Short communication

Inter– and intra–population variability in physical dormancy along a precipitation gradient

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ABSTRACT

Physical dormancy enables plants that occur in temporally stochastic and harsh environments to survive; thus, within the distribution area of a species, the percentage of seeds with physical dormancy is expected to increase towards more arid and unpredictable areas. There have been no previous studies evaluating both inter and intra-population variability in physical dormancy along a precipitation gradient. The aim of this study was to determine the inter- and intra-population variability in physical dormancy of seeds of *Vachellia aroma* (Fabaceae) along a precipitation gradient. We collected mature fruits from four localities along a precipitation gradient in central Argentina for an imbibition experiment using controlled germination chambers. Changes in physical dormancy were not found along the precipitation gradient; however, a trend toward higher percentage of seed imbibition (lower PY) at the most humid extreme of the gradient was observed. On the contrary, we did observed intra-population variability in three of the four populations, suggesting that most of the populations of *V. aroma* might have the ability to deal with the environmental variability encountered at local scale. This study highlights the importance of studying both inter- and intra-population variability in physical dormancy.

Keywords: Fabaceae, inter-population variability, intra-population variability, physical dormancy, precipitation gradient, *Vachellia aroma*

Plants have evolved dormancy mechanisms to optimize the time of germination, avoiding germination in periods that are only ephemerally favourable and, therefore, ensuring seedling survival (Baskin & Baskin 2014; Willis *et al.* 2014). Seed dormancy, one of the earliest traits expressed in the life of plants, can be a critical determinant of plant population dynamics through a direct effect on colonization and establishment success (Willis *et al.* 2014). Physical dormancy (hereafter, PY), one of the types of seed dormancy, is caused by one or more layers of palisade cells in the seed or fruit coat; such layers are impermeable to water and, once broken, dormancy cannot be reversed (Baskin & Baskin 2014). This type of dormancy is most commonly found in species occurring in arid and semi-arid regions (Funes & Venier 2006; Baskin & Baskin 2014) and in fire-prone areas, especially in species with a persistent seed bank (Ooi *et al.*

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2014; Liyanage & Ooi 2015). Furthermore, some studies have suggested that PY variability allows seeds to persist in variable environments (Ooi *et al*. 2012; Fernández-Pascual *et al*. 2013). However, there is scarce knowledge about the degree of PY variability, both among and within populations, especially in natural ecosystems (Cochrane *et al*. 2015; Hudson *et al*. 2015).

Some authors agree that PY has evolved to enable plants to survive in temporally stochastic and harsh environments (Baskin & Baskin 2014; Hudson *et al*. 2015). Consequently, in the context of climate change, it has been suggested that PY would prosper under warmer, more variable and unpredictable climates (Hudson *et al*. 2015). Natural gradients (e.g., precipitation, temperature, altitude) offer great opportunities for evaluating inter-population variability and might provide information about how species may cope with environmental variability (Ooi *et al*. 2012; Fernández-Pascual *et al*. 2013). Variation along precipitation gradients might be highly important for seed traits such as dormancy, because changes in humidity during seed development are related to the degree of seed dormancy (Jaganathan 2016). Within species, a review of studies comparing populations in sites with different precipitations found that low rainfall during seed development favours the occurrence of PY (Hudson *et al*. 2015 and reference therein). In addition, some degree of intra-population variability has been observed in regeneration traits (Moreira *et al*. 2012; Baskin & Baskin 2014). Particularly, studies that analysed PY variation within populations found important differences among individuals (Pérez-García 1997; Lacerda *et al*. 2004; Masaka & Yamada 2009). Nonetheless, a recent study focussing on five Fabaceae woody species found significant intra-population differences in initial dormancy only in one of the species (Liyanage & Ooi 2015). Intra-population variability could be a strategy that buffers species against local unpredictable events such as fire and drought (Hudson *et al*. 2015; Liyanage & Ooi 2015) allowing a staggered seed germination.

Recent reviews (Hudson *et al*. 2015; Jaganathan 2016) expressed the need for jointly evaluating inter and intra-population variability in PY. Determining the extent of PY variation may help us to fully understand how species cope with environmental heterogeneity, ensuring that at least some seedling emergence, and potentially recruitment, will occur (Ooi *et al*. 2014; Liyanage & Ooi 2015). However, studies considering both sources of variability together are scarce (Hudson *et al*. 2015). One example is the work of Lacerda *et al*. (2004) who compared two tropical Fabaceae woody species from two distant populations differing in their degree of disturbance but presenting similar precipitation regime, founding inter- and intra-population variability in PY levels, with notable high intra-population variability. Furthermore, to our knowledge, there are not previous studies analysing together inter- and intra-populations variability in PY along a precipitation gradient which may improve our understanding of the possibilities that a species may have to cope with unpredictable changes such as those expected by climate change (Hudson *et al*. 2015).

Moreover, most studies focused on PY variation among populations and individuals were performed in agricultural species, mainly in annuals and short-lived herbaceous perennials, but very few have dealt with woody species (Cochrane *et al*. 2015; Hudson *et al*. 2015). *Vachellia aroma* (Gillies ex Hook. & Arn.) Seigler & Ebinger (ex *Acacia aroma* Gillies ex Hook. & Arn.) (Seigler & Ebinger 2005), a perennial woody species belonging to the family Fabaceae, is an important component of the subtropical seasonal xerophytic forest of central Argentina (Giorgis *et al*. 2011). Seeds of this species present PY due to a very thick and compact seed coat with a sclerified parenchyma (Funes & Venier 2006; Venier *et al*. 2012a). It is not clear how this species breaks PY in nature, however previous studies suggested that passing through the digestive tract of cattle might break seed dormancy in this species (Venier *et al*. 2012b) while fire and other native dispersal agents such as foxes might not be involved in this process (Ferreras *et al*. 2015). In this region, *V. aroma* is present in different plant communities along a precipitation gradient ranging from 306 to 710 mm. Along the gradient, rainfall is concentrated in the warm season (October to April, Ebinger *et al*. 2000), nevertheless in the dry sectors of the gradient the rain events becomes much more unpredictable. Hence, this precipitation gradient represents an excellent natural experiment to study inter- and intra-populations variability together.

The aim of this study was to determine inter- and intra-population variability in PY of *V. aroma* seeds along a precipitation gradient. We specifically attempted to answer the following questions: does PY increase towards the driest and most unpredictable extremes of a precipitation gradient? Does PY vary among individuals within populations?

Four localities differing in annual precipitation along a precipitation gradient in central Argentina were selected (Tab. 1). In each of these localities, described in Table 1, a *V. aroma* population was identified. At each population mature fruits from at least 10 individuals were collected. When the fruits were mature in all the populations fruit collection was performed in two consecutive days in May 2015 (18-19 of May, 2015). Fruits from each individual were kept separate in paper bags. At the laboratory, the fruits were opened and the seeds were extracted; seeds with no signs of predation and whose external morphology were the typical for the species (Funes & Venier 2006) were stored in sealed papers bags at room temperature for less than two weeks until the start of the imbibition experiment.

The imbibition experiment was conducted using controlled germination chambers under a 12/12-h daily photoperiod and an alternating temperature regime of 25/15 °C (Funes & Venier 2006). For each individual, three replicates of 25 seeds each were used, resulting in at least 30 Petri dishes per population (10 individuals per

Table 1. Collection site, population number ordered by increasing aridity, coordinates, annual precipitation and annual mean temperature of each locality along a precipitation gradient.

Collection sites	Population number[*]	Coordinates	Annual precipitation (mm)[§]	Annual mean temperature (°C)[§]
Mendiolaza	Population 1 (10)	31º15´44.16´´S; 64º18´56.3´´W	710	16.5
Tuclame	Population 2 (10)	30º45´18.91´´S; 65º13´22.27´´W	502	19.4
Chamical	Population 3 (11)	30º28´41.44´´S; 66º5´45.69´´W	433	20.1
Patquía	Population 4 (11)	30º2´22.86´´S; 66º52´34.64´´W	306	20.2

[*] The number of individuals collected from each population is indicated between parentheses.
[§] representing the average annual precipitations/temperatures from 1960 to 1990 (http://www.worldclim.org/).

population x 3 Petri dishes per individual= 30 Petri dishes per population). In each Petri dish (9-cm diameter) seeds were put on filter paper and moistened with distilled water when necessary. During one month, the number of seeds that showed clear signs of imbibition (i.e., softening and changes in seed size) were recorded (Lacerda *et al.* 2004).

To test if the populations differed in seed imbibition percentage along the precipitation gradient, a generalized linear mixed model (GLMM) with a binomial error structure and logit link function was performed, using the populations (four levels) as fixed factor and individuals as random factor. To compare seed imbibition percentages among individuals within each population, a generalized linear model (GLM) was performed using the individuals as fixed factor. A quasibinomial error structure and logit link function was used due to overdispersion. When differences among individuals within populations were significant, the DCG a posteriori test was used for multiple comparisons. The GLMM and graphs were performed with R version 3.1.1 (R Development Core Team 2014) using the packages *mass* and *nlme* and GLM in Infostat (Di Rienzo *et al.* 2013) and its interface to R (R 3.1.1, R Development Core Team 2014).

No significant differences were observed among populations along the precipitation gradient (Z=-1.74; p=0.089; Fig. 1). However a trend toward a higher imbibition percentage at the most humid extreme could be observed (Fig. 1).

We observed intra-population variability in seed imbibition percentage in most of the populations (Fig. 2), with significant differences being detected among individuals belonging to Populations 1, 2 and 4 (ranges of seed imbibition percentages: 2.67-29.33 %; 0-21.33 %; and 1.33-26.66 %, respectively) (Fig. 2A-C). Population 3 did not show significant differences among individuals, with all of them exhibiting low seed imbibition percentages (values of seed imbibition ranged from 0 % to 4 %; Fig. 2C).

The assembly of plant species in an area is the result of biotic and abiotic processes operating at different spatial and temporal scales (Woodward 1987). At the regional scale, these processes act as filters, selecting those species
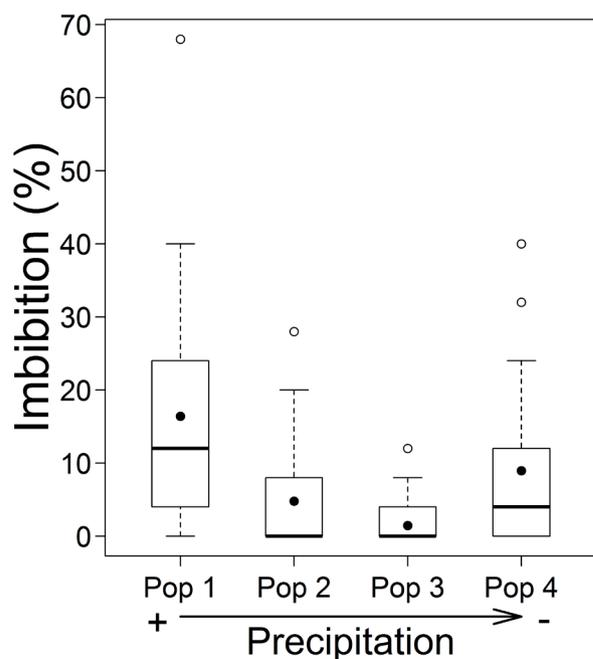
Figure 1. Seed imbibition percentages of four *Vachellia aroma* populations. Populations are ordered according to their location in the precipitation gradient, with Population 1 (Mendiolaza) being at the wettest extreme and Population 4 (Patquía), at the driest extreme. The black dot inside each box represents the median.

with suitable traits to withstand those filters (Keddy 1992). According to Keddy (1992), climatic conditions are the first filter that species have to pass through. Seed dormancy is a key trait involved in the first steps of species establishment and a higher percentage of seeds with PY is expected at sites with lower precipitation (Hudson *et al.* 2015). In our study, even though a trend toward higher imbibition in the most humid population was observed, no statistically significant differences were found among populations arranged along the precipitation gradient considered. On the contrary, we did observed intra-population variability in three of the four populations, suggesting that most of the populations of *V. aroma* might have the ability to deal with the environmental variability that could be found at local scale.
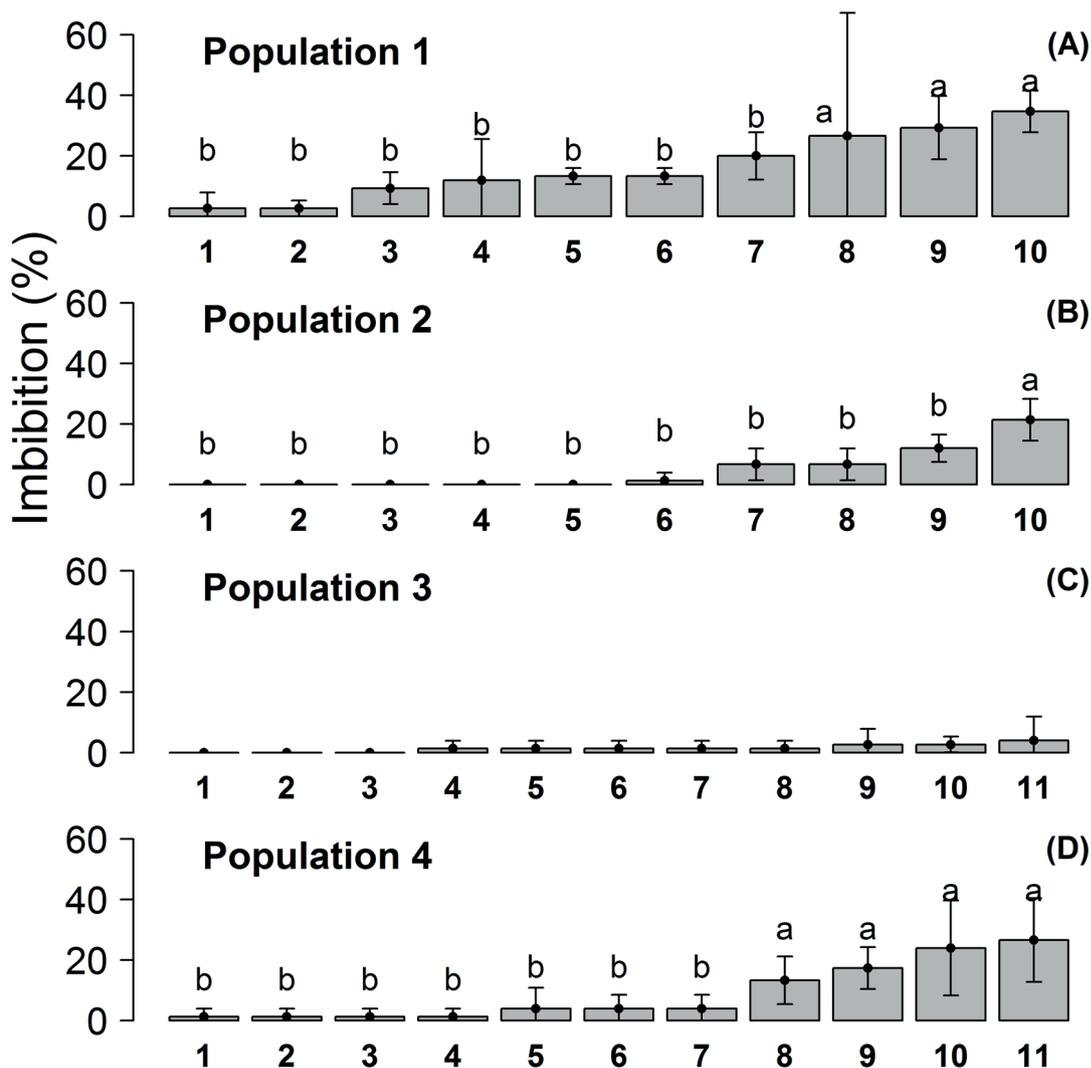
Figure 2. Mean seed imbibition percentages for individual plants of four *Vachellia aroma* populations distributed along a precipitation gradient in central Argentina. (A), 10 plants of Population 1 (Mendiolaza); (B), 10 plants of Population 2 (Tuclame); (C), 11 plants of Population 3 (INTA Chamical); and (D), 11 plants of Population 4 (Patquía). Mean seed imbibition percentage for each population is represented by a dashed horizontal line. Different letters indicate significant differences in mean seed imbibition percentage.

The trend toward higher imbibition (lower PY) at the most humid extreme of the gradient could be related to the fact that in that portion of the gradient it would be favourable to have always an amount of seeds that could rapidly germinate and take advantages of the rainy events. In this sense, Jaganathan (2016) suggested that when in the maternal environment the relative humidity and rainfall are high, seeds could develop a seed coat permeable to water. However, although the lack of significant differences found in our study could be due to the extent of our precipitation gradient, previous studies obtaining the expected pattern in seed with PY were performed in narrower or similar precipitations gradients (Norman *et al.* 2006; Gresta *et al.* 2007). In particular, the study performed

by Norman *et al.* (2006) on three populations of *Trifolium subterraneum* in a precipitation gradient similar than our did find differences in initial dormancy but as the authors mention the differences were less marked than might be expected in view of the substantial precipitation differences among sites. Therefore, this previous study and our results suggested that maintaining high amount of dormant seeds along this type of precipitation gradients might be the most favourable strategy, although more studies are needed to confirm this trend. Finally, it has to be mention that temperature could be also involved in changes in the percentage of PY among populations –i.e., populations with higher temperatures generally exhibit higher percentages of seeds with PY (Jaganathan 2016). However, in our

study populations were chosen considering their notable differences in precipitations and not in temperatures. So, if temperature is the main responsible for the changes in PY, it remain to be explored what would occur in populations with greater differences in average temperatures.

The presence of intra-population variability might not only provide the species with a strategy to cope with local extreme events such as fires, it might also be a way of establishing in portions with particular microclimatic conditions –e.g., higher amount of humidity (Baskin & Baskin 2014). Along the precipitation gradient selected in this study three of the four populations showed significant differences in intra-population variability in PY (i.e., Populations 1, 2 and 4). In this region fires are common (Jaureguiberry & Díaz 2015), particularly of anthropogenic origin. Therefore, the intra-population variability observed in most of the populations may provide the capacity to respond to the variability of heat produced by fire in the soil (Liyanage & Ooi 2015). Similar results were observed by Pérez-García (1997) in other Fabaceae species of fire-prone environments. Nonetheless, Liyanage & Ooi (2015) did not find intra-population differences in initial PY in four of the five species of fire-prone environments studied. This discrepancy between studies indicates that the ability to cope with local environmental heterogeneity may change among species, suggesting that in the future those species or populations that showed higher intra-population variability may have a higher capability to deal with climate change.

All in all, our study did not show inter-population variability in initial PY, however high intra-population variability was observed in most of the populations analysed. This intra-population variability could be hiding the main pattern reported in the literature in which at sites with lower precipitations the highest PY is observed (Hudson *et al.* 2015). The intra-population variability could indicate a higher ability to cope with changes in environmental heterogeneity at the local scale, enhancing the chances of persisting under local extreme events. In addition, as far as we know this is the first work that evaluates inter- and intra-population variability along a precipitation gradient; therefore, more experiments should be conducted in other species, which would provide valuable information to enhance our understanding of the mechanisms regulating physical dormancy and how they might be related to species occurrence along different environmental gradients.

Acknowledgements

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