Short communication

Response of rhizomes of the invasive *Hedychium coronarium* J. König (Zingiberaceae) to different soil moisture conditions

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ABSTRACT

The ability to maintain high competitiveness under a wide range of conditions is common among successful invasive species. The performance of rhizomatous macrophytes in different habitats is closely related to morphological and physiological adaptations in the rhizome system. We investigated the effects of soil moisture conditions on the microstructure of rhizomes of *Hedychium coronarium* (Zingiberaceae), an aggressive invader of Neotropical riparian sites. We collected rhizome fragments of *H. coronarium* and soil samples in wet and dry regions of riparian areas dominated by this species in southeastern Brazil. We measured soil moisture content gravimetrically and rhizome fragments were fixed, sectioned, and stained for histological analysis. Only rhizomes from wet regions exhibited aerenchyma, whereas amyloplasts were much more abundant and larger in rhizomes from dry regions than in rhizomes from wet regions. Even though low starch content in wet soils indicates the occurrence of anaerobic metabolism, the presence of aerenchyma may contribute to the typically high performance of *H. coronarium* in waterlogged soils. Although further studies are needed to assess how responses at the rhizome level affect the competitive ability of *H. coronarium*, our findings show that they may play a role in the dominance of this species in Neotropical riparian sites.

Keywords: aerenchyma, amyloplasts, invasive macrophytes, riparian floodplains, soil hypoxia

The successful establishment of aquatic macrophytes depends on their tolerance to the excess and lack of water (Levitt 1980). This tolerance is often associated with morphological and physiological adaptations to cope with changes in the aquatic environment (Sultan 2003), which may originate from adaptive evolution to local conditions,

phenotypic plasticity, or a combination of both mechanisms (Davidson *et al.* 2011; VanWallendael *et al.* 2018). Among invasive macrophytes, the ability to spread and become dominant in new habitats is often closely related to rhizome adaptations (Weber 2011). Therefore, investigating morphological and physiological responses of invasive species to variability in the introduced range is essential to understand the drivers of invasion success (Williams *et al.* 2008). We investigated the influence of differences in soil moisture on the microstructure of rhizomes of *Hedychium coronarium* J. König (Zingiberaceae). *Hedychium coronarium*, commonly known as white ginger lily, is an up to 2 m high rhizomatous macrophyte native to tropical Asia (Kissmann 1997; Macedo 1997). The species has been widely introduced elsewhere as an ornamental plant (Kissmann 1997) and is an aggressive invasive species in Brazil's riparian sites (Chiba-de-Castro *et al.* 2016). Given the success of *H. coronarium* in Neotropical wetlands, we expected that its rhizomes would show histological adaptations to cope with soil hypoxia in response to high soil moisture.

The study was carried out in five floodplains of first or second order streams near the city of São Carlos (21°30'-22°30' S; 47°30'-48°30' W), São Paulo state, southeastern Brazil. The sites were at least 5 km apart and located on different river systems bordered by riparian vegetation and fragments of semi-deciduous mesophytic forest and of savanna vegetation (cerrado) (Soares *et al.* 2003, see Figures 3 to 8 in Almeida (2015) for further site details). The regional climate is a transition between Cwa.i and Aw.i (Alvares *et al.* 2013 *sensu* Köppen-Geiger), with a dry season from April to September and a rainy season from October to March. The annual average temperature is 21.8 °C and the annual average precipitation is 1,440 mm (Ferrari 2012). We randomly collected three rhizome fragments and three soil samples (5 to 10 cm deep) in the wet region (in the stream margin) and dry region (approximately 20 m distant from the stream) of each invaded site, resulting in 30 rhizome fragments and 30 soil samples overall. We mixed the three soil samples from each site to create a composite sample, so that there were 10 composite soil samples overall (five from wet regions and five from dry regions). We measured the soil moisture content gravimetrically after drying 50 g of each humid soil sample at 60 °C for 48 h and compared values between wet and dry regions using a paired t-test. Rhizome fragments were cut into 5 cm segments, fixed in Formalin-Acetic-Alcohol, progressively dehydrated in ethanol series (15 to 95 %) and embedded in glycol methacrylate historesin (Sims 1974). We transversely sectioned each processed rhizome at 4 to 7 μm with a rotary microtome and stained the resulting sections with 1 % thionine solution and Lugol reagent to detect starch. We photographed each rhizome section with an Olympus BX41 microscope. The percentage of cross-sectional area occupied by aerenchyma and amyloplasts was then quantified for each image using the ImageJ software. We calculated average percentages for

the three rhizome fragments from each site, resulting in five values for wet regions and five for dry regions.

Soil moisture was two times lower in dry (39.8 % ± standard deviation = 4.27) than in wet (85.2 % ± 5.06) regions (N=5, t=11.43, p<0.001). For all sites, we found large histological differences between rhizomes removed from the dry and wet region (Fig. 1). The percentage of rhizome cross-sectional area occupied by aerenchyma tissue was close to 0 % in rhizomes from dry regions (mean ± standard deviation- 0.002±0.008 %, Fig. 1A) but was on average 11.4 % (±0.024) in rhizomes from wet regions (Fig. 1B). By contrast, amyloplasts occupied over 20.9 % of the cross-sectional area in rhizomes from dry regions (±0.033, Fig. 1C), but occupied a very small area in rhizomes from wet regions (0.004±0.018 %, Fig. 1D).

We found that rhizomes of *H. coronarium* exhibited both aerenchyma lacunae and low abundance of amyloplasts in response to high soil moisture conditions. The production of aerenchyma is stimulated by ethylene accumulation and facilitates oxygen conduction from aerial to submerged organs (Armstrong *et al.* 2000; Jung *et al.* 2008). Accordingly, aerenchyma is commonly found in hygrophytic species subjected to hypoxic or anoxic conditions (Chen *et al.* 2002; Sultan 2003; Leite *et al.* 2012), including the emergent macrophyte *Typha angustifolia* L. (Typhaceae), found throughout Brazil (Corrêa *et al.* 2015). Likewise, Maricle & Lee (2002) reported that the highly invasive intertidal grass *Spartina alterniflora* Loisel (Poaceae) produced more aerenchyma in underground organs under flooding than in well-drained soils on the west coast of North America. However, in their study, the amount of aerenchyma shown by *S. alterniflora* in well-drained soils (10 %) was similar to what we found in rhizomes of *H. coronarium* collected in the wet region of riparian sites. *Spartina* grasses often grow vigorously under stressful conditions, such as saline and anoxic estuarine environments (Teal & Teal 1969), and tolerates high H_2S levels (Lee 2003). Therefore, we believe that this discrepancy between the findings of Maricle & Lee (2002) and our results could be attributed to differences in responses to oxygen depletion between the target species and/or in the local hydrological conditions.

Pan *et al.* (2012) associated low starch content in roots of emergent macrophytes subjected to flooding with increased consumption of carbohydrate due to fermentative metabolism. The most likely mechanism for this relationship is an increase in the activity of alcohol dehydrogenase and in the concentration of ethanol originated from ethylene as a result of flooding (Chen & Qualls 2003). The demand for soluble sugar is expected to increase due to the inefficient use of glucose in this alcoholic fermentation, so that roots under flooding tend to have high concentration of soluble sugars and low concentration of starch (Chen *et al.* 2005). This mechanism is consistent with the low number of amyloplasts in rhizomes of *H. coronarium* from wet regions compared to those from dry regions, as the main source
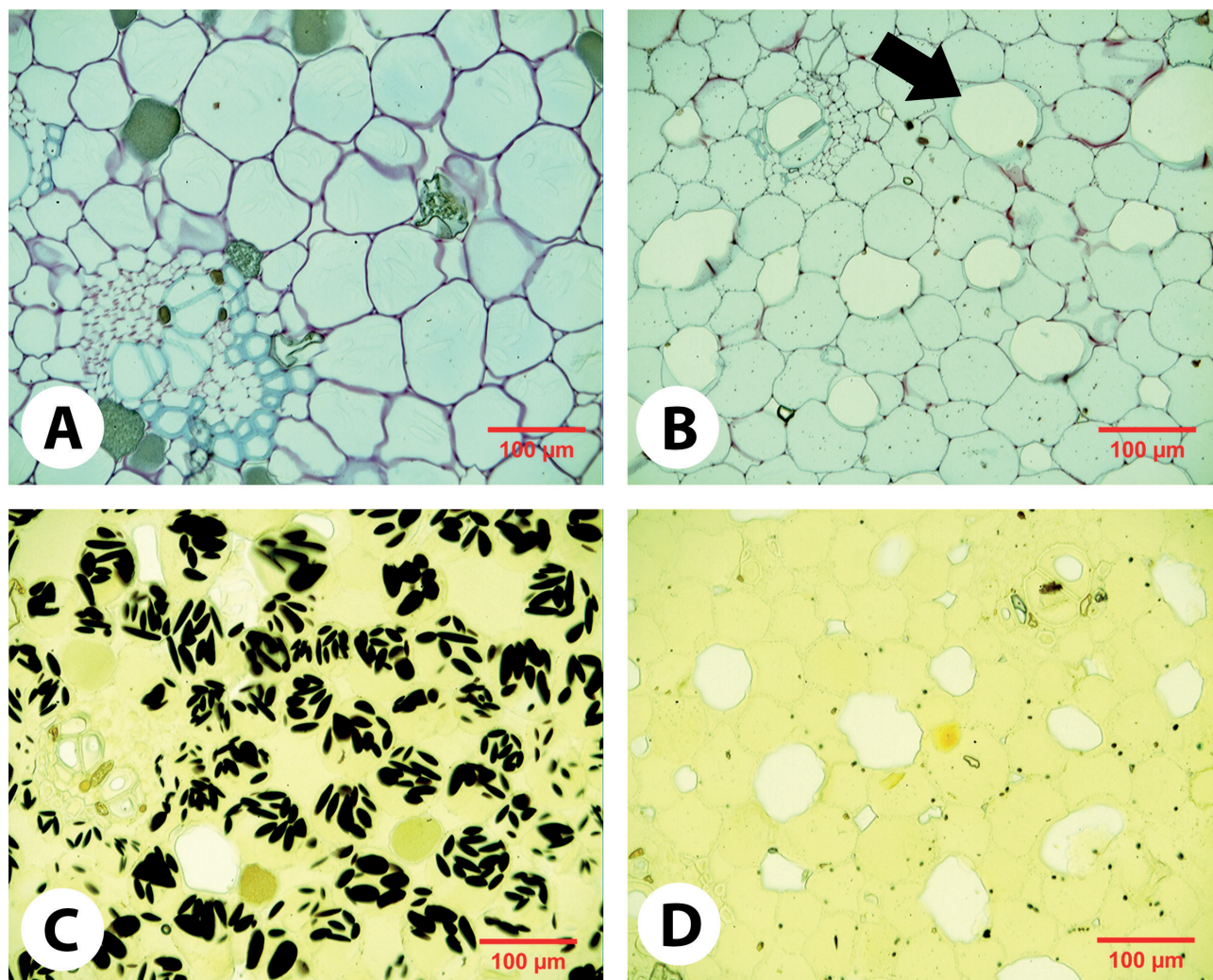
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Figure 1. Transverse sections of rhizomes of *H. coronarium* collected under different soil moisture conditions in ten riparian sites in southeastern Brazil. Rhizome sections were stained either with a 1 % tionine sollution (**A** and **B**) or lugol reagent (**C** and **D**). (**A**) Rhizome collected dry regions without aerenchyma. (**B**) Rhizome collected in wet regions showing aerenchyma. (**C**) Rhizome collected in dry regions showing large and numerous amyloplasts. (**D**) Rhizome collected in wet regions showing small and sparse amyloplasts.

of non-structural carbohydrates is the starch located in rhizome amyloplasts.

The occurrence of anaerobic metabolism in rhizomes of *H. coronarium* subjected to high soil moisture suggests that flooding can decrease the performance of this species. However, Neotropical riparian sites subjected to soil waterlogging are commonly dominated by this species and show low plant diversity and sparse tree cover (Chiba-de-Castro 2014), possibly because negative effects of hypoxic conditions on *H. coronarium* are alleviated by the production of aerenchyma in the rhizomes (Mendelssohn *et al.* 1981). Although further studies are needed to assess the role of these mechanisms of tolerance to soil reduction in the competitive ability of *H. coronarium* and coexisting native macrophytes, we conclude that specific rhizome responses to soil moisture conditions may contribute to the success of this invader in the Neotropics.

Acknowledgements

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