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Short communication

Germination of six native perennial grasses that can be used as potential soil cover crops in drip-irrigated vineyards in semiarid environs of Argentina

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Native desert plants have structural adaptations that maximize photosynthesis rates and minimize water loss. They can be successfully utilized as soil cover crops in drip-irrigated vineyards where water availability is low. The objective of this paper is to study seed germination conditions and to recommend which best species is most apt as soil cover crop in drip-irrigated vineyards. Seed weight (the higher the seed weight, the greater the success of mechanical seeding) and germination tests (at 15, 20, 25, 30, and 35 °C in light and dark conditions) were carried out for six species native to Mendoza, Argentina. Germination percentage (GP) and mean time germination (MTG) were calculated. *Digitaria californica* (C_4) had the highest GP (97% in light condition), which is recommended as a cover crop because of its seed germination potential. *Pappophorum phillippianum* (C_4) had a 70% GP in light conditions (regardless of temperature) and a high seed weight. *Leptochloa dubia* (C_4) and *Sporobolus cryptandrus* (C_4) reached the highest GP at the highest temperature, although *S. cryptandrus* had the lowest seed weight. *Nassella tenuis* (C_3) averaged 54% GP at 25 °C in light conditions. The GP of *Setaria leucopila* (C_4) was not affected by temperature (26% in light, and 16% in darkness). Based on the GP results, *S. leucopila* was the worst choice of the six species. Hence, during seeding, soil temperature should be high (>20 °C) to ensure a rapid plant establishment of all species.

1. Introduction

Several native herbaceous species grow in arid regions of Argentina, most of which are drought-tolerant perennial grasses such as *Sporobolus*, *Nassella*, *Setaria*, *Digitaria*, *Pappophorum*, and *Leptochloa*, among others (Ruiz Leal, 1972). These species have structural and physiological adaptations and water-conserving strategies that maximize photosynthesis rate and minimize water loss (Gibson, 1998). They are well suited to drip-irrigated vineyards (Ferrari and Parera, 2014; Uliarte, 2013) where there is less humidity than in surface-irrigated vineyards. Non-native species are commonly used as vegetal cover in surface-irrigated crops, but they cannot be used with drip-irrigated crops due to reduced water availability (Logan, 2009).

Cover crops in vineyards help preserve soil structure, improve water infiltration, increase soil biological activity, aerate roots, reduce soil compactness, add organic matter, and improve traction for agricultural machinery after rains or irrigation (Ingels et al., 1998). These advantages lead wine growers to use cover crops, either by seeding or encouraging spontaneous vegetation.

Seeds of native species are sold for use as cover crops in vineyards in the USA and Canada (Ingels et al., 2005). In Australia, projects for studying native species and their use in soil management in commercial vineyards are currently being carried out (Penfold et al., 2005). Experiments in Argentina have not successfully established native species in surface-irrigated vineyards (Cavagnaro and Dalmasso, 1986), but in drip-irrigated vineyards, spontaneous (without seeding) and native species (with seeding) have been successfully established in no-till soils (Uliarte, 2013). Seeds of these species are not commercially available, partly because there is not enough information about their growth, reproductive development, and germination capacity.

Seeds of *Setaria lachnea*, *Setaria leucopila*, and *Sporobolus cryptandrus* have a very low germination percentage (GP) (Sartor

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and Marone, 2010; Schrauf et al., 1998). However, when *S. lachnea* seed coats are removed, GP increases significantly, since inhibitors in the seed coat are eliminated (Schrauf et al., 1998). Sartor and Marone (2010) report that *S. cryptandrus* and *S. leucopila* disperse a large amount of dormant seeds; that seed dormancy ensures favourable conditions for reproductive success (Harper, 1977). The optimal temperature for germination of *Sporobolus spicatus* was 35 °C (El-Keblawy et al., 2009) and for *Sporobolus ioclados,* 20–30 °C (Khan and Gulzar, 2003). *Stipa longiglumis* (Phil.) reached almost 100% GP at 22–27 °C when anthecium (caryopses with glumes and glumellas) were seeded on the surface (Hernández, 1999). This demonstrates the positive effect of light on germination of this species. The same effect was also verified for *Digitaria ciliaris* (Vivian et al., 2008) and *Leptochloa chinensis* (Benvenuti et al., 2004). A high GP has been documented for *Digitaria californica* and *Pappophorum caespitosum* at 30 °C (Sartor and Marone, 2010).

The aim of this research was to determine the optimal germination conditions of six native species and recommend the best species for reliably establishing as soil cover crops with mechanical seeding, based on the comparison of GP and dispersal-unit weight.

2. Materials and methods

Six predominant species from native vegetation spontaneously established in drip-irrigated vineyards (Uliarte, 2013) were evaluated: *S. leucopila* Phil., *D. californica* (Benth.) Henrard *var. californica*, *Pappophorum phillippianum* Parodi, *Leptochloa dubia* (Kunth) Nees, *S. cryptandrus* (Torr.) A. Gray, and *Nassella tenuis* (Phil.) Barkworth. *N. tenuis* is a C_3 species with winter–spring growth and the other five are C_4 species with summer growth.

The trial was conducted in the experimental field of INTA (National Institute of Agricultural Technology), Mendoza, Argentina (33°00′21″S; Long. 68°51′53″W; 929 masl). Soils are typically alluvial with loamy-clay texture; rainfall occurs mainly in summer (200–400 mm annual), and evaporation rates are 6–7 mm d^{-1} in January (Catania et al., 2012). Areas between vines in Mendoza's vineyards are managed with cover crops or bare soil. The use of cover crops is widespread, either by seeding or spontaneous developing.

Seeds were harvested from plants cultivated in 30 L pots located in INTA, EEA (Agricultural Experimental Station) Mendoza in November to December 2011. Average seed weight was based on 1000 seeds. Germination was done in a Jacobsen growing chamber at 15, 20, 25, 30, and 35 °C. All seeds were disinfected with sodium hypochlorite (active chlorine 2 g L^{-1}) and rinsed three times with distilled water. Each treatment was conducted in a 90 mm diameter Petri dish with 25 seeds arranged on moistened filter paper over a layer of cotton. There were six repeated treatments for each species at each temperature: three were in light conditions, that is, for each 24-h cycle, 15 h of light and 9 h of darkness; three were in continuous darkness, which were covered by a sheet of aluminium foil (Funes et al., 2009).

Seed germination in light conditions was monitored every two days for a period of 28 days. At the end of this period, germination in darkness was verified. Seeds were considered germinated by the presence of a radicle (Sartor and Marone, 2010). After 28 days, GP was calculated and germination speed was estimated based on the mean time of germination (MTG) (Brenchley and Probert, 1998). The relative light germination index (RLG) was calculated with the following equation: RLG = LG/(LG + DG), where LG is the light germination percentage and DG is the darkness germination percentage (Milberg et al., 2000).

The caryopses (simple dry indehiscent fruit) of *S. cryptandrus* and *S. leucopila* do not germinate easily since they have some physical or chemical dormancy. This may be due to the impermeability of the seed coats (Jackson, 1928) or the presence of inhibitors (Schrauf et al., 1998). Hence, germination tests were carried out with scarified seeds for these two species, rubbed about 15 times between two pieces of sandpaper.

As the first step, an ANOVA was performed with GP and MTG. Then, GP regression based on temperature was determined for each species; only the statistically significant ones were graphed. Finally, an MTG regression was carried out for each carbon fixation pathway (C_3 and C_4) based on temperature.

3. Results and discussion

Each species' GP was affected by light and temperature ($p < 0.001$). The statistical interaction between the fixed factors (species, temperature, and light) on GP was significant ($p < 0.001$); therefore, each species was evaluated individually. Different responses were observed in GP' ANOVA of each species. *S. leucopila* and *D. californica* were not affected by temperature or the interaction of temperature and light; the remaining four species were affected by the interaction of both variables (Table 1).

The germination of scarified *S. leucopila* seeds was marginally affected by light condition ($p = 0.0597$), but not significantly affected by temperature nor the interaction of the two variables (Table 1). According to Funes et al. (2009) seed germination was no affected by light conditions since the RLG was 0.62. The average GP for *S. leucopila* was 26 ± 13.9% in light conditions, and 16 ± 10.9% in darkness (Fig. 1A). In a test performed in Arizona, USA, *S. macrostachya* and *S. leucopila* seeds had high and similar germination rates at winter, spring, and summer temperatures (Roundy and Biedenbender, 1996). In this study, each *S. leucopila* seed-dispersal unit, a two-floret spikelet, weighed 1.61 mg.

Germination of *D. californica* seeds was not significantly affected by temperature, but it was by light (Table 1). The RLG index was 0.52, so according to Funes et al. (2009), the seed germination of this species was no affected by light conditions. However, statistically more seeds germinated in light (97 ± 3.5% GP) than in darkness (89% ± 6.8% GP) (Fig. 1B). Vivian et al. (2008) found that the GP

Table 1
Degrees of freedom (df) and F-values with level of significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.06$; ns: $p > 0.06$) of the effect of temperature (*T*), light condition (*L*), and the statistical interaction of both variables (*T* * *L*) on the germination percentage for each species.

Variable	*S. leucopila*		*D. californica*		*S. cryptandrus*		*P. phillippianum*		*L. dubia*		*N. tenuis*	
	df	F	df	F	df	F	df	F	df	F	df	F
Model	9	2.0 ns	9	2.3 ns	9	27.7 ***	9	9.2 ***	9	16.0 ***	9	15.2 ***
T	4	2.0 ns	4	0.7 ns	4	58.1 ***	4	5.2 **	4	24.7 ***	4	13.6 ***
L	1	4.0 *	1	15.6 ***	1	1.8 ns	1	43.2 ***	1	17.5 ***	1	58.2 ***
T * *L*	4	1.3 ns	4	0.4 ns	4	3.2 *	4	4.7 **	4	6.8 **	4	6.1 **
Error	18		20		19		20		20		20	
Total	27		29		28		29		29		29	

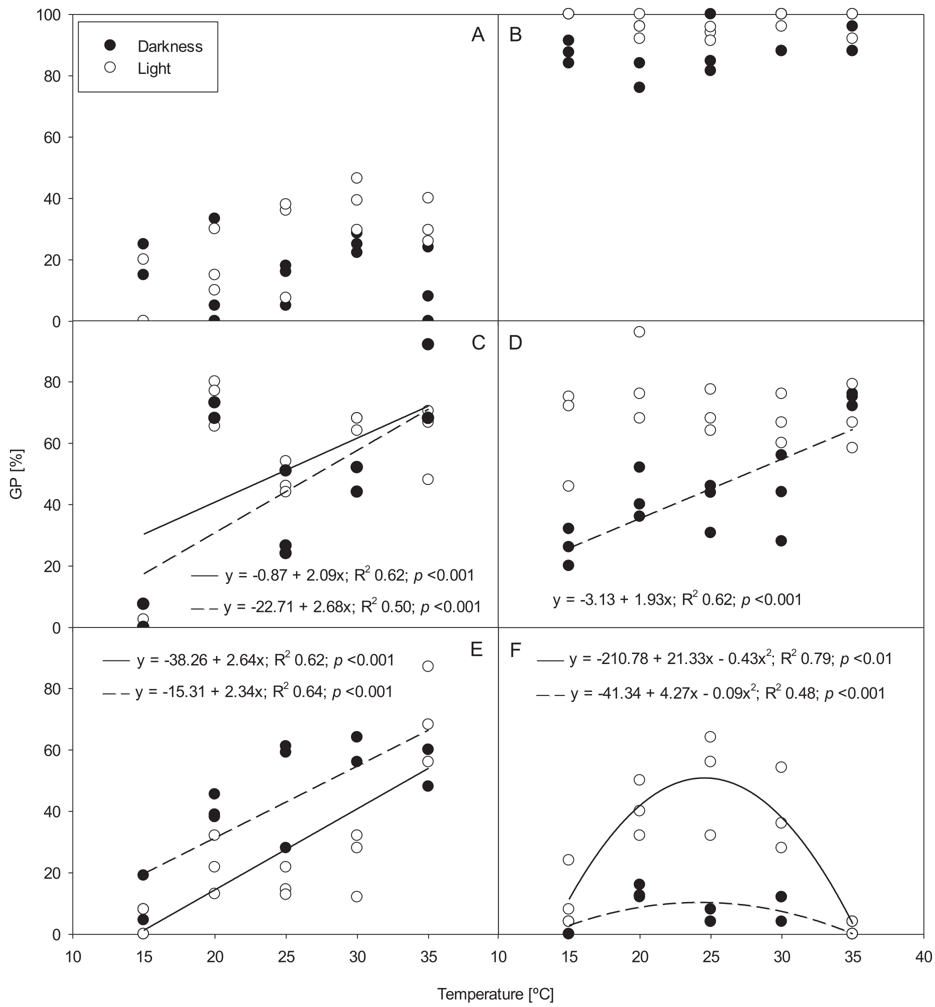

Fig. 1. Germination percentage (GP) at different temperature in light (white circles) and in dark conditions (black circles) for *S. leucopila* (A), *D. californica* (B), *S. cryptandrus* (C), *P. phillippianum* (D), *L. dubia* (E), and *N. tenuis* (F). Significant linear and quadratic regressions are shown for light (solid lines), and darkness (short dashed lines).

of *D. ciliaris* was positively affected by light and registered a significant effect of temperature, the optimum being 20–30 °C. Hence, higher germination can be achieved by placing the spikelet directly on the soil surface or seeding shallowly. The one-floret spikelet was the dispersal unit of *D. californica*; each one weighed on average 0.76 mg. For successful crop establishment by mechanical seeding, it is necessary to use heavy seeds with uniform shape for a good distribution (Ingels et al., 1998).

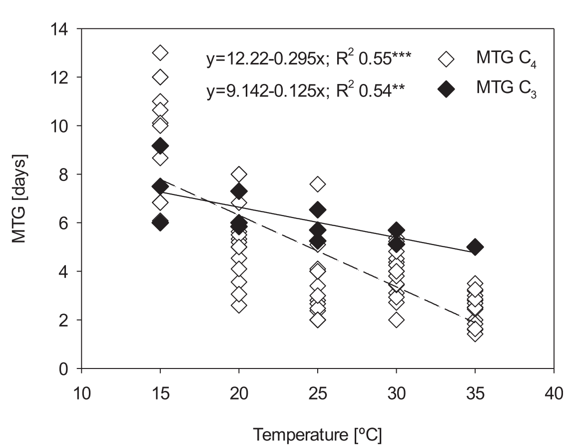

Fig. 2. Mean time of germination (MTG) at different temperatures (°C) for C_4 and C_3 species. Dashed and solid lines show C_4 and C_3 linear regressions, respectively.

The ANOVA of GP for *S. cryptandrus*, *P. phillippianum*, *L. dubia*, and *Ntenuis* showed a significant interaction between temperature and light condition (Table 1). GP was assessed against temperature, showing different effects of light and darkness on each species.

According to Funes et al. (2009) the seed germination of *S. cryptandrus* was no affected by light conditions (RLG 0.55). These seeds were scarified before germination, as they otherwise they would have had a very low GP (<10%) (Sartor and Marone, 2010). In Fig. 1C, the linear regression of GP to temperature is shown to each light condition (light and darkness were both statistically significant). This species germinates better at high temperatures; similar results were obtained by El-Keblawy et al. (2009) for *Sporobolus spicatus* and by Khan and Gulzar (2003) for *S. ioclados.* The caryopses were the lightest seed-dispersal units of the tested species. Seeds weighed a very low 0.10 mg, which would make mechanical seeding difficult, as stated above. However, this could be solved with seed treatments such as pelleting.

In the presence of light, *P. phillippianum* germinated similarly under all temperatures, reaching an average 70 ± 11.3% GP (p 0.5651). Seed germination was not affected by light conditions; the

RLG was 0.61 (Funes et al., 2009). On the other hand, a linear relationship was observed in seeds germinated in darkness ($p < 0.001$): the higher the temperature, the higher the GP (Fig. 1D). Sartor and Marone (2010) reported an average GP of 70% in darkness at 30 °C for *P. caespitosum*. The seed-dispersal units or anthecium were one of the heaviest, averaging 2.57 mg.

The GP of *L. dubia* or *Diplachne dubia* (Kunth) Scribn had a statistically significant relationship with temperature in both in light and darkness ($p < 0.001$). *Diplachne* are native to warm and semi-warm climates. In light and dark conditions, GP increased significantly at higher temperatures (Fig. 1E). GP was always greater in darkness than light; however, considering an RLG of 0.35 the seed germination would not be affected by light conditions. Benvenuti et al. (2004) had similar results with *L. chinensis*, where the highest GP was at 25–35 °C in light conditions. The weight of these seed-dispersal unit or anthecium was light; each weighed 0.38 mg.

The GP response to temperature in *N. tenuis* caryopses was significantly different in light ($p < 0.01$) and darkness ($p < 0.001$), as shown by quadratic regressions (Fig. 1F). Seed-dispersal units (caryopses with awns) were the heaviest of the six species (2.82 mg). *N. tenuis* has a winter–spring growing cycle. Thus, increased temperature reduced GP considerably. High temperatures (above 30 °C) are unfavourable for growth (Cavagnaro, 1988). Similarly, the optimal range of germination temperatures for *S. longiglumis* was 22–27 °C (Hernández, 1999) and the GP was much higher in light. *S. longiglumis* and *N. tenuis* belong to the same taxonomic group, Stipeae. *N. tenuis*, for all temperatures, had a higher GP in light than darkness. It was the only species whose seed germination was positively affected by light (RLG 0.85); according to Funes et al. (2009) it could be classified as positive photoblastic. Hernández (1999) also concluded that *S. longiglumis* germinated better under light conditions, so the seeds should be shallowly seeded to achieve higher germination rates.

Only seeds germinated in light were considered in the MTG, as calculating MTG required partial counts of germinated seeds. The temperature significantly affected MTG ($p < 0.001$), carbon fixation pathway or type of species ($p = 0.013$), and type of species–temperature statistical interaction ($p = 0.004$). Fig. 2 shows the mean linear response of MTG to temperature to C_4 ($p < 0.001$) and C_3 species ($p = 0.004$). When temperature was increased from 15 to 35 °C, germination accelerated and MTG decreased six days in C_4 species, but only two days in C_3 species. Both MTG and GP of *N. tenuis* (C_3) were slightly modified by the increase of temperature because unlike the other species, it has a winter–spring growth.

As lower temperatures cause slower germinations, longer periods with sufficient soil moisture are necessary to ensure seed germination and plant establishment (Roundy and Biedenbender, 1996). However, long periods of time with sufficient soil moisture are rare in Mendoza, Argentina, especially between rows of vines in drip-irrigated vineyards, as annual rainfall is only 200–300 mm. Therefore, the faster seed germinate, the more successful the establishment of the cover crop. This research suggests that soil temperature should be above 20 °C during seeding to ensure rapid establishment, that is, MTG <5 days for C_4 species and MTG <6 days for C_3 species.

4. Conclusions

Soil temperature should be above 20 °C during seeding to ensure rapid and successful plant establishment (MTG < 5 days in C_4 species). *D. californica* achieved a high GP under almost all tested conditions. On the basis of its high GP, it is the species most recommended to seed as a cover crop. A previous seed treatment is recommended to facilitate mechanical seeding (*e.g.* pelleting), and should be seeded superficially to obtain the highest GP.

P. phillippianum should be seeded under light conditions near the surface or at high temperatures in darkness (deeply buried) to ensure high GP. Heavier seed-dispersal units would make mechanical seeding more effective. *L. dubia* had a high GP in darkness at the highest tested temperature. *S. cryptandrus* had a strong GP (highest at the maximum assessed temperature), but the lowest seed weight. This could make mechanical seeding difficult and the uniform establishment of plants less successful, unless seeds are treated before seeding. Pelleting, for example, increases seed weight and leads to better plant establishment, but the reduction of light reaching the seeds should be monitored. *N. tenuis* has winter–spring growth; it reached a moderate GP at moderate temperatures in light conditions. Temperature did not considerably affect germination velocity, and at 20 °C the MTG would be 6 days or fewer. It had heavy seed-dispersal units, but some seed treatment may be necessary to facilitate mechanical seeding (*e.g.* awns elimination). Finally, *S. leucopila* seeds had an average weight. A large number of seeds would be needed to obtain a high vegetal cover percentage (~100%) because of its lower GP. Based on the results presented here, it would be the least advisable species to seed as a cover crop in drip-irrigated vineyards.

To build on these results, other attributes of these species could be compared, for example, seed cost, seed treatments, success of mechanical seeding, biomass production, root development, management ease, and reseeding success.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2014.09.002.

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