were observed 82.00 (±19.91) meters from the external limit of the protected forest plot.

The propagation of palms for agricultural and ornamental purposes is accomplish typically by seeds. Most palms seeds take 100 days or more to germinate, with an average germination rate of less than 20% [11]. Although it is possible to find some young palm seedlings in their natural protected forest plot (unpublished data), we did not achieve germination of *T. campestris* seeds after six months at either 25° or 35℃ (Table 2). For subtropical palms, 27℃ appears to be an optimal germination temperature [12] although some desert palms can be germinated best at 34°-37℃ [13-16]. Our experimental design comprise the suggested temperature range for palm germination.

Meerow [11] indicated that the greatest cause of poor germination is dead seeds but this would not be the case in *T. campestris* because seeds kept higher viability percentages at the end of the experiments (Table 2).

A seed that does not germinate after harvest is a dormant seed [17]. Dormancy is considered the principal cause of the low germination rates seen in many palm species [18,19]. One of the main external reason for seed dormancy is the presence of impermeable teguments, which limit seed water absorption [11,20]. However, in *T. campestris* seeds this possibility must be ruled out because of the rapid increase in seed fresh weight under water imbibition observed in both 'large' and 'small' seeds (Fig. 3B) at either 25° or 35℃ (Fig. 3A).

Another source of seed dormancy is the presence of immature seed embryos [21], but this can be overcoming with different pre-treatments [17,22,23]. However, most of pre-treatment tested have been tested in *T. campestris* seeds have led to no positive results (data not shown). However, embryo immaturity must not be ruled out in *T. campestris* seeds.

T. campestris inflorescences emerge during April every two years and seeds fall to the ground during December (data not shown). However, during the last three developing months before harvest, we found a great increase in fresh weight and size of both fruits and seeds (Table 3).

During germination, water uptake by a mature dry palm seed is triphasic, with a rapid initial uptake followed by a plateau phase. Water uptake further increase only after germination is completed, as the embryonic axes elongate [24]. Within a short time of rehydration, the membranes return to their more stable configuration, at which time solute leakage is curtailed [25]. However, our results showed that the leakage of solutes (presumably from the embryo axe) in mature seeds was extremely high during the first ten days of imbibition (Fig. 3). Our findings suggest that seed invigoration by hydration treatments (data not shown) did not result from the fast repair of previously sustained deterioration and would be delaying embryo maturation. However, this hypothesis must be tested in future experiments.

The key question is why *T. campestris* forests established and grew through a prolific seed production and dispersal in the past but failed during the last decades.

Based on the higher solute leakage data (Fig. 3), we speculate that the decrease in germination of *T. campestris* seeds would be associated with a decrease in solute reserves, which are needed for embryo maturation. The maternal environment is an important determinant of seed yield and quality [26].

The analysis of daily temperature and rainfall records between 1930 and 2010 (Table 5) showed no changes in both mean maximum and minimum temperatures but a sustained increase in total rainfall, especially from the 1970s according with previous reports in other Argentinean countries [27,28,29].

WGEN has been used for generate long-term weather data for crop simulations [30,31] including for Argentina [32]. Our results showed that by decreasing the values of the α of the Gamma distribution becomes more skewed to the left, it moves towards lower values of precipitation and, somehow compensates for the increase in the probability of having a wet day, so as to be more likely to have wet days, but daily precipitation amounts tend to be lower. Similar results were presented by Keller et al. [33] who found that the precipitation sums at all stations of Switzerland exhibited a decrease in summer precipitation towards the end of the 21st century, which was predominantly caused by a decrease in the number of wet days, while the intensity remained similar. Rajczak et al. [34] also projected patterns in mean precipitation and wet-day frequency, which showed spatial and seasonal variations in Europe.

Many species, including palms, are currently expanding their ranges in response to climate change [35]. The recent climate warming has led to changes in the behaviour and distribution of species, in the composition of communities and interactions among them and in the structure and dynamics of ecosystems in numerous types of habitats [36]. However, palm crop limitations are associated with a temperature increase [37] or drought [38]. There is no report showing a change in survival possibilities related to rainfall increase in the native environment such as observed in *T. campestris*.

5. CONCLUSIONS

The geographic distribution of *T. campestris* in Entre Ríos province (Argentina) has shown a significant decrease due to the advance of the agricultural frontier; only between 2,000 and 2,500 plants remain reproductive (unpublished data). However, the lack of new palm seedlings would be related to an extremely low seed germination. The high solute leakage from *T. campestris* seeds at the beginning of the imbibition period and the change in rainfall since the 1970s would be limiting the survival possibility of this palm in its native environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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