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Seed germination and seedling establishment of the rare *Carex helodes* Link depend on the proximity to water

Eduardo Narbona*, Ana Delgado, Fernando Encina, Mónica Miguez, Maria L. Buide

Departamento de Biología Molecular e Ingeniería Bioquímica, Universidad Pablo de Olavide, Ctra. Utrera Km 1, 41013 Sevilla, Spain

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ABSTRACT

Seed germination, seedling emergence and survival of *Carex helodes* Link were assessed in laboratory and field experiments. This rare, endangered sedge inhabits intermittent streams of the South Iberian and North African cork woodlands. Dry storage (1.5–6 months) rather than low temperature was found to break the innate dormancy. In the field, germination occurred during fall and winter, and seeds survived continuous flooding during this period. The mean percentages of seed germination and seedling emergence in seeds located in the stream were four times higher than those of the bank, but a considerable percentage did not germinate and appeared to have developed a secondary dormancy. All of the seedlings died in the first summer due to drought stress; however, seedling survival was higher and delayed in the stream versus the bank microhabitat. Our findings suggest that the survival of this rare and endemic species is closely linked to conservation of intermittent streams.

1. Introduction

In contrast to permanent ones, temporary streams are waterbodies that experience a dry phase and can be classified as intermittent or episodic depending of the predictability of the dry phase (Williams, 2006). Mediterranean streams undergo seasonal changes: high flows begin with the first rains in the fall, whereas the flow declines gradually over a period of several months in late spring and summer (Gasith and Resh, 1999). Thus, most south European streams can be considered intermittent (Gasith and Resh, 1999; Costa Pérez, 2003).

It is well documented that hydrological and morphological factors, such as seasonal changes in flooding or the depth of the water table, affect the composition and distribution of the riparian vegetation on permanent rivers (Parker and Leck, 1985; Bendix and Hupp, 2000). The vegetation that grows in intermittent streams is exposed to high flows and flooding in winter and suffers the effects of extreme drought in summer caused by the seasonal loss of the water (Lake, 2003; Stromberg et al., 2005; Lite et al., 2005). Intermittent streams also show a great spatial heterogeneity and temporal variability in environmental conditions (Salinas and Casas, 2007; Sandercock et al., 2007). Thus, the riparian vegetation of intermittent streams is highly diverse (Moreno et al., 2001; Salinas and Casas, 2007), and hence conservation targets (Gómez et al., 2005). For example, Katz et al. (2012) found that intermittent streams can have more plant species than adjacent water courses with permanent flow.

Species that grow in highly specific habitats, such as deserts, sandy solids, or tree crowns, have seeds that are highly adapted to these habitats, showing good germination and establishment (Baskin and Baskin, 1998; Winkler et al., 2005; Narbona et al., 2007a; Cruz-Mazo et al., 2010). Thus, plants that inhabit intermittent channels should have a germination ecology that is adapted to this highly specialised habitat. In an intermittent stream of California, McBride and Strahan (1984) found that the sediment texture in which seeds germinated clearly affects the seedling establishment of some riparian species. In a dryland river channel of southeast Spain, damage due to flows caused a high mortality in plants that are not specifically adapted to this habitat (Sandercock et al., 2007). For the endangered tree *Frangula alnus* subsp. *baetica*, a species that grows on South Iberian mountain streams, winter flow displaced the seeds to unfavourable sites where the seedlings died due to desiccation (Hampe and Arroyo, 2002).

In this study, we investigated the seed and seedling ecology of the perennial *Carex helodes*, a rare plant endemic to the western Mediterranean region (Luceño and Escudero, 2008; Moreno, 2008). Molecular studies show that this species is a relict of the Pliocene period (Escudero et al., 2008a), when the climate was warmer and

* Corresponding author. Tel.: +34 954349350; fax: +34 954349151.
E-mail addresses: enarfer@upo.es (E. Narbona), aniuskydp@hotmail.com (A. Delgado), lacasaderwalu@gmail.com (F. Encina), mmigrio@upo.es (M. Miguez), mlbuirea@upo.es (M.L. Buide).

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wetter than the current Mediterranean conditions. Nowadays, this sedge is restricted to a few small intermittent streams with the circumstance that these habitats are disappearing as a result of human alteration (Gómez et al., 2005; Ferreira and Aguiar, 2006; Luceño et al., 2007a). If this sedge shows a seed and seedling ecology highly dependent on intermittent streams, the fact that the habitat is disappearing may seriously compromise the conservation of the species. Aspects of the seed dormancy, germination and survival of *Carex* species are well documented (Schütz and Rave, 1999; Schütz, 2000; Vellend et al., 2000; Leck and Schütz, 2005; Kettenring and Galatowitsch, 2007). However, these studies focus on mesic temperate regions. The objectives of this study were as follows: (1) to investigate the germination capacity of the seeds of different individuals of *C. helodes*; (2) to identify differences in the seed germination in relation to the water proximity by considering two different microhabitats (stream and bank); and (3) to investigate whether the seedling emergence and establishment depends on the microhabitat.

2. Materials and methods

2.1. Species description and study sites

C. helodes Link (sect. *Spirostachyae*) is a caespitose perennial herb, without the capacity for clonal expansion via creeping rhizomes, it is a morphologically, cytogenetically, and phylogenetically well-supported species (Luceño and Escudero, 2008; Escudero et al., 2008a; Escudero and Luceño, 2009). Approximately five populations have been recorded in south-western Portugal, three in the south of Spain and three in the north of Morocco as a result of recent visitations of historically know sites or newly discovered populations (Luceño et al., 2007a,b; Escudero et al., 2008b). In Spain, *C. helodes* has been considered to be extinct (AA.VV., 2000), but three new small populations have been discovered in 2006 (Luceño et al., 2007a,b); thus, according the IUCN standards, the species has been reclassified as endangered (Moreno, 2008). The number of individuals per population is quite variable, ranging from only a few in the Moroccan populations to hundreds in the Portuguese populations. In a recent molecular study on this anemophilous sedge, Escudero et al. (2008b) found that there is no genetic structure among the populations within the continents which suggests active gene flow although the Strait of Gibraltar has been an effective gene-flow barrier.

C. helodes grows in a very specific habitat: streams and temporarily inundated acidic soils of open *Quercus suber* L. forests at elevations of 50–500 m a.s.l. (Luceño and Escudero, 2008). Flowering occurs in early spring, and dispersal occurs in early to mid-summer. The mature plants produce between 3 and 60 floral stems (mean ± S.E. = 11 ± 3) and between 99 and 16,560 fruits (hereafter called seeds, mean ± S.E. = 1590 ± 210) (E. Narbona and M.L. Buide, unpub. results). When the seeds are mature, they fall to the ground; the seeds float on water (E. Narbona and M. Miguez, pers. obs.).

Using one of the newly discovered populations of Spain, the study was conducted in the El Gago stream located in Aznalcóllar (Seville province, Spain; 37°35′24″ N, 6°21′30″ W; 316 m a.s.l.; Fig. 1A). The vegetation consists of sparse *Q. suber* trees and dense scrub dominated by *Cistus salviifolius* L., *Cistus ladanifer* L. and *Lavandula stoechas* L. Other species more typical of water courses are found within the stream, including *Pulicaria odora* (L) Rchb., *Sanguisorba hybrida* (L.) Nordborg, *Juncus capitatus* Weigel, and *Isolepis cernua* (Vahl) Roem. & Schult. Individuals of *C. helodes* mainly grow within the stream channel but they occasionally are located along the stream margins (Fig. 1). This intermittent stream generally contains flowing water only from October–November to April–May, and the stream may become dry even during this period in drought years. This site has a typical dry-summer Mediterranean climate, with a mean annual temperature of 16.4 °C and a mean annual rainfall of 713 mm (weather station Minas de Riotinto, 421 m a.s.l. at approximately 16 km). In the period in which experiment 2 was performed (winter–spring of 2008), the accumulated rainfall was 8% lower than the mean, whereas the accumulated rainfall in the period for experiment 3 (fall 2008–spring 2009) was only 61% of the mean for this period.

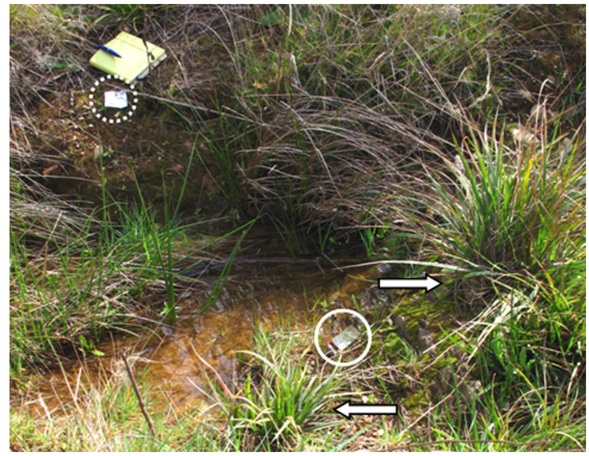

Fig. 1. The different microhabitats on the stream used in experiment 2. Broken white circle, aluminium tag used for locating the buried seeds in the “bank” microhabitat; solid white circle, aluminium tag used for locating the buried seeds in the “stream” microhabitat; white arrows; mature plants of *Carex helodes*.

2.2. Experiment 1: seed dormancy and inter-individual germination

In July 2007, approximately 300–500 mature seeds from each of 10 plants were collected. The seeds were visually inspected to discard apparently nonviable ones, i.e., thin seeds that have no embryo and endosperm (<0.5% of the total collected seeds). A total of 150 seeds were sown (three replicates of 50 seeds each) immediately. The seeds were placed on permanently moist Whatman #1 filter papers in Petri dishes and incubated in a chamber at 14/24 °C in the dark/light (35 μmol m^{-2} s^{-1} PAR) for 11/13 h, respectively. These conditions are considered optimal of the germination for other Mediterranean species (Baskin and Baskin, 1998). The dishes were inspected every 3–6 days, for a total period of 45 days. As no germination was observed, we left the dishes unirrigated for another 45 days in the darkness under laboratory conditions (approximately 20 °C; relative humidity 50–70%). Watering was resumed following this treatment, and the germination was monitored again for 49 days. The dishes were inspected every 3–6 days, and the number of germinated seeds was recorded (i.e., those with an emerged radicle greater than 0.2 mm). Another three replicates of 50 seeds per plant were stored for 6 months in paper envelopes in the dark under laboratory conditions and then germinated as above. After the trials, the non-germinated seeds were pinched with forceps to confirm their viability (Baskin and Baskin, 1998).

2.3. Experiment 2: seed germination in different microhabitats

To determine whether the seed germination of *C. helodes* is affected by the proximity of water, the seeds were placed at two different sites: in the stream and on the bank, the stream being the microhabitat with water for most of the wet period, and the bank being the microhabitat outside of the water channel (20–40 cm up,