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Original Research Article

Burial depth and diameter of the rhizome fragments affect the regenerative capacity of a clonal shrub

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ABSTRACT

Clonal plants in highly disturbed habitats are often broken into small fragments of various sizes and buried at various soil depths. As a storage organ, rhizome fragments play an important role in enabling plants to survive in such habitats. But few studies have been concerned about the regenerative capacity of rhizome fragments of clonal shrubs of different rhizome diameter and at different burial depths. Here, we investigated whether deeper burial decreased, and diameter of the rhizome fragment increased, the regenerative capacity of a clonal shrub. Research samples of rhizome fragment (rhizome diameters of 2, 5, 10, 15, and 20 mm) of the clonal shrub *Calligonum arborescens* were buried at different depths (0, 1, 5, 10, and 20 cm). Increasing the diameter of the rhizome fragments significantly increased the survival rate of fragments, and increased the above-ground, below-ground and total biomass production of fragments. Vegetative reproduction ability also increased with an increase in diameter of the rhizome fragments. With an increase in sand burial depth, above-ground, below-ground, total biomass production and vegetative reproduction ability first decreased and then increased, and no fragments survived at the 0 cm burial depth. These results indicate that sand burial depth and diameter of the rhizome fragments significantly affected the regeneration capacity of *C. arborescens*. Sand burial is one of the essential prerequisites for *C. arborescens* rhizome fragments' survival. Moderate burial depth (5 cm) and larger fragment diameter (20 mm diameter) were more suitable for biomass production and vegetative reproduction. These results indicate that reserves stored in rhizome fragments can contribute greatly to the regeneration capacity of the *C. arborescens*—responses that are very important for *C. arborescens* survival and establishment in frequently disturbed habitats.

1. Introduction

Disturbances such as grazing, trampling, fire, flood and landslides are common features of natural habitats and an important component of ecosystems, and occur at various spatial and temporal scales (Grime, 2002; van Kleunen, 1996). Such disruptions are particularly stressful for clonal plants, whose stolons or rhizomes, located in the shallow soil layer, are likely to be fragmented, further destabilizing soils to varying degrees and burying different-sized clonal fragments at different depths (Dong et al., 2011; Stuefer and Huber, 1999). In response, clonal plants growing in frequently disturbed habitats have developed some coping strategies (Bornette et al., 2008; Puijalon et al., 2008; Latzel and Klimešová, 2009). For example, solon internodes may help fragmented clones of stoloniferous plants withstand the fragmentation, and survive at deeper sand burial depths (Stuefer and Huber, 1999; Dong et al., 2011).

In recent decades, numerous studies have dealt with the effects of disturbances on the germination and growth of clonal plant fragments, or with the effects of fragmentation on the survival and growth of whole communities of herbs (Dong et al., 2010a,b; Sakamaki and Ino, 2006; Wang et al., 2014). These clonal herbs are of particular interest, because they comprise a large subset of clonal plants and are distributed across a wide range of habitats (Klimeš et al., 1997). For example, juvenile ramets can survive clone fragmentation better if they remain attached to an internode after being severed from the rest of the clone (Stuefer and Huber, 1999). *Uniola paniculata* tiller emergence declined with increasing length of air exposure and decreasing size of rhizomes (Miller et al., 2003). The node position of fragments significantly affected regeneration rate and subsequent growth of the *Alternanthera* species (Song et al., 2014). However, few studies have tested the

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effects of disturbances on the regeneration capacity of clonal fragments of shrubs, and little is known about the responses of rhizome clonal fragments to soil burial, or the role of rhizome fragments in the regeneration capacity of shrub fragments after burial (Dong et al., 2011; West et al., 2012). In this study, therefore, we selected a typical rhizomatous perennial shrub to study the effects of fragment size and burial depth on the regenerative capacity of clonal shrubs.

Disturbances of different intensities can yield clonal fragments of different sizes (Bornette et al., 2008), and the size of fragments is an obvious and likely factor in their performance (Lin et al., 2012). The size of clonal fragments, however, is genetically determined and varies among species (Liu et al., 2007). As important clonal fragments, stolons or rhizomes are likely to fulfill a storage function that may be of ecological importance in disturbed habitats (Stuefer & Huber, 1999). Larger stolon or rhizome sizes may also positively affect the amount of resources stored in the stolons or rhizomes, and in turn increase survival and regrowth after clone fragmentation (Huber et al., 2014). Larger fragments therefore tend to grow better than smaller ones (Lin et al., 2012), because larger fragments are able to build more carbohydrate reserves than smaller ones can. The regenerative capacity of root fragments also increases with enlargement of fragment size (West et al., 2012). The optimal internode size of fragments, though, can depend on a multitude of environmental conditions (Hutchings et al., 1997; Weijschede et al., 2008). Although internode length and diameter may be largely determined by optimal ramet spacing and morphological features (Huber et al., 2014), previous studies have estimated fragment sizes by their length (Dong et al., 2010b; Lin et al., 2012; West et al., 2012), and no studies have used the diameter of rhizomes or stolons to estimate fragment sizes. In this study, therefore, we estimated fragment sizes based on their diameter, and tested the hypothesis that larger rhizome fragments have higher emergence ability, survival rates, growth, morphology and rate regenerative capacity.

Sand burial is a common disturbance for plants in arid and semi-arid land. Burial can change both biotic (e.g., pathogen-activity) (Maun, 1998; Perumal and Maun, 1999) and abiotic (e.g., light, temperature and moisture) (Klimeš et al., 1993; Brown, 1997) conditions, and can also create a physical barrier that can retard shoot emergence (Yu et al., 2002, 2004). Numerous studies have shown that sand burial influences the germination of seeds (Zhang and Maun, 1990; Huang and Gutterman, 1998; Huang et al., 2004) and the growth of both seedlings (Maun, 1994; Li et al., 2010) and adult plants (Maun, 1996; Li et al., 2010). A few studies also have tested the effects of burial on the regeneration capacity of clonal fragments (Chen et al., 2010; Dong et al., 2011). Increasing burial depth significantly reduced survival rates of *Alternanthera philoxeroides* plants and increased root-to-shoot ratio and total stolon length, but did not change growth (Dong et al., 2011). Deeper burial markedly decreased the emergence and survival of rhizome fragments (Klimeš et al., 1993; Shen et al., 2005). Similarly, the regenerative capacity of root fragments increased with an increase in burial depth (West et al., 2012).

The survival and growth of deeper-buried clonal fragments may rely mainly on the utilization of plant reserves—storage organs—when carbohydrates cannot be provided through photosynthesis (Harris and Davy, 1986; Klimeš et al., 1993; Dong et al., 2010b). However, the fragments will face the risk of non-survival if reserves stored in plant organs are depleted before the new shoots come out (restoring the plant's ability to photosynthesize) (Stuefer and Huber, 1999; Dong et al., 2010a,b). The duration and conditions of exposure, and soil moisture, are the two most important factors affecting tiller emergence from rhizome fragments. Exposed rhizome fragments fail to produce tillers unless the soil is moistened by rainfall during exposure (Miller et al., 2003). Hence moderate sand burial maybe necessary for rhizome fragments to survive and regenerate in a natural environment. Here we tested the hypothesis that moderate sand burial may increase the emergence ability, survival rate, growth, morphology and rate regenerative capacity of small clonal fragments, but deep sand burial may lead to the opposite result.

2. Materials and methods

2.1. The species

Calligonum arborescens Litv. is a dominant perennial shrub in active sand dunes and stabilized sand fields in the northern deserts of China. It can grow in mobile sand dunes in extreme drought (Mao and Pan, 1986; Ren, 2001), making it appear to be suitable for revegetating deserts, due to its high tolerance of water deficit. *C. arborescens* can generate by both seed and rhizomes, and is a typical rhizomatous clonal perennial shrub (Zhuang et al., 2008). The optimal temperature for *C. arborescens* seeds to germinate is between 18 °C and 22 °C, and seeds germinate faster at higher constant temperatures (Ren et al., 2005). The most suitable sand burial depth for *C. arborescens* seeds to germinate is 5 cm (Li and Zhao, 2006). However, most of these studies focused on the seeds and seedlings; only a few paid attention to the clonal growth of *C. arborescens* (Zhuang et al., 2008). In our early observation, rhizome fragments of *C. arborescens* can be easily broken and readily split into segments of different size due to the disturbance such as mowing, trampling and wind interference. However, no studies have illustrated the correlation between fragment size or burial depth and the regenerative capacity of *C. arborescens*. Such responses, however, may play an important role for *C. arborescens* establishment and germination in frequently disturbed habitats.

2.2. Material preparation and experiment design

On 18 April 2014, we collected 750 *C. arborescens* rhizome fragments in desert land near the Linze Inland River Basin Research Station of Cold and Arid Regions Environmental and Engineering Research Institute in Zhanagye province, China. The initial rhizome fragments of *C. arborescens* was 20 cm long, and the dry weight of the 2, 5, 10, 15 and 20 mm-diameter fragments were 4.64 ± 0.13, 8.92 ± 0.20, 10.81 ± 0.24, 24.80 ± 0.32, 31.51 ± 0.44 g (mean $\pm$ *SE*, $N = 20$); the number of buds on each fragment were 32.80 ± 1.09, 32.70 ± 1.23, 32.80 ± 0.89, 33.30 ± 0.92, 33.60 ± 1.14 individuals (mean $\pm$ *SE*, $N = 20$), respectively. Each diameter group contained 150 replicates.

The experiment took a split-plot design with burial depth as a whole plot factor and rhizome diameter as a subplot factor (Dong et al., 2011). There were five burial depths (0, 1, 5, 10 and 20 cm) and five rhizome diameters (2, 5, 10, 15 and 20 mm). A total of 25 plastic containers (60 cm long $\times$ 30 cm wide $\times$ 40 cm high) were used and divided into five groups (each group has five containers). Each container was filled with sandy soil. Each group assigned to one sand burial depth, the five containers in each group were randomly assigned five rhizome diameters, and in each container thirty fragments were placed horizontally in evenly spaced positions (Dong et al., 2011). All the containers were placed in the greenhouse at the Linze station. Tap water (approximately 1.0 L per container per day) was applied to keep the soil moist. The experiment began on 20 April 2014 and concluded on 20 August 2014.

2.3. Measurements

During the experiment, we recorded the emergence status of clonal fragments in each treatment every other day. A fragment