Flora 249 (2018) 31–39

Contents lists available at ScienceDirect

Flora

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

Are alien species necessarily stress sensitive? A case study on *Lemna minuta* and *Lemna minor*

Simona Paolacci[a,*], Simon Harrison[a,b], Marcel A.K. Jansen[a,b]

[a] School of Biological, Earth and Environmental Sciences, University College Cork, Enterprise Centre, Distillery Field, North Mall, Cork, Ireland
[b] Environmental Research Institute, University College Cork Enterprise Centre, Distillery Field, North Mall, Cork, Ireland

ARTICLE INFO

Edited by Hermann Heilmeier

Keywords:
Lemnaceae
Invasive species
Stress tolerance
Photosynthesis
Metal toxicity
Oxidative stress
Drought

ABSTRACT

It is widely assumed that environmental stressors contribute to the protection of habitats from invasion by alien species, and that native species are better stress-tolerators. This assumption was tested by comparing the performance of the invasive alien *Lemna minuta* Kunth with that of the co-generic native *L. minor* Linnaeus, under several environmental stressors. The effects of temperature and drought, important determinants of the distribution of Lemnaceae, on growth and photosynthesis were explored. Also, tolerance to, and accumulation of aluminium and copper were studied. Finally, tolerance to Reactive Oxygen Species (ROS) was compared by growing the plants at different concentrations of the ROS generator paraquat (methyl-viologen). The present study shows that specific stressors (such as low temperature in this study) disproportionally affect growth of alien *L. minuta*. Yet, in the case of three other stressors (aluminium, copper, drought), effects on biomass growth are similar for the two species, or they are even less severe on *L. minuta*. Remarkably, *L. minuta* dries out faster, and accumulates more metals than *L. minor*, suggesting that the former species has a greater physiological tolerance, whilst the latter species has an avoidance strategy. Thus, the current study on the role of environmental stressors in facilitating alien invasions does not support the notion that the presence of stressors impedes alien invasions, but rather shows that differences between an alien and a native species are multi-faceted, and stressor-specific.

1. Introduction

Invasive alien species comprise a major threat to biodiversity across the globe (Gaertner et al., 2009). These species are responsible for a decline in native species richness (Galil, 2007; Stiers et al., 2011), they modify habitats (Didham et al., 2007) and can also have a serious economic impact (Pimentel et al., 2001). Invasive aquatic plants have disproportionate effects on specific ecosystems such as freshwater habitats (Dudgeon et al., 2006). Physico-chemical characteristics of freshwater environments can be altered by invasive, alien macrophytes which may, indirectly, result in a further negative impact on native plants, fish and macroinvertebrate communities. For example, when invasive macrophytes form dense floating mats, these species may compromise the natural exchange of oxygen between atmosphere and water column and cause hypoxia stress to other aquatic organisms (Masifwa et al., 2001; Troutman et al., 2007; Villamagna and Murphy, 2010). Dense mats of floating plants also shade the submerged environment, limiting algal growth and consequently affecting the aquatic community and food web structure even more (Villamagna and Murphy, 2010). Aquatic alien species can also impact on human activities such as fisheries, navigation and water-based leisure pursuits (Caffrey, 1993; Caffrey et al., 2010).

The dispersal of viable propagules of an alien species into an ecosystem does not necessarily result in an actual invasion. Rather, the invasion of particular ecosystems is a consequence of both ecosystem and plant characteristics (Alpert et al., 2000). In order to predict the invasiveness of a particular species, plant growth strategies in particular have been scrutinised. Grime (1974) identified three major growth strategies adopted by plants, and divided plant species into competitive, stress-tolerant and ruderal species. A number of sub-categories cater for plants with intermediate characteristics. Ruderal species are typically abundant in severely disturbed but potentially productive environments. Competitive species are particularly abundant in environments characterised by low stress and low disturbance. In contrast, stress-tolerant species are more successful in environments in which one or more stressors limit plant growth (Grime, 1974; Alpert et al., 2000). Species following a competitive/ruderal strategy are often early successional, short lived, lack mechanical structures, display a high

* Corresponding author.
E-mail address: spaolacci@ucc.ie (S. Paolacci).

https://doi.org/10.1016/j.flora.2018.09.004
Received 25 January 2018; Received in revised form 10 August 2018; Accepted 7 September 2018
Available online 11 September 2018

Relative Growth Rate (RGR), and respond strongly to environmental change. Conversely, species following a competitive/stress tolerant strategy are often late successional, long lived, possess mechanical structures, display a low RGR, and do not respond strongly to environmental change (Bussotti, 2008). Thus, knowledge of the growth strategy of a species not only helps to understand its distribution, but can also help to estimate the invasiveness of a species in a particular habitat. For example, Alpert et al. (2000) associated invasive species with ruderal traits. Conversely, the presence of specific stressors in the environment is often associated with low levels of invasibility as invasive species are considered less tolerant to stress than native species (Tilman, 1997; Davis et al., 2000, 2005; MacDougall et al., 2006).

Lemna minuta Kunth is a floating freshwater Lemnaceae that originates in temperate areas of North and South America (Stace, 2010). In Europe, the species was first recorded in France in 1965 (Jovet and Jovet-Ast, 1966). Since then, it has progressively spread around Europe becoming naturalised in several countries such as the United Kingdom (Bramley et al., 1995), Ireland (Cotton, 1999), Italy (Conti et al., 2005; Ceschin et al., 2016), Poland (Wójciak and Urban, 2009), Malta (Misfud, 2010), Germany (Hussner et al., 2010), Belgium (Halford et al., 2011), and Hungary (Lukács et al., 2014). A pertinent question concerns the traits that make *L. minuta* so successful as an invader in Europe. In this study we used a comparative approach to assess the traits of alien *L. minuta* relative to those of native *L. minor*, a species which commonly shares the same habitat. Such a comparative approach has been used successfully in the past to identify the traits that make some alien species so successful (Mack, 1996). This approach is even more effective if the comparison involves co-generic species as the identification of the differences between two closely related, similar species is easier and it is likely that observed differences impact on the invasiveness (Daehler, 2003; Lloret et al., 2005).

In previous studies, the ability of these two species to use resources such as nutrients and light (Paolacci et al., 2016, 2018) was investigated. The next step, in order to identify differences in survivor and dispersal strategies between the two species, is to investigate how they cope with different stressors. The aim of this study was to assess the growth strategies of native *Lemna minor* and alien *L. minuta*, in relation to environmental stress. Previously, it was observed that *L. minuta* displayed characteristics of a ruderal species, such as a high growth rate, and an ability to take advantage of high resource availability (Njambuya et al., 2011; Paolacci et al., 2016). For this reason, it was hypothesized that *L. minuta* is less efficient at tolerating stressors than the native *L. minor,* and this was tested by assessing growth following exposure to a range of abiotic stressors. Drought stress tolerance was tested as it is considered a particularly important determinant of the distribution of Lemnaceae (Crawford et al., 2006) and also as it plays a role in duckweed dispersal (Coughlan et al., 2015). Growth performance of the two species at either low or high temperatures was analysed as this is another major determinant of both distribution as well as competitive ability (Crawford et al., 2006). Lemnaceae have been extensively demonstrated to be accumulators of various metals (Axtell et al., 2003; Kanoun-Boulé et al., 2009); therefore, two common metal pollutants present in freshwater, copper and aluminium, were used as stressors. Finally, as the production of Reactive Oxygen Species (ROS) is a common factor in virtually all types of plant stress, the impact of the ROS generator paraquat (methyl-viologen) (Blackburn and Weldon, 1965) was explored on both species of Lemnaceae.

2. Material and methods

2.1. Plant growth

Lemna minor and *L. minuta* strains were collected from the same pond in Blarney, Co. Cork, Ireland (51.940476 latitude, −8.563637 longitude). The *L. minor* strain has since been registered in the Rutgers Duckweed Stock Cooperative database as strain number 5500 "Blarney". The plant material used for all the experiments was cultured under sterile conditions, in glass flasks, on 100 ml of half-strength Hutner's nutrient solution (Hutner, 1953). Plants were kept in a growth room at a constant temperature of 20 °C and exposed to a photosynthetic photon flux density (PPFD) of 40 μmol $m^{-2} s^{-1}$ (cool-white fluorescent tubes) with a light: dark cycle of 16:8 h.

All the growth experiments lasted just one week in order to avoid nutrient depletion to affect the results. All the experiments, except the drought exposure experiment, started with nine fronds (3 colonies) of each species (4.62 ± SE 0.79 mg fresh weight for *L. minuta* and 11.32 ± SE 0.9 mg for *L. minor*), grown separately, and were replicated four times.

2.2. Drought exposure experiment

Lemna minuta and *L. minor* desiccation rate, survival rate and ability to recover after drought stress were tested by removing fronds of these two species out of the growth medium and exposing them to the air in a growth room at a constant temperature of 20 °C, a PPFD of 40 μmol $m^{-2} s^{-1}$ and a relative humidity of 35% for 10, 20, 30, 40 or 50 min.

Ten fronds of *L. minuta* (5 mg on average) and ten fronds of *L. minor* (12 mg on average) were placed in ten Petri dishes on a layer of dry cellulose filter paper (thickness 180 μm, pore size 11 μm). Every ten minutes fronds were withdrawn from two Petri dishes and weighed in order to measure the loss of weight due to desiccation. The experiment was replicated four times.

The same experimental design was used to determine the survival rate and the ability to restart growth after drought exposure. After exposure to desiccation, fronds were placed in flasks containing 100 ml of growth medium inside a growth room. After one week the green fronds and the fronds that had completely lost their pigments (becoming white) were counted. The white fronds were considered dead and were deducted from the number of fronds initially placed in the medium (ten fronds) to calculate the survival rate. The difference between the initial number of fronds and the white fronds was used to calculate the Relative Growth Rate of the surviving fronds (see Section 2.6.1).

2.3. Temperature experiment

The effect of temperature on the growth of the species was assessed by keeping the medium at temperatures ranging between 0 and 35 °C (0, 10, 15, 20, 25, 30, and 35 °C). This range of temperature was selected as it reflects the actual temperatures that can occur in Europe and at which Lemnaceae can be found. The temperature of the medium under standard growth conditions was 18–20 °C. For the lower temperature treatments the flasks containing the plants were placed on a cold plate connected to a thermostat. To test the growth of the plants at temperatures higher than 18 °C the flasks were placed in a warm water bath.

2.4. Aluminium and copper exposure experiments

The toxic effect of aluminium sulphate on the plants was analysed by growing the two species in 100 ml of growth medium with added $Al_2(SO_4)_3{\cdot}H_2O$ (Sigma Aldrich, A7523-500 G, grade > 98%). Six different concentrations of aluminium were used, ranging between 0.08–30 ng [Al]$\cdot l^{-1}$. This range was selected on the basis of previous results detailing toxic effects of Al on growth of *L. minor* (Radić et al., 2010). As previous results for *L. minuta* were not available, the experimental range was broadened in order to identify differences in tolerance between the two species. The standard growth medium does not contain any aluminium and was used as a control. The addition of $Al_2(SO_4)_3{\cdot}H_2O$ resulted in an increase in sulphur in the medium, which constituted only 0.3% at the lowest aluminium concentration tested and 30% at 0.8 ng [Al] $\cdot l^{-1}$. At the highest concentration tested (30 ng