Environmental and Experimental Botany 123 (2016) 59–67

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Reproductive output, seed anatomy and germination under water stress in the seeder *Cistus ladanifer* subjected to experimental drought

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ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form 2 November 2015
Accepted 4 November 2015
Available online 23 November 2015

Keywords:
Maternal environment effects
Seed coat
Micropyle
Physical dormancy
Fire cues
Hydrotime model
Climate change

ABSTRACT

Reproductive output and seed traits can be altered by water availability during seed formation and maturation, which could affect the population recovery after fire of seeders (i.e., species regenerating from seeds). This is important for species in fire-prone, dry areas that are projected to encounter reduced total precipitation and longer annual drought with climate change. Here we determine the sensitivity of several reproductive processes to drought in *Cistus ladanifer*, a plant widely distributed in the shrublands of the western part of the Mediterranean Basin. Three levels of annual drought were simulated in a shrubland by means of a rainout shelter and an irrigation system in 6 × 6 m plots. Fruits and seeds from drought-exposed mother plants were collected, and reproductive output, seed size and anatomy studied. Seeds non-exposed/exposed to fire cues (heat plus smoke) were germinated at five levels of water stress (Ψ_s = 0.0 to −0.50 MPa). Hydrotime modeling was applied to germination under water stress. Plant growth was sensitive to drought, but reproductive output, seed size, dormancy and viability were not. Drought significantly affected seed anatomy, increasing the palisade layer at the micropyle. Drought in the maternal plants, in interaction with seed exposure to fire cues, significantly reduced final germination. Water stress during germination decreased final germination, independent of maternal plant drought, and interacted with fire cues to decrease germination when exposed. Hydrotime modeling confirmed that fire cues made seeds highly sensitive to water stress (Ψ_b (50) = −0.25 MPa). Post-germination viability was reduced in seeds from drought-treated maternal plants that were exposed to fire cues and germinated under water stress. Reproductive output showed low plasticity in response to drought. However, the effects of drought in the mother plant affected seed anatomy and germination in interaction with fire cues. The conclusion is that exposing *C. ladanifer* maternal plants to drought arguably increases seed sensitivity to water limitations during germination after fire.

1. Introduction

Seed production and seed characteristics, including size, dormancy and germination responses to various environmental cues, are key plant reproduction that have evolved driven by habitat characteristics, and make a major contribution to plant fitness (Venable and Brown, 1988; Leishman et al., 2000; Donohue et al., 2005). Water availability in the mother plant at the time of seed formation (i.e., maternal effects) (Roach and Wulff, 1987) can affect both these components (Fenner, 1991, 1992; Gutterman, 2000; Donohue, 2009). It is well known that, in general, water stress negatively affects plant growth, ovule formation and maturation, and thus fruit and seed production effort. Moreover, not all seeds produced under water stress are viable, so the overall reduction in regenerative potential from seed can be large (Albert et al., 2001). Water stress in maternal plants can also affect important seed characteristics including provisioning and size, usually negatively (Fenner, 1992). Alteration of seed formation and traits by drought could affect germination rates and ultimately have consequences on plant regeneration, especially under future climate conditions (Walck et al., 2011). Therefore, understanding the effects of drought on plant reproduction is of utmost importance in water-limited areas around the world that are projected to encounter further limitations due to climate change.

Not all plants are equally sensitive to water stress. Some species are able to partially modify allocation patterns between growth and reproduction, relatively increasing the latter to favor greater reproductive outputs in spite of water limitations (Sultan, 2003). In addition, some species can reduce the number of fruits produced and/or the number of seeds per fruit (and therefore total seeds), but maintain seed size or even increase it under water stress; these

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http://dx.doi.org/10.1016/j.envexpbot.2015.11.002
0098-8472/

include both annual (Sultan, 1996; Gorecki et al., 2012) and perennial species (Stromberg and Patten, 1990). This plasticity in seed size is important: seed size has often been related to germinability and seedling survival once germination has occurred (Sultan, 1996; Moles and Westoby, 2004). A reduction in the number of seeds but an increase in their size could still ensure a relatively large number of established seedlings.

Water availability of the maternal plant can also affect seed dormancy, depending on its type. While seeds with physiological dormancy (i.e., a physiological inhibiting mechanism in the embryo prevents germination) often exhibit a negative relationship with plant water availability (i.e., fewer seeds are dormant as water stress increases) (Meyer and Allen, 1999; Tielbörger and Valleriani, 2005), seeds with physical dormancy (i.e., a water-impermeable coat prevents imbibition and thus germination) exhibit the opposite trend (i.e., more seeds are dormant at higher water stress) (Clua et al., 2006; D'hondt et al., 2010). Physical seed dormancy can also be related to seed size, whereby larger seeds have lower dormancy than smaller seeds (Delgado et al., 2001). In fire-prone environments, where a long-lived seed bank is advantageous to produce a large flush of germination after fire (Moreno et al., 2011), reduced dormancy would imply prompt germination once the seed is moistened, which would be detrimental to this strategy. Missing the opportunity to regenerate after fire to outcompete other plants would threaten the dominance and/or persistence of plants in such environments. Determining the impact of water stress on dormancy is therefore of utmost importance in plants that respond to fire by means of a long-lasting soil seed bank.

The testa of the seed originates from the mother plant, and starts differentiating from the ovule teguments right after pollination (Beeckman et al., 2000). Arguably, variations in the environment experienced by the mother plant until seed maturation could affect seed anatomy. There is evidence that changes in maternal temperature can affect total seed mass and testa mass, but not endosperm or embryo mass (Lacey et al., 1997). Moreover, reduced seed germination can be related to structural changes in the testa, such as greater thickness (Wada et al., 2011). Release from physical dormancy is dependent on the breaking of the testa to allow seed imbibition (Gama-Arachchige et al., 2013). In fire-prone environments, exposure of seeds to the heat of fire cracks the testa in various places (Aronne and Mazzoleni, 1989), making imbibition possible. However, little is known about the possible effects of variations in the anatomical characteristics and dormancy break factors in relation to the conditions during seed formation.

The germination process is also highly sensitive to water stress (Baskin and Baskin, 2014), but sensitivity varies within species and between species (Fady, 1992; Thomas et al., 2010). In studies on gradients, variations within species have been related to the environment of the mother plant (Fady, 1992; Tilki and Dirik, 2007); however, the cause of such relationships is not known. They could be due to trait selection driven by the local environment along the gradient (i.e., be genetically fixed), and/or variations in the maternal environment during seed development and maturation along the gradient (i.e., phenotypic plasticity) (Roach and Wulff, 1987). In seasonal habitats with large interannual variability in precipitation, a plastic germination response to water stress due to genetic or phenotypic effects could be advantageous to cope with variations in water availability from seed formation through seed germination and initial establishment. However, maternal effects have been studied mainly in annual plants. These being short-lived, the sensitivity of the mother plant to the environment could have a large adaptive value (Herman and Sultan, 2011). Little is known, however, about maternal effects on the sensitivity of the seeds of long-lived perennial species, including shrubs, to water stress in germination.

Mediterranean environments are fire-prone and have limited water, and precipitation variability is common, particularly where precipitation is low (Lionello et al., 2006). With climate change, reduced precipitation is projected for this region in spring, at the time of flowering, and fall, lengthening the summer drought (Christensen et al., 2013). Changes in precipitation patterns consistent with these projections have already been observed (De Luis et al., 2010). Understanding the factors that control plant regeneration under limited water availability is very important for species that regenerate from seeds (i.e., seeders) after fire, since this is the only mechanism they have for recovering their population after the blaze.

The objective of this work was to determine the response in terms of reproductive output (fruit and seed biomass), seed number, size, anatomy, dormancy, viability, and germination to water stress in the germination environment in seeds of *Cistus ladanifer* non-exposed/exposed to fire cues (heat and smoke) at a Mediterranean-climate continental site in Central Spain. To assess the sensitivity of the various reproductive processes in mother plants to different precipitation regimes, a mature shrubland was subjected to a fully replicated rainfall manipulation experiment during the growing season, exposing the vegetation, including mature *Cistus ladanifer* plants, to various levels of drought.

2. Material and methods

2.1. Study area and rainfall manipulation experiment

The rainfall manipulation experiment was carried out in a mature shrubland dominated by several woody perennial shrubs (mainly *Cistus ladanifer* and *Erica* species), located at the Quintos de Mora range station (39° 25′ N, 4° 04′ W). The climate is typically Mediterranean, with a mean annual temperature of 14.9 °C and a mean annual precipitation of 622 mm (Los Cortijos meteorological station, 39°19′ N, 4°04′ W) (AEMET, Spain). The experiment was implemented using a set of automatic rainout shelters and an irrigation system that allowed the desired level of rainfall in each treatment to be controlled at two-week intervals. Four rainfall treatments were implemented: (1) EC: environmental control (i.e., natural rainfall); (2) HC: historical control (simulation of long-term rainfall patterns, including two months of drought [July and August]); (3) MD: moderate drought (total rainfall reduction of 25% from HC [i.e., to percentile 8 of the historical record], with five months of drought from May to September); and (4) SD: severe drought (reduction of 45% from HC [to percentile 2 of the long-term historical record], with seven months of drought from April to October). Treatments were implemented as follows: at the beginning of each two-week period shelters were open to natural rainfall until the established levels for the period were reached. After these were reached, the shelters were deployed at every rain event to preclude rainfall. If rainfall was insufficient to match the set target at the end of the period, irrigation was applied up to that level. These treatments were applied on a total of 16 plots (6×6 m), four plots per treatment, following a randomized complete block design with four blocks. Rainfall manipulations started in late March, 2009, and continued until late September of that year, when the shelters and tubing were removed for a few days to allow experimental burning of the plots. The system was reinstalled shortly after burning and the treatments continued for several years. Here, we report data from the first growing season after treatment implementation on the pre-fire mature shrubland. *C. ladanifer* flowers in May, and seed dispersal starts in mid-July. The precipitation since the beginning of the hydrological year (October 1, 2008) until the start of rainfall manipulations (March