



UNIVERSIDADE FEDERAL DE SÃO CARLOS  
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE  
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

ROSANE OLIVEIRA COSTA

ESTRATÉGIAS DE INVASÃO DE *Hedychium coronarium* J. KÖNIG  
(ZINGIBERACEAE)

Orientadora: Prof. Dr<sup>a</sup> Dalva Maria da Silva Matos

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São Carlos

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Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos/SP, como parte dos requisitos necessários para obtenção do título de Doutora em Ciências, área de concentração Ecologia e Recursos Naturais.

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# UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde  
Programa de Pós-Graduação em Ecologia e Recursos Naturais

## Folha de Aprovação

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*“Coragem, coragem, se o que você quer é aquilo que pensa e faz*

*Coragem, coragem, eu sei que você pode mais”*

*Raul Seixas*



## APRESENTAÇÃO

Este trabalho refere-se à tese de doutorado de Rosane Oliveira Costa, realizada sob orientação da Prof. Dr<sup>a</sup>. Dalva Maria da Silva Matos (PPGERN/UFSCar) e coorientação de Maria Tereza Grombone-Guaratini (Instituto de Botânica, SP), apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos/SP, como parte dos requisitos necessários para obtenção do título de Doutora em Ciências, área de concentração Ecologia e Recursos Naturais. A tese, com o título “Estratégias de invasão de *Hedychium coronarium* J. König (Zingiberaceae)”, está estruturada em uma introdução geral, apontando a problemática da invasão biológica e as principais características da espécie estudada, seguida por quatro capítulos em formato de artigos científicos, apresentando os resultados dos experimentos realizados e uma conclusão geral, resumindo as principais inferências e implicações desse trabalho.

No primeiro capítulo, “Density effect on *Hedychium coronarium* J. König (Zingiberaceae) growth under different light conditions”, avaliamos o investimento energético da planta invasora *H. coronarium* em parte aérea e subterrânea, sob diferentes condições de densidade e luminosidade. O segundo capítulo, “Competition between an invasive and a native plant under different light conditions”, aponta os resultados do efeito da competição acima e abaixo do solo entre uma planta invasora e uma nativa sob diferentes condições de luminosidade. O terceiro capítulo, “Phytotoxicity of essential oil from the invasive *Hedychium coronarium* J. König (Zingiberaceae) on seeds of native riparian trees”, apresenta a composição do óleo essencial extraído de rizomas de *H. coronarium* e seu efeito na germinação de sementes de espécies arbóreas nativas. No quarto capítulo, “Toxicity of the invasive *Hedychium coronarium* J. König (Zingiberaceae) on aquatic species”, avaliamos a toxicidade do extrato aquoso de *H. coronarium* na sobrevivência e crescimento de organismos aquáticos.

Os capítulos aqui apresentados apontam algumas estratégias e consequências da invasora *H. coronarium* sobre comunidades invadidas em diferentes condições ambientais, reforçando sua plasticidade e seu poder de alteração do ambiente.

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## RESUMO

A invasão dos ecossistemas naturais por espécies exóticas ameaça a biodiversidade global, causando a exclusão de espécies nativas e perda de interações ecológicas, com prejuízo das funções e serviços ecossistêmicos. Espécies invasoras tem grande resistência a variações ambientais e são capazes de desenvolver estratégias que aumentam sua habilidade competitiva. *Hedychium coronarium* J. König (Zingiberaceae) é uma macrófita herbácea nativa do Himalaia, considerada invasora agressiva nas Américas. Neste estudo investigamos os mecanismos associados à dominância dessa espécie e os efeitos desta invasão sobre as comunidades terrestres e aquáticas associadas. Avaliamos 1) o efeito da competição intraespecífica (densidade de rizomas) na rebrota e no crescimento das partes aérea e subterrânea de *H. coronarium* sob diferentes condições de luminosidade; 2) a competição interespecífica, estimando o crescimento de plântulas de uma espécie arbórea nativa (*Anadenanthera macrocarpa*) na presença/ausência da invasora; 3) a fitotoxicidade do óleo essencial do rizoma de *H. coronarium* na germinação de espécies arbóreas ripárias nativas; e 4) a toxicidade do extrato aquoso de *H. coronarium* em organismos aquáticos (algas, macrófitas, cladóceros, insetos e peixes). *H. coronarium* apresentou desempenho e capacidade de ocupação similares em ambientes abertos e sombreados, sugerindo a existência de um mecanismo de compensação na alocação de recursos em diferentes condições de luminosidade e densidade. A presença de *H. coronarium* prejudicou o crescimento das plântulas de *A. macrocarpa* independentemente da retirada da parte aérea da invasora. Os compostos secundários do óleo essencial dos rizomas de *H. coronarium* afetaram a germinação de sementes de espécies arbóreas nativas. Observamos efeito tóxico do extrato aquoso nos organismos aquáticos testados, indicando alterações na dinâmica de cadeias tróficas de ecossistemas aquáticos invadidos. Nossos resultados sugerem que *H. coronarium* usa diferentes estratégias de invasão e impõe múltiplos efeitos negativos nas comunidades nativas, destacando seu alto potencial de invasão e capacidade de promover mudanças nos ecossistemas.

**Palavras-chave:** áreas úmidas, competição, invasão, plasticidade, toxicidade



## ABSTRACT

The invasion of natural ecosystems by exotic species threatens world biodiversity, causing competitive exclusion of native species and loss of ecological interactions, compromising ecosystem functions and services. Invasive species often resist to environmental variations and are capable to develop strategies that increase their competitive ability. *Hedychium coronarium* J. König (Zingiberaceae) is an herbaceous macrophyte native from Himalaya, considered an aggressive invader on Americas. We investigated its dominance mechanisms and the effects on associated terrestrial and aquatic communities. We evaluated 1) the effect of intraspecific competition (rhizomes density) on sprouting and growth of aerial and subterraneous parts of *H. coronarium* on different light conditions; 2) the interspecific competition, by estimating the seedling growth of a native tree species (*Anadenanthera macrocarpa*) on the presence/absence of the invader; 3) the phytotoxicity of the essential oil from *H. coronarium* rhizomes on the germination of native riparian tree species; and 4) the toxicity of aqueous extract of *H. coronarium* rhizomes on aquatic organisms (algae, macrophyte, cladoceran, insect and fish). *H. coronarium* showed similar performance and occupation capacity on opened and shaded environments, suggesting the existence of a compensation mechanism of resource allocation on different light and density conditions. The presence of *H. coronarium* harmed the growth of *A. macrocarpa* seedlings independently of the removal of aerial part of the invader. The secondary compounds of essential oil of *H. coronarium* rhizomes affected the germination of native riparian tree species. The aqueous extract was toxic for all tested aquatic organisms, indicating possible alterations on trophic chain dynamic of invaded aquatic ecosystems. Our results suggest that *H. coronarium* use different invasion strategies and impose multiple negative effects on native communities, highlighting their high invasion potential and capacity of promote ecosystem changes.

**Key words:** competition, invasion, plasticity, toxicity, wetlands

# INTRODUÇÃO GERAL

## CONTEXTUALIZAÇÃO

Diversos fatores limitam a distribuição das espécies ao longo do globo, como o clima, o tipo de solo, a topografia e a capacidade de dispersão (Woodward & Williams 1987). No entanto, espécies são frequentemente transportadas de forma proposital ou acidental para fora de suas regiões de origem. Algumas espécies introduzidas se adaptam às novas condições locais, são capazes de se reproduzirem espontaneamente e sustentar populações por várias gerações (Simberloff & Rejmanek 2011). Outras, além de se adaptarem são capazes de produzir número elevado de descendentes, exercer dominância no habitat e ainda excluir competitivamente espécies nativas, alterando a estrutura e o funcionamento do ecossistema invadido (Wolfe & Klironomos 2005), sendo classificadas como invasoras.

A invasão dos ecossistemas naturais por espécies exóticas e as consequências desse processo representam uma importante ameaça à biodiversidade (Mooney & Hobbs 2000; Simberloff & Rejmanek 2011). Em escala global, os impactos da invasão biológica estão diretamente relacionados à redução da diversidade biológica, perda de interações ecológicas e consequente degradação de habitats (Olden et al. 2004). Esses desequilíbrios comprometem o provimento de serviços ecossistêmicos, afetando a qualidade de vida humana, saúde, segurança e causando prejuízos econômicos (Charles & Dukes 2007; Pejchar & Mooney 2009). Assim, a ecologia da invasão é uma área de estudo crescente, com vasto corpo de teorias propostas e exploradas acerca do assunto (Catford et al. 2009). Basicamente, essas teorias buscam compreender os processos de invasão investigando tanto o potencial invasor das espécies (Marco et al. 2002) quanto a vulnerabilidade dos ecossistemas (Davis et al. 2000).

As comunidades biológicas oferecem resistência à invasão através da ação de inimigos naturais, como competidores, herbívoros e patógenos, que limitam a capacidade de colonização e a persistência de espécies não nativas ("*Biotic resistance hypothesis*", Levine et al. 2004). Quanto maior a biodiversidade local, maior a resistência biótica exercida pela comunidade e menor propensão à invasão (Kennedy et al. 2002). Em oposição, quanto menor a diversidade e maior ocorrência de distúrbios, maior a vulnerabilidade do habitat. Áreas úmidas são particularmente vulneráveis a invasões, onde distúrbios, umidade e nutrientes se acumulam, tornando-se mais propensas à ocorrência de espécies dominantes (Zedler & Kercher 2004).

Espécies de plantas estão entre as mais representativas entre os invasores (Luque et al. 2014). Geralmente plantas invasoras apresentam ampla tolerância ambiental, altas taxas de germinação e crescimento, capacidade de reproduzir vegetativamente e impor dominância sobre a diversidade biológica das comunidades invadidas, alterando as características do ambiente natural (Simberloff & Rejmanek 2011). Vários estudos relatam o efeito negativo de espécies de plantas invasoras sobre comunidades nativas (Levine et al. 2003; Gaertner et al. 2009; Hejda et al. 2009), as quais podem afetar espécies já estabelecidas e suprimir o estabelecimento de novos indivíduos dessas espécies (Stork & Turton 2008). As invasoras podem impactar diretamente os ecossistemas, alterando a composição da comunidade e a ciclagem de nutrientes, aumentando a produtividade e depósito de serapilheira, além de modificar as relações com microrganismos, competidores, herbívoros (Zedler & Kercher 2004) e os processos evolutivos (Mooney & Cleland 2001). Além disso, são capazes de reduzir a diversidade de plantas e animais e alterar o funcionamento da teia alimentar, modificando a qualidade dos recursos disponíveis (Werner & Zedler 2002).

A competição por luz afeta diretamente a composição das comunidades vegetais em ambientes naturais (Pagès et al. 2003). Determinadas espécies invasoras provocam sombreamento de extensas áreas impedindo o crescimento de espécies nativas dependentes de luz. Além disso, a grande produção de matéria vegetal morta (serapilheira) modifica as condições do microambiente da superfície do solo, alterando a amplitude térmica, a interceptação de luz e de chuva, bem como a transferência de calor e água entre o solo e a atmosfera (Facelli & Pickett 1991).

Espécies invasoras clonais produzem grande número de descendentes e ocupam vastas regiões, inibindo o crescimento de espécies nativas. No entanto, os rametos produzidos por espécies clonais tendem a ocupar os espaços próximos à planta mãe e competir entre si (Silvertown & Charlesworth 2001). Dessa forma, deve existir um *trade-off* entre produção de descendentes e competição por recursos. Apesar da competição entre coespecíficos, o crescimento clonal é vantajoso no sentido de mobilidade, disputa por espaço e armazenamento de recursos sem os custos e riscos da reprodução sexuada (Silvertown & Charlesworth 2001). Além disso, rametas de plantas clonais conectadas a uma mesma estrutura subterrânea podem compartilhar recursos e, assim, usar mais eficientemente nutrientes distribuídos heterogeneamente no solo (Zhou et al. 2012). Tais

características, além de contribuir para o sucesso do invasor, podem afetar negativamente o crescimento de outras espécies.

Espécies invasoras geralmente enfrentam menos inimigos naturais nas áreas invadidas em relação à sua região de origem (“*Enemy release hypothesis*”, Elton 1958). Assim, essas espécies podem destinar menos recursos à defesa e desenvolver características que aumentam suas habilidades competitivas (Callaway & Ridenour 2004). Por exemplo, várias plantas invasoras produzem compostos secundários tóxicos (Callaway & Ridenour 2004) que podem inibir a germinação e o crescimento de outras plantas (Callaway et al. 2008; Inderjit et al. 2011). A interferência química mediante a liberação de aleloquímicos tem sido apontada como um mecanismo chave do processo de invasão biológica, afetando profundamente a caracterização das interações interespecíficas, a estruturação da comunidade e a dinâmica da vegetação nativa (Inderjit & Ducke 2003). Os efeitos desses compostos podem atingir diversos níveis: individual, populacional, comunitário e ecossistêmico (Wardle et al. 1998). Ou seja, os efeitos tóxicos não alteram apenas o sucesso de outras plantas, mas também podem afetar outros grupos, como micro-organismos do solo e herbívoros, resultando em mudanças nos processos e funções dos ecossistemas (Reinhart & Callaway 2006).

Nesse sentido, estudos que avaliam a influência dos aleloquímicos produzidos por plantas invasoras consideram a hipótese de *novel weapons* (novas armas) (Callaway & Ridenour 2004), em que espécies invasoras exercem forte efeito fitotóxico contra espécies nativas através da adição de novos compostos químicos ao ambiente e, assim, contribuir para a sua dominação e homogeneização dos ecossistemas. Esses compostos podem agir de múltiplas maneiras, por exemplo, reduzindo a germinação de espécies nativas (Bais et al. 2003), repelindo herbívoros e patógenos (Callaway & Ridenour 2004) e ainda controlar populações de outros organismos que vivem em ambiente semelhante. Em áreas ripárias, esse efeito pode se aplicar a espécies terrestres e aquáticas e alterar a dinâmica de teias tróficas em áreas invadidas (Brown et al. 2006).

## CARACTERIZAÇÃO DA ESPÉCIE

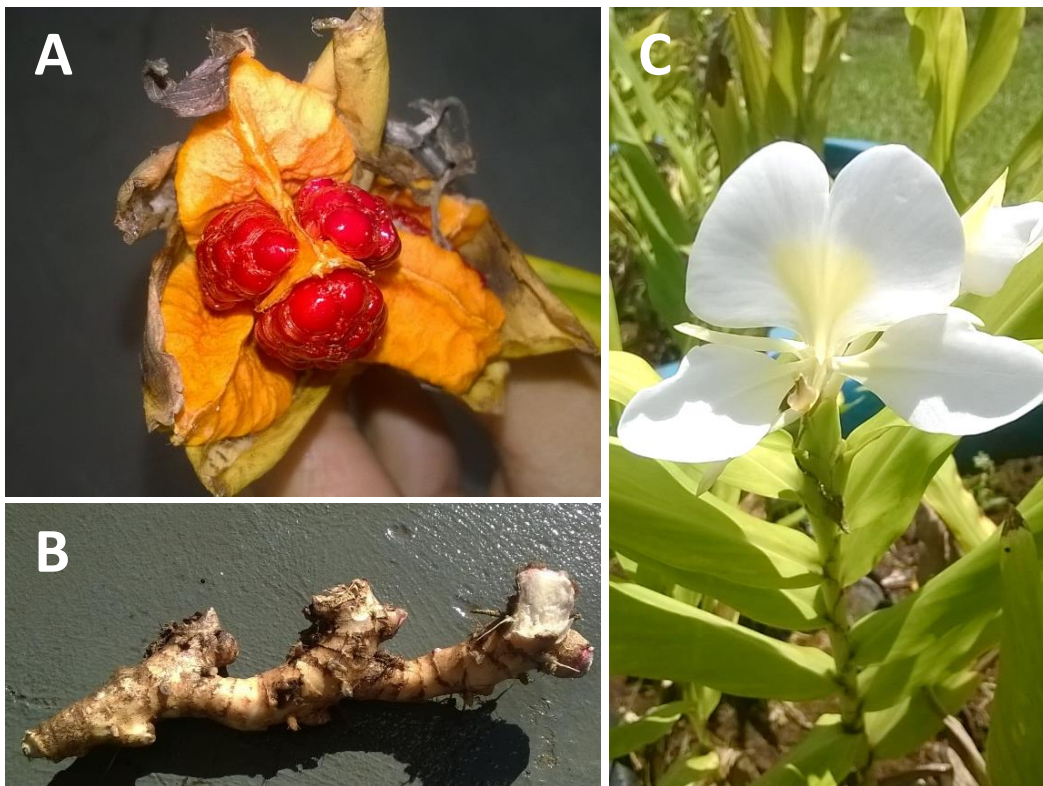
*Hedygium coronarium* J. König (Zingiberaceae) é uma macrófita herbácea nativa da região do Himalaia, na Ásia tropical (Macedo 1997). Em sua região de origem, é

considerada ameaçada e possui amplo uso farmacológico, sendo conhecida por suas propriedades antifúngicas, antimicrobianas, anti-inflamatórias, analgésica, antioxidante, dentre outras (Pachurekar & Dixit 2017). Por outro lado, é invasora nas Américas, desde os Estados Unidos até a Argentina, tendo sido introduzida no Brasil em 1819 (Kissmann & Groth 1999). Recebe os nomes comuns de lírio-do-brejo, mariazinha-do-brejo e gengibre-branco (Kissmann & Groth 1999; Macedo 1997). No Brasil, a espécie tem ampla distribuição, estando presente inclusive em unidades de conservação federais (Sampaio & Schmidt 2014).

*H. coronarium* é considerada agressiva devido ao seu desenvolvimento rápido em locais de elevadas umidade e temperatura e à profusão de rizomas, formando densas populações em áreas úmidas brasileiras (Kissmann & Groth 1999) (Fig. 1). Ocorre em forma de manchas, apresenta tanto reprodução sexuada, por formação de sementes, quanto assexuada, pela produção de hastes aéreas a partir de pedaços do rizoma, que se rompem e são dispersos pela água (Stone et al. 1992) (Fig. 2). Apresenta folhagem densa e alta, que sombreia as camadas inferiores de vegetação, diminuindo a ocorrência de espécies nativas (De Castro et al. 2016). Acumula grande quantidade de biomassa aérea e conseqüentemente cria camadas espessas de serapilheira, além de apresentar compostos alelopáticos nas folhas, flores, caules e rizoma (Miranda et al. 2015; Ray et al. 2018). A presença da espécie pode também alterar a estrutura e qualidade de áreas úmidas, reduzindo a diversidade local e a ocorrência de espécies animais (Del-Rio et al. 2017; Saulino & Trivinho-Strixino 2017).



**Fig. 1** Área úmida invadida por *Hedychium coronarium* no município de São Carlos (SP), Brasil.



**Fig. 2** Detalhes das estruturas vegetativas/reprodutivas de *Hedychium coronarium*. A) fruto maduro com sementes, B) rizoma, C) rameta com flor.

## OBJETIVO

Considerando os impactos negativos causados por *H. coronarium* nos ecossistemas invadidos, o objetivo deste estudo foi investigar os mecanismos de dominância utilizados por esta invasora e quais os possíveis efeitos desta invasão sobre as comunidades terrestres e aquáticas associadas.

## OBJETIVOS ESPECÍFICOS

- 1- Avaliar o efeito da competição intraespecífica, representada pela densidade de rizomas, na rebrota e crescimento da parte aérea e subterrânea de *H. coronarium* sob diferentes condições de luminosidade.
- 2- Avaliar o efeito da competição interespecífica no crescimento inicial de uma espécie arbórea nativa (*Anadenanthera macrocarpa*), na presença/ausência de *H. coronarium*, sob diferentes condições de luminosidade.
- 3- Avaliar o efeito fitotóxico do óleo essencial dos rizomas de *H. coronarium* na germinação de espécies arbóreas nativas de área ripária.
- 4- Avaliar a toxicidade do extrato aquoso de *H. coronarium* em organismos aquáticos produtores, invertebrados e vertebrados.

## HIPÓTESES

As hipóteses de estudo foram:

- 1- Ocorrência de maior produção de biomassa aérea e subterrânea em condições de maior luminosidade (maior oferta de recursos) e menor densidade (menor competição intraespecífica).
- 2- A presença da invasora *H. coronarium* afeta o crescimento da espécie nativa *A. macrocarpa*, principalmente sob menor luminosidade.
- 3- Os compostos presentes nos rizomas de *H. coronarium* afetam a germinação de espécies nativas arbóreas presente em áreas ripárias, contribuindo para sua dominação.
- 4- Os compostos presentes nos rizomas de *H. coronarium* são tóxicos a organismos aquáticos, afetando diretamente as comunidades ripárias.



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# CAPÍTULO I

DENSITY EFFECT ON *Hedychium coronarium* J. KÖNIG (ZINGIBERACEAE)  
GROWTH UNDER DIFFERENT LIGHT CONDITIONS

ABSTRACT

Invasive species can affect ecosystems functioning by outcompeting native plants, but invader success depends on both biotic and abiotic attributes of invaded communities. Therefore, it is important to investigate how invasive species respond to the increase of their own abundance under different conditions during the dominance process. In this sense, intraspecific competition and their interactions with environmental conditions remain poorly studied for invasive species. We assessed density effects on ramet and rhizome growth on the invasive *Hedychium coronarium* (Zingiberaceae), establishing two paired density treatments: high density (25 rhizomes) and low density (five rhizomes) with five replicates per treatment under natural light and 50% of shade. We counted the number of ramets and leaves per ramet, measured the ramet diameter, the volume of each rhizome, the fresh and dry weight of rhizomes and ramets. We used linear mixed models to test the influence of light, density and their interaction on rhizome biomass, ramet production, the relation ramets/rhizomes, number of leaves, diameter and mean dry weight of ramets. On natural light condition, *H. coronarium* produced more ramets, whereas under shadow there were fewer but higher ramets and more investment on rhizome production. *H. coronarium* maintained constant biomass production under different light conditions and intraspecific competition levels. There was no interaction of density and light on evaluated parameters. Our results suggest a trade-off between rhizome growth to avoid light competition on shaded sites and investment on aboveground biomass production to rapid colonization in favorable sites. These strategies of energy allocation may have a key role on the colonization of different habitats, such as opened wetlands and closed, forested sites.

**Key Words-**Biomass, intraspecific competition, invasion

## INTRODUCTION

Invasive species can affect ecosystems through changes in the habitat, biological and ecological processes of the community (Elton 1958). The understanding of how exotic species become invaders may avoid their proliferation and reduce their impact on communities (Kolar and Lodge 2001). The success of invasive species depends on their biological traits, biotic and abiotic characteristics of invaded communities (Simberloff and Rejmanek 2011). Several researches described attributes and traits of invader species and their capacity of occupation and dominance (Marco et al. 2002), while other studies investigated habitat conditions and its susceptibility to invasion (Davis et al. 2000). However, associate aspects of invasive species and environmental factors is important to improve the knowledge about the invasion process.

The competition between invasive and native species has been investigated to explore invaders dominance strategies (Vila and Weiner 2004; Herrera et al. 2016), however intraspecific competition for invaders remains poorly studied (Huang and Peng 2016). Conspecific individuals are similar and compete for resources, which can affect their growth, survival and reproduction (Wang et al. 2014). These effects are as greater as higher the density of conspecific individuals as a result of intraspecific or environmental pressures (Wang et al. 2014). Generally, species respond negatively to density effect, which can regulate their population size (Müller-Schärer et al. 2004).

Invasive plants usually show high phenotypic plasticity (DeWalt et al. 2004; Davidson et al. 2011), which allows them to occupy a wide range of habitats. Nevertheless, environmental factors such as nutrient availability, temperature and light can regulate their spatial distribution (El-Keblawy and Al-Rawai 2005; Lannes et al. 2016). For instance, light radiation can increase the photosynthesis rate until the saturation on some macrophyte species, but excessive light intensity can inhibit their development, suggesting optimal intensity bands (Cosby et al. 1984). Although the distribution of most of invasive species can be constrained by environmental factors, some clonal plants present compensation mechanisms as adaptive strategy to growth under different abiotic conditions (Ming 1996; Ikegami et al. 2007).

*Hedychium coronarium* J. König (Zingiberaceae) is a successful invader from tropical Asia, with rapid growth and efficient dispersion in Brazilian riparian zones, causing impact on the maintenance of biodiversity and ecosystem services (Kissmann and Groth 1999; De Castro et al. 2016). It presents efficient vegetative reproduction through

rhizomes from which small fragments can be dispersed by water flow (Stone et al. 1992), originating new plants (Kissmann and Groth 1999).

To understand internal and external (environmental) regulatory mechanisms of invasive plant populations is useful to predict fluctuations in their birth rates, growth and mortality (Davis et al. 2000). Here, we experimentally assessed the effects of rhizome density on sprouting and growth of *H. coronarium* under different light conditions in a controlled experiment. We expect higher ramet biomass and greater rhizome production under higher light (more resources) and lower density (reduced intraspecific competition).

## MATERIALS AND METHODS

### *Experimental Design*

We conducted the experiment at an external area in the Center of Hydric Resources and Environmental Studies of University of São Paulo (CRHEA-USP) in Itirapina, São Paulo State, southeastern Brazil (22°10'08"S, 47°53'59"W). The climate is humid subtropical with hot summer and dry winter (Cwa according Köppen), mean monthly temperature 19.3 °C and annual rainfall around 1400 mm (Alvares et al. 2013). The experiment was carried out using ten 500 L tanks with drain exhaust system at the bottom. We divided each tank in half with impermeable canvas and both sides were filled with a soil layer of ~40 cm height (Fig. I-1). In each half, we transplanted *H. coronarium* rhizomes of ~20 cm length, establishing two paired density treatments: high density (25 rhizomes) and low density (five rhizomes). We choose these densities according a previous study of *H. coronarium* population dynamics in the region (De Castro et al. 2016).

To assess environmental condition effects on *H. coronarium* growth, we distributed five tanks in each light condition: 50% (hereafter “shadow”) and 100% of light (under natural light). We constructed the shadow treatment covering the tanks with 50% black screen. In the first six months of the experiment, we used a dispersion irrigation system over the tanks, for better rhizome establishment and stimulate the production of new ramets. After 22 months (April 2016), we counted the number of ramets and leaves per ramet, measured the ramet diameter at basis (to the nearest 0.01 cm using a manual caliper) and length (to the nearest 0.5 cm using a ruler). We measured the volume of each rhizome and weighed rhizomes and ramets using a digital scale (precision 0.01 gr) to



obtain fresh weight. We dried the ramets and rhizomes at  $60\text{ }^{\circ}\text{C} \pm 2$  for one week to obtain their dry weight.



**Fig. I-1** Experimental design of rhizome density effect on *Hedychium coronarium* development under different environmental conditions.

### *Statistical Analyses*

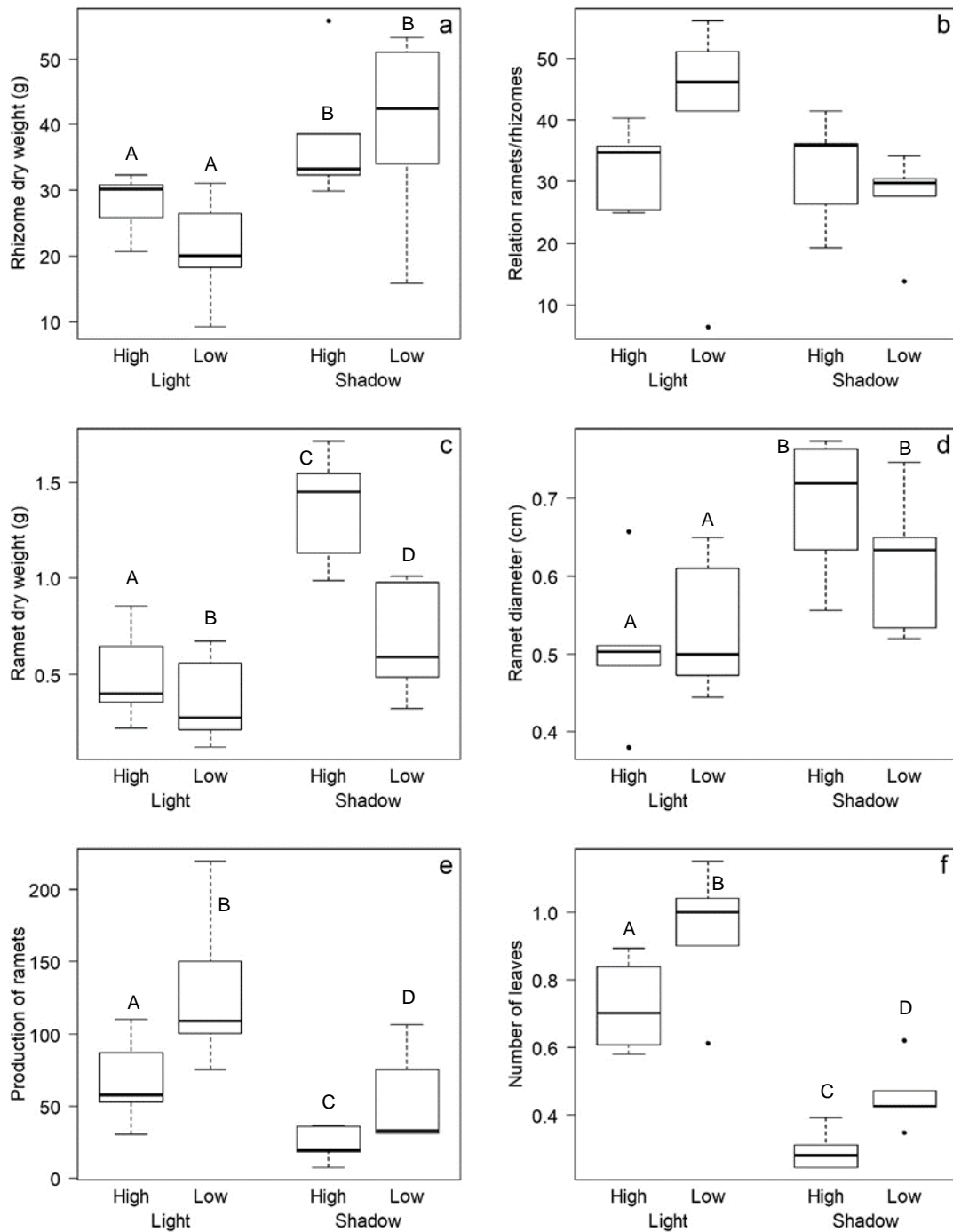
We estimated the number of leaves per ramet dividing it by ramet length. We used the ramet dry weight as a proxy to ramet length since they were strongly correlated (data not shown). For each replicate, we estimated the mean rhizome dry weight (total rhizome dry weight/number of rhizomes), the ramet production (number of ramets/total rhizome dry weight in kg), the relation ramet/rhizome biomass (total ramet dry weight/total rhizome dry weight), the total number of leaves, the mean diameter and the mean dry weight of ramets. Through linear mixed models we tested the influence of light, density and their interaction (fixed factors) on rhizomes (mean dry weight), ramet production, the relation ramets/rhizomes, number of leaves, diameter and mean dry weight of ramets. We used the tank number as random effect. All analysis and graphs were performed in the R environment (R Development Core Team 2017), using “*lmer*” function of “*lme4*” package (Bates et al. 2015).

## RESULTS

We found that 318 ramets of *H. coronarium* emerged during the experiment. We found negative influence of light on dry weight of rhizomes (Table I-1), but there was no significant effect of density treatment neither of its interaction with light (Fig. I-2a). The relation ramet/rhizome biomass was not affected by light neither by density (Table I-1, Fig. I-2b). However, the mean ramet dry weight was positively affected by rhizome density and negatively by light. Their interaction was also statistically significant, where the positive effect of density was higher under shadow condition (Fig. I-2c). The diameter of ramets was just negatively influenced by light (Fig. I-2d). Both the production of ramets and the total number of leaves were negatively affected by rhizome density and positively by light, but were not influenced by their interaction (Fig. I-2e, f).

**Table I-1.** Results of ANOVA of linear mixed models testing the effects of light treatment (Light), density treatment (Density) and their interaction (Density  $\times$  Light) on mean rhizome dry weight, production of ramets, number of leaves, ramet diameter, mean ramet dry weight and relation ramet/rhizome of *Hedychium coronarium*. The column (+/-) represents the positive (+) or negative (-) effects. \*statistical significance ( $\alpha = 0.05$ ).

	Sum sq.	df	F	P	+/-
<i>Rhizome dry weight</i>					
Density	39.51	8	0.486	0.505	
Light	609.88	8	75.034	0.025*	-
Density $\times$ Light	87.26	8	10.735	0.330	
<i>Production of ramets</i>					
Density	11296	16	8.3000	0.011*	-
Light	17890	16	13.451	0.002*	+
Density $\times$ Light	1220	16	0.897	0.358	
<i>Number of leaves</i>					
Density	0.179	8	11.670	0.009*	-
Light	0.728	8	47.369	<0.001*	+
Density $\times$ Light	0.004	8	0.232	0.643	
<i>Ramet diameter</i>					
Density	0.002	8	0.3198	0.587	
Light	0.068	8	88.368	0.018*	-
Density $\times$ Light	0.013	8	16.466	0.235	
<i>Ramet dry weight</i>					
Density	0.829	8	29.325	0.001*	+
Light	0.399	8	14.123	0.006*	-
Density $\times$ Light	0.395	8	13.972	0.006*	
<i>Relation ramets/rhizomes</i>					
Density	14.397	8	0.181	0.682	
Light	88.243	8	110.831	0.323	
Density $\times$ Light	197.673	8	248.273	0.154	



**Fig. I-2** Rhizome dry weight (a), relation ramets/rhizomes (b), ramet dry weight (c), ramet diameter (d), production of ramets (e) and the number of leaves (f) of *Hedychium coronarium* on different conditions of density (High and Low) and light (Light and Shadow). Capitalized letters indicate significant differences.

## DISCUSSION

We assessed the energetic investment of *H. coronarium* on rhizomes and ramets under different conditions of density and light. Contradicting our initial hypothesis, the rhizome growth was similar between high and low density treatments. One likely reason for this is that the rhizome growth reached during the experiment was not enough to observe the effect of intraspecific competition. On the other hand, our results demonstrated that the rhizome biomass production is higher on shaded areas, where there is greater competition for light. Considering that rhizomes are an important propagule (Lange and Marshall 2016), the higher production of rhizomes under shadow conditions may be associated to subterranean propagation and the effort to colonize new areas, an attempt to escape competition for light. Alternatively, rhizomes are able to sustain the continued production of new ramets to compensate the higher mortality in open areas, although it limits their own growth and consequently subterranean propagation.

The production of ramets decreased with rhizomes density under either natural light or shaded condition because of intraspecific competition. Other studies show that neighborhood density affects the growth, survival and fitness of clonal species (de Kroon and Kwant 1991; Kleunen et al. 2001; Van Kleunen et al. 2005). The ramet diameter was lower under natural light, probably due to competition between ramets for water. In contrast, the production of ramets and the number of leaves were higher under natural light, showing that there was a higher investment in the number of ramets rather than in ramet size (De Castro et al. 2016). This result suggest that light stimulate sprouting of *H. coronarium*, a remarkable strategy of invasive clonal plants to colonize open areas (Ikegami et al. 2007).

In our study, ramet dry weight was higher in shadow treatment than under natural light at both density treatments. In fact, height of *H. coronarium* ramets can be positively affected by shading levels (Santos et al. 2005). Furthermore, some clonal species can exhibit higher growth rates in shadow environments, increasing investment on height under greater light condition (Huber and Wiggerman 1997; Ikegami et al. 2007). Similarly, the increase in rhizome biomass was higher in shaded areas. Considering the invasiveness of *H. coronarium*, as registered in Brazil and other American countries (Kissmann and Groth 1999), our results indicate that closed canopy forests, as riparian forests, can facilitate the growth of rhizomes and ramets, being more susceptible to the spatial expansion of invasion range. Besides, these areas may be “key areas” for *H.*

*coronarium* invasion, once in this condition is possible to save energy (less investment in production of ramets) and invest on subterranean propagation (greater production of rhizome biomass) to colonize new areas.

We expected to find a negative effect of rhizome density on aerial biomass as an intraspecific competition effect (Müller et al. 2016). However, although individual parameters have differed between the treatments, the total productivity of aerial biomass by the rhizomes did not vary between them. Our results suggest the existence of division of labor (Ikegami et al. 2008) as mechanism of compensation to maintain the relation of aerial/subterranean biomass. These strategies of energy allocation may have a key role on the colonization of different habitat types. *H. coronarium* can keep biomass production constant on varied environmental conditions and intraspecific competition levels, establishing a compensation mechanism of energy investment in ramets: higher and fewer ramets on the shade, smaller and more ramets on the light. These traits may confer an important competitive advantage and spatial expansion to different areas, such as opened water lands and closed, forested sites.

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# CAPÍTULO II

# COMPETITION BETWEEN AN INVASIVE AND A NATIVE PLANT UNDER DIFFERENT LIGHT CONDITIONS

## ABSTRACT

Invasive plants negatively affect the invaded communities, reducing their biodiversity by changing environmental conditions and outcompeting native species. Thus, to understand the way in which ecological compartments and requirements (i.e. light and space) modulate interspecific competition is important to elucidate dominance mechanisms of invasive plants. Here, we tested the effect of above- and belowground competition between an invasive amphibious macrophyte (*Hedychium coronarium*) and seedlings of a native pioneer riparian tree (*Anadenanthera macrocarpa*) on their growth under different light conditions. We disposed five tanks under natural light and five tanks under partial shade. We transplanted *H. coronarium* rhizomes and sowed *A. macrocarpa* seeds to establish four competition treatments per tank: invasive only, native only, both species together with clipping of invasive aerial parts and both species together without clipping. The length growth of the native seedlings was not affected by light or by the interaction competition  $\times$  light, but was higher in invader absence. *H. coronarium* ramets instead had higher length growth in the presence of native seedlings and under natural light. We observed no difference in length growth of native seedlings between before and after the removal of invasive aerial parts. Our results suggest that the negative effect of this invader was related mainly to belowground competition rather than to shading by aerial parts. The invasion by *H. coronarium* may create a barrier to the development of native seedlings, harming the recolonization by native species and consequently the ecological succession in riparian invaded areas.

**Key Words**-*Anadenanthera macrocarpa*, belowground competition, biological invasion, *Hedychium coronarium*, riparian ecosystems

## INTRODUCTION

Invasive plants cause negative effects on the plant diversity of invaded communities (Levine et al. 2003; Gaertner et al. 2009; Hejda et al. 2009). The interspecific competition is an important factor in this process because it can affect negatively the reproduction, growth and germination of native species (Vila and Weiner 2004), which may cause the competitive exclusion of natives (Dickson et al. 2012). Invasive species usually face less natural enemies on invaded regions, enabling them to translocate resources from defense to mechanisms that improve their competitive ability (“evolution of increased competitive ability hypothesis” Blossey and Rolf 1995; Keane and Crawley 2002). As a consequence, several invasive plants, which are not good competitors on their native range, develop this ability on invaded communities (Callaway and Aschehoug 2000). However, the ecological requirements responsible for invasive species outcompete native ones is still a barely explored issue.

Plants compete for light, water, space and soil nutrients, and simultaneously for combination of these resources (Aschehoug et al. 2016). For this reason, they develop multiple competitive abilities to tolerate, respond to and also suppress their neighbor (Bennett et al. 2011). The interference competition inhibit neighbor’s ability to obtain resources and grow (Aschehoug et al. 2016) and may occur either above and belowground (Casper and Jackson 1997). Invasive plants in particular have high vegetative growth rates so they can rapidly colonize and dominate invaded habitats (Van Kleunen et al. 2010). The physical effects of the large biomass of invasive plants have a key role in promoting the dominance of invasive species by affecting the established natives and suppress their recruitment (Stork and Turton 2008).

The shading and accumulation of litter produced by the high vegetative growth rates of dominant invaders cause deep changes in the environmental local conditions, altering the thermic amplitude and the interception of light and rain (Facelli and Pickett 1991). These microhabitat alterations form a barrier that harm seed germination and the emergency of seedlings (Facelli and Pickett 1991; Inderjit et al. 2008; Currie et al. 2014) affecting the growth of light dependent species, mostly. Some exotic species change the availability of light differently of natives, in the same community, by modifying the quantity and quality of available light (Reinhart et al. 2006), which suggests that the shading is a strategy that enhance their dominance.

Here, we assessed the effect of aboveground competition between the invasive amphibious macrophyte *Hedychium coronarium* J. König (Zingiberaceae) and seedlings of a native pioneer riparian tree *Anadenanthera macrocarpa* (Benth) Brenan (Fabaceae). We evaluate their growth rate under different light conditions. We hypothesized that 1) the presence of the invasive reduce the growth rate of native seedlings; 2) the growth rate of native seedlings is higher when the aerial part of the invasive species is removed; 3) the negative effect of the invasive on the seedlings growth is greater under low light condition.

## MATERIALS AND METHODS

### *Studied Species*

*H. coronarium* is native from tropical Asia and became invader on Brazilian riparian areas. This species has fast growth and its rhizomes have high sprouting capacity, forming dense populations and depositing great amount of litter on the soil (Kissmann and Groth 1991). It occurs on shaded and open areas and along a wide range of soil moisture. *H. coronarium* produce allelochemical compounds on its leaves, flowers and rhizomes (Pachurekar and Dixit 2017), which avoid the germination and growth of native species, reducing the local biodiversity (De Castro et al. 2016). *A. macrocarpa* is a native pioneer tree species that occurs on Brazilian riparian areas (Assis et al. 2013). It presents rapid growth and reaches up to 20 m height, produces large amount of seeds and has high germination rate (Lorenzi 2000).

### *Experimental Design*

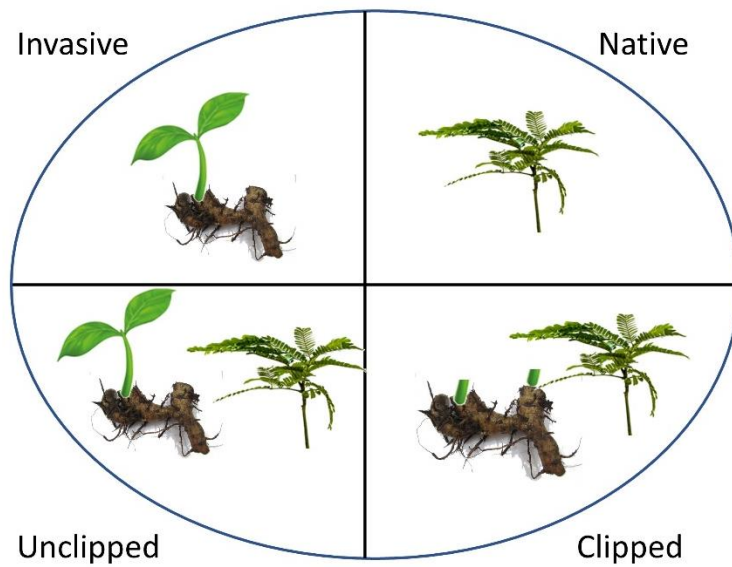
The experiment was conducted between September 2016 to March 2018 at the Center of Hydric Resources and Environmental Studies of University of São Paulo (CRHEA-USP) in Itirapina, São Paulo State, southeastern Brazil (22°10'08"S, 47°53'59"W). We used ten circular 500 L tanks with drain exhaust system at the bottom. Each tank was splitted with impermeable canvas in four equal semicircles. All semicircles were similarly filled with soil layer of ~40 cm height. In order to assess the influence of *H. coronarium* on seedling development we established four competition treatments per

tank: (1) only the invasive species was planted (“Invasive”), (2) invasive and native species were planted (“Unclipped”) (3) invasive and native species were planted and the invader was cut off after four months of native germination (“Clipped”) and (4) only native was planted (“Native”) (Fig. II-2). In September 2016, we transplanted ten *H. coronarium* rhizomes of ~20 cm length into the following treatments: Invasive, Clipped and Unclipped. In September 2017, one year after the establishing of ramets, we planted seeds of the native species *Anadenanthera macrocarpa*. We sowed 10 seeds in the treatment Clipped, 10 seeds on the treatment Unclipped, and 20 seeds on the treatment Native.

To assess the influence of light, we disposed two groups of five tanks to different light conditions: 50% of light (shadow treatment) and 100% of light, under natural light (Fig. II-1). We watered the plants daily using an aspersion irrigation system over the tanks. We marked all ramets of *H. coronarium* and all individuals of *A. macrocarpa* and measured them for diameter and length in November 2017, January and March 2018. In January and March, we cut the aerial parts of *H. coronarium* from the treatment “Clipped” of each tank. At the end of the experiment, we estimated the fresh weight of ramets that have grown from January to March.



**Fig. II-1** Experimental design of competition between *Hedychium coronarium* and *Anadenanthera macrocarpa* under different environmental conditions.



**Fig. II-2** Treatments established in the semicircles of the tanks.

## Statistical Analyses

To test whether the removal of aerial parts of the invader affected native seedling development, we performed linear mixed models using growth in length and in diameter as continuous dependent variables, and competition treatments (Native, Clipped and Unclipped), light treatments (shaded and natural light) and their interaction as fixed explanatory variables. We also used linear mixed models to assess the effect of native presence (Unclipped and Invasive treatments), light (shaded and natural light treatments) and their interaction in *H. coronarium* ramet growth (in length and in diameter). In all linear mixed models, the dependent variables (growth in length and in diameter) were obtained from the difference between the measurements in January and March for each ramet or for the whole individual, and tank was set as random factor.

We tested whether the length growth of native seedlings varied between the periods before (Nov-Jan) and after clipping (Jan-Mar) of invader aerial parts using the paired Student t-test for those of Unclipped treatment (normally distributed data, Shapiro-Wilk test,  $P = 0.101$ ) and paired Wilcoxon test for those of Clipped and Native treatments (not normally distributed data,  $P = 0.039$  and  $P = 0.007$  respectively). To assess if the length growth of native seedlings was intrinsically related to the invasive abundance rather than its presence/absence, we employed a within-treatment approach. We selected three variables representing the invasive abundance on the Unclipped treatment (mean ramet height, number of ramets and mean ramet length growth). We first tested if these variables were correlated using Pearson correlation test. As the ramet height and mean ramet length growth were negatively correlated (see Results), we conducted a multiple regression model to evaluate the influence of mean ramet height and number of ramets on the native seedling growth. We also performed a linear regression model to test the effect of total fresh weight of ramets on seedling length growth within the treatment Clipped. All analyses were performed in the R environment (R Development Core Team 2017), using the packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova 2016).

## RESULTS

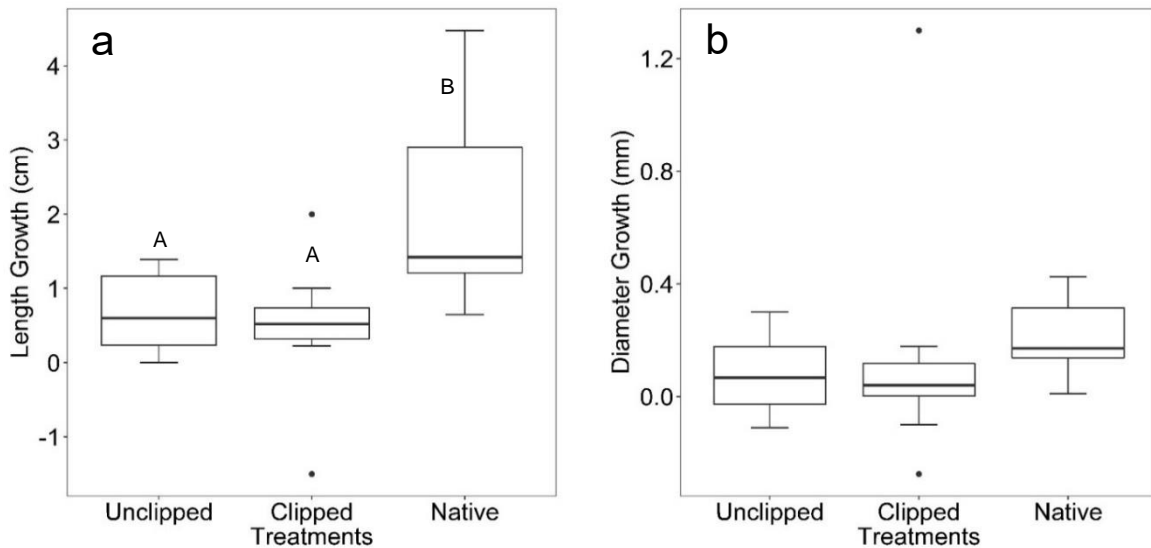
The length growth of *A. macrocarpa* differed between treatments, but was not affected by the light and by their interaction (Competition  $\times$  Light) (Table II-1). The



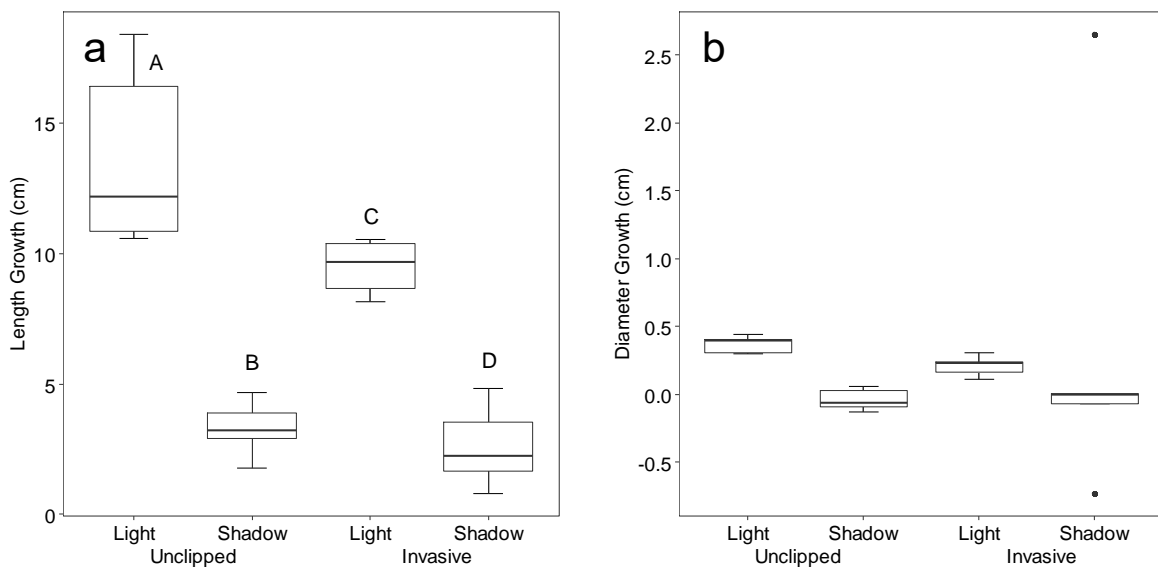
length growth on the treatment Clipped did not differ from the treatment Unclipped (Tukey *post-hoc*,  $df = 23$ ,  $t = 0.426$ ,  $P = 0.905$ ), but the length growth differed between Unclipped and Native treatments ( $df = 23$ ,  $t = -2.910$ ,  $P = 0.021$ ), and between Native and Clipped treatments ( $df = 23$ ,  $t = -3.323$ ,  $P = 0.008$ ) (Fig. II-3a). The diameter of *A. macrocarpa* did not differ between the treatments (Table II-1, Fig. II-3b). The length growth of *H. coronarium* differed between Unclipped and Invasive treatments and was positively affected by the light, but not by their interaction (Competition  $\times$  Light) (Table II-1, Fig. II-4a). The diameter of *H. coronarium* was not affected by the competition and light treatments (Fig. II-4b).

**Table II-1.** Results of ANOVA of linear mixed models testing the effects of competition and light treatments and their interaction (Competition $\times$ Light) on length growth and diameter growth of *Anadenanthera macrocarpa* and of *Hedychium coronarium*. \*statistical significance ( $\alpha = 0.05$ ).

	Sum Sq	Mean Sq	DenDF	F	P
Length growth ( <i>A. macrocarpa</i> )					
Competition	14.006	7.003	23	6.442	0.006*
Light	0.085	0.085	23	0.078	0.782
Competition $\times$ Light	0.171	0.085	23	0.079	0.925
Diameter growth ( <i>A. macrocarpa</i> )					
Competition	0.085	0.043	15.720	0.580	0.572
Light	0.031	0.031	8.142	0.424	0.533
Competition $\times$ Light	0.182	0.091	15.720	1.235	0.318
Length growth ( <i>H. coronarium</i> )					
Competition	29.783	29.783	8	9.907	0.014*
Light	200.606	200.606	8	66.732	0.038*
Competition $\times$ Light	15.335	15.335	8	5.101	0.054
Diameter growth ( <i>H. coronarium</i> )					
Competition	0.079	0.079	16	0.183	0.675
Light	0.079	0.079	16	0.183	0.675
Competition $\times$ Light	0.407	0.407	16	0.939	0.347

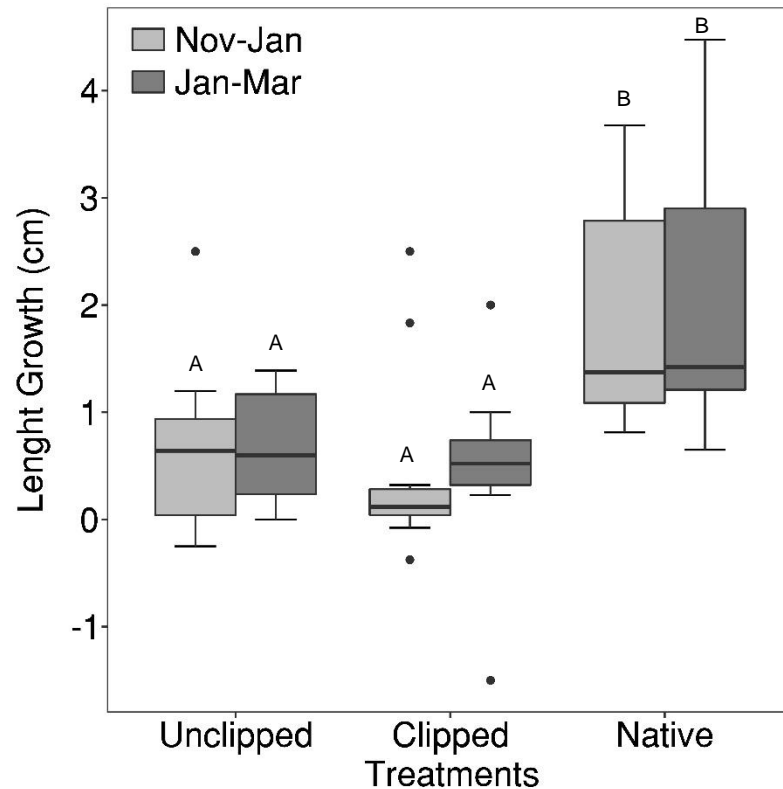


**Fig. II-3** Length growth (a) and diameter growth (b) of *Anadenanthera macrocarpa* seedlings under different competition treatments: without the invader (Native), with the invader (Unclipped), and with the invader without its aerial parts (Clipped). The letters (A and B) indicates significant differences.



**Fig. II-4** Length growth (a) and diameter growth (b) of *Hedychium coronarium* ramets in the presence (Unclipped) and absence (Invasive) of the native *Anadenanthera macrocarpa* under natural light (Light) and % shading (Shadow). Capitalized letters indicate significant differences.

There was no difference on native length growth between the periods November–January and January–March in the treatments Unclipped (t-test,  $t = -0.018$ ,  $df = 9$ ,  $P = 0.986$ ), Clipped (Wilcoxon,  $V = 10$ ,  $P = 0.084$ ) and Native (Wilcoxon,  $V = 12$ ,  $P = 0.25$ ) (Fig. II-5).



**Fig. II-5** Length growth of *Anadenanthera macrocarpa* on different time periods and competition treatments: without the invader (Native), with the invader (Unclipped), and with the invasive without its aerial parts from January to March (Clipped). Capitalized letters indicate significant differences.

The height of *H. coronarium* ramets were positively correlated to their length growth (Pearson,  $t = -2.551$ ,  $P = 0.034$ ), but was not correlated to the number of ramets ( $t = -0.916$ ,  $P = 0.387$ ). The multiple regression model revealed that the length growth of *A. macrocarpa* seedlings was not influenced by the number of ramets of *H. coronarium* (linear model,  $t = -1.256$ ,  $F = 1.578$ ,  $P = 0.245$ ), neither by mean ramet height ( $t = -0.560$ ,  $F = 0.313$ ,  $P = 0.591$ ) or total *H. coronarium* fresh weight ( $t = 1.539$ ,  $F = 2.369$ ,  $P = 0.162$ ).

## DISCUSSION

We assessed the performance of a native and an invasive plant growing separately and in mixture under different light conditions. We did not observe an increase in the growth of the native species after the removal of aerial part of the invasive, which indicate that the competition between these species could be for underground resources or space. Several studies report that the presence of invasive species reduce the performance of native plants (reviewed by Vilá and Weiner 2004), but few studies estimate individually the role of aerial and subterraneous interspecific competition (e.g., Gorchov and Trisiel 2003, Pereira et al. 2013). Aerial competition occurs basically for light, while the competition in the soil level involves a number of limiting resources as water and several essential nutrients (Casper and Jackson 1997). In fact, only removal of aerial parts of the invasive grass *Urochloa brizantha* did not result on better establishment of tree seedlings, but removal of the grass roots was effective (Pereira et al. 2013). Belowground interspecific competition can have been underestimated in understanding the dominance of alien plants, mainly for rhizomatous herbaceous species such as *H. coronarium*.

The direct competition is the major factor underlining the competition between native and invasive plants, such as observed for *Holcus lanatus* (invasive) and *Erigeron glaucus* (native) (Bennett et al. 2011). However, other associated effects, such as herbivory pressure, allelopathy and changes on the soil community, promote indirect competition. The allelopathic compounds present on subterraneous structures of invasive species may modify the soil microbial community (Bennett et al. 2011) and reduce the fitness of native species. *H. coronarium* is known by its powerful chemical compounds, which are phytotoxic (Miranda et al. 2015) and antimicrobial (Ray et al. 2018). The invader is benefited when its toxic compounds disrupt mutualistic relationships involving native plants (Callaway et al. 2008). For example, *Acer saccharum*, *Acer rubrum* and *Fraxinus americana* showed less arbuscular mycorrhizal fungi colonization of roots and slow growth on the presence of the invasive *Alliaria petiolate* (Stinson et al. 2006).

The growth rate of *A. macrocarpa* in the Native treatment was not stimulated by higher light (natural light treatment). Contradicting our hypothesis, the lower light availability on the shaded treatment did not intensified the competition between studied species. These results agree with that of the Clipped treatment, indicating that the extra light provided by the removal of invasive aerial parts was insufficiently to increase native

growth. Furthermore, the absence of correlation between the growth of native seedlings and number of ramets, ramet height, and ramet biomass in a within-treatment approach confirmed that none of these aspects affected the development of native seedlings. Although *A. macrocarpa* is a pioneer species, it grows well under shading conditions (30% of light) during its initial development (Cunha et al. 2009). So, we believe that these treatments were not enough to decrease light beyond the optimal range of light of *A. macrocarpa* growth and consequently reduce seedling growth. Furthermore, the loss in light caused by the aerial parts of *H. coronarium* may have been counterbalanced by lower evapotranspiration levels, reducing the water stress in the seedlings.

Unlike the native seedlings, *H. coronarium* had greater growth rate in the higher light condition. These findings suggest that the invader uses the light more efficiently, showing their greater competitive ability. The high vegetative growth rate is a remarkable trait of invasive plant species (Vila and Weiner 2004). *H. coronarium* growth was higher when growing in mixture with the native seedlings. We suppose that as *A. macrocarpa* is a Fabaceae species that composes symbiotic association with nitrogen fixing bacteria, it may have favored the growth of the invasive species by increasing the nitrogen availability in the soil. Similarly, Maron and Connors (1996) demonstrated that the nitrogen fixation by the shrub *Lupinus arboreus* (Fabaceae) facilitate the invasion by *Bromus diandrus*. In the same way, the invasive *Taraxacum officinale* is benefited by the nurse effect of *Azorella monantha* (native) on an alpine Chilean region (Cavieres et al. 2005). Our results indicate that some native species may facilitate invaders establishment rather than promote resistance to plant invasions in certain circumstances, but this topic requires further studies.

Overall, we found that belowground competition is apparently more important than aboveground effects on the interspecific negative interaction between *H. coronarium* and seedlings of a native riparian tree. Considering 1) the lower growth rate of riparian tree species growing in mixture with *H. coronarium*, without cutting its leaves and 2) the apparently positive association of *H. coronarium* growing in mixture with the native species, we may state that this invasive plant is an important barrier to native plant recolonization in invaded areas, reducing the biodiversity and harming ecological succession. Although this research has evaluated only a relatively short time period, the studied life stage (early seedling development) plays an important role for plant population dynamics (Eriksson and Erlhén 1992). We suggest that the negative impacts

of invasive plants on native species should be addressed in a broad context given the variety and complexity of mechanisms involved in these interactions (Schlaepfer et al. 2010; Van Kleunen et al. 2010; Bennett et al. 2011).

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# CAPÍTULO III

PHYTOTOXICITY OF ESSENTIAL OIL FROM THE INVASIVE *Hedychium coronarium* J. KÖNIG (ZINGIBERACEAE) ON SEEDS OF NATIVE RIPARIAN TREES

ABSTRACT

Allelopathy is one of the mechanisms that facilitate the invasion by plants due to their phytotoxicity. The allelochemical compounds released may affect directly the germination and the development of other plant species. Biological invasion has been considered one of the most important process of biodiversity loss, and its impact can be even worst in riparian areas affecting the water provision. However, little is known about this in the neotropics, especially for the impact of invasive plants on native tree species from riparian forests. In this study, we evaluated the effect of the *Hedychium coronarium* oil, extracted from the rhizomes, on the speed germination index and percentage of germination of four native riparian tree species (*Anadenanthera macrocarpa*, *Peltophorum dubium*, *Mimosa bimucronata* and *Sesbania virgata*), and on the radicle growth of *S. virgata*. The essential oil was extracted, chemically characterized, and germination bioassays were performed using concentrations of 0.01%, 0.1% and 1%, distillate water and Tween 80 (1%) as controls. The concentration 1% of the essential oil inhibited the germination of tested species and the radicle growth of *S. virgata*. Our results showed that the invasive *H. coronarium* can affect the germination of native species, which may negatively affect the local regeneration and the succession process on riparian forests.

**Key Words**-Allelopathy, germination, invasion, riparian forest

## INTRODUCTION

Biological invasion is the process of ecosystems degradation occasioned by the out-of-control proliferation of an introduced species (Vitousek et al. 1997; Simberloff and Rejmanek 2011). Several studies have reported the negative effect of invaders on native community (Levine et al. 2003; Gaertner et al. 2009; Hejda et al. 2009), affecting established species, homogenizing plant communities (Wiser et al. 1998) and suppressing the colonization by new individuals (De Castro et al. 2016). Dominant invasive species can modify environmental conditions in different ways, such as shading or by high litter production and liberation of secondary compounds, creating barriers that hamper the germination and emergence of native seeds (Facelli and Pickett 1991; Inderjit et al. 2008; Currie et al. 2014).

Invasive plant species usually have less natural enemies on invaded areas, as postulated by the enemy release hypothesis (Keane and Crawley 2002; Jeschke 2014). Thus, they can allocate less energy on defense mechanisms and consequently invest in other ways to increase their competitive capability, such as the production of secondary compounds (Hierro and Callaway 2003; Callaway and Ridenour 2004; Barney et al. 2005). The “novel weapon hypothesis” proposes that the addition of new phytotoxic biochemicals by invasive plants are highly effective on suppressing the growth of “naïve native species” (Callaway et al. 2008; Inderjit et al. 2011). These compounds may inhibit the germination and growth of other plants (Cummings et al. 2013), influencing interspecific relations, community structure and native vegetation dynamics (Inderjit and Duke 2003). Several studies have shown the allelopathic potential of compounds commonly found in the essential oil of a number of invasive plants, such as  $\alpha$ -pinene, o-cymene, 1,8-cineole (Romagni et al. 2000; Scrivanti et al. 2003; Gniazdowska and Bogatek 2005; Verdeguer et al. 2009; Zahed et al. 2010).

The amphibious macrophyte *Hedychium coronarium* J. König (Zingiberaceae) is an invasive herbaceous species native from Himalayan but nowadays is dispersed worldwide, with high dominance on Brazilian riparian forests and wetlands (Kissmann and Groth 1999; Zenni and Ziller 2011). This species presents rapid growth and efficient vegetative reproduction, high environmental tolerance, forming dense populations (Kissmann and Groth 1999), which negatively affect the diversity of riparian zones (De Castro et al. 2016). Riparian zones - described as an interface between aquatic and terrestrial ecosystems - are areas of permanent protection in Brazil. Due to expansion of

agricultural activities, these areas are extremely devastated and endangered (Grombone-Guaratini et al. 2004).

The chemical characterization of the essential oil of *H. coronarium* was previously described for its leaves and rhizomes (Taveira et al. 2005; Miranda et al. 2015), and the inhibitory effect of these compounds was reported over indicator species such as lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*) (Rodrigues and Lopes 2006; Miranda et al. 2015). However, for conservation purposes we should also look for its impacts on native species, identifying the more vulnerable and resistant species to use this information in the restoration of invaded areas (Silva Matos and Belinato 2010).

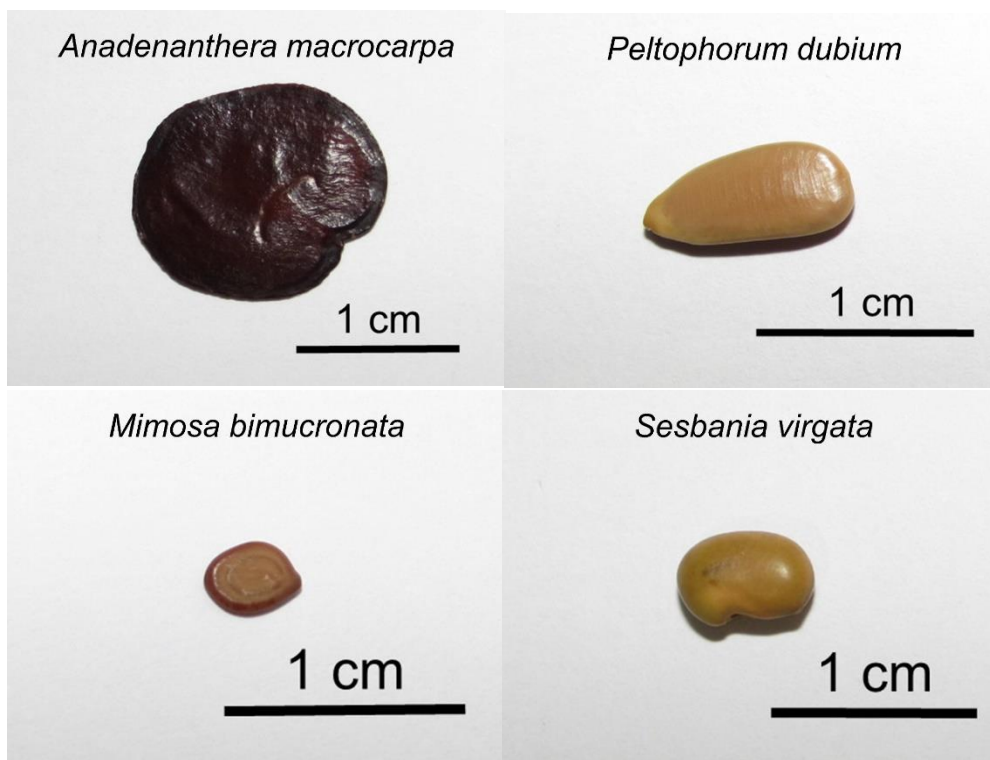
Here, we evaluate the phytotoxic effect of *H. coronarium* essential oil on germination of native tree species co-occurring in riparian forests where *H. coronarium* is invading (De Castro et al. 2016). Specifically, we tested the effect of different essential oil concentrations on the germination speed index (GSI) and germination percentage (GP) of four riparian pioneer tree species, and on the radicle length of one dominant species. We hypothesized that *H. coronarium* have an inhibitory effect on seed germination of native tree species that may also explain the formation of dense and mono-specific stands in riparian zones.

## MATERIALS AND METHODS

### *Studied Species*

We assessed the effect of different concentrations of *H. coronarium* oil on seeds of four native trees, selected from a set of 10 species based on previous tests of seed viability and germination. The species whose seed viability and germination were null or less than 10% were discarded. All selected species are pioneers commonly found in riparian forests and frequently used for restoration (Assis et al. 2013; Barbosa et al. 2015): 1) *Anadenanthera macrocarpa* (Benth) Brenan is a deciduous plant that reaches up to 20 meters high and produces large quantities of viable seeds annually (Lorenzi 2000); seed weight is about  $0.123 \pm 0.015$  g (mean  $\pm$  SD, n = 20). 2) *Peltophorum dubium* (Spreng.) Taub. is a deciduous plant that reaches up to 20 meters high in both dense primary forest and in secondary formations (Medri et al. 1998); seed weight is about  $0.048 \pm 0.007$  g (n = 20). 3) *Mimosa bimucronata* (DC.) is a semi-deciduous plant that reaches up to 10

meters high and produces large quantities of small seeds ( $0.010 \pm 0.001$  g,  $n=20$ ). 4) *Sesbania virgata* (Cav.) reaches up to 6 meters high and is dominant in some riparian areas where it associated to Rhizobium (Souza et al. 2011; Mignoni et al. 2017); seed weight is about  $0.072 \pm 0.008$  g ( $n = 20$ ) (Fig. 1). For *P. dubium* and *S. virgata* we overcame integumentary dormancy by mechanical scarification.



**Fig. III-1** Native seeds (Fabaceae) from riparian areas.

#### *Extraction and Chemical Characterization*

We collected *H. coronarium* rhizomes in a riparian zone within the campus of Federal University of São Carlos ( $22^{\circ}58'16''$  S,  $47^{\circ}53'15''$  W). A voucher specimen (R. Costa 1) was deposited in the herbarium of Federal University of São Carlos (SPSC; acronym according to Thiers 2018). The rhizomes were dried in a greenhouse under natural conditions. Dried rhizomes were weighed, washed, chopped and crushed in an industrial blender and divided in 5 replicates of 1 Kg. The *H. coronarium* essential oil was extracted by hydrodistillation with a Clevenger-type apparatus adapted to round-bottom flasks of 6 l for 3 h (until no more essential oil was obtained). The essential oil

was collected and dried with anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) to remove traces of moisture. We used a vacuum system to evaporate the pentane. The essential oil was weighed to determine the extraction yield and stored in a freezer ( $-22\text{ }^\circ\text{C}$ ) for further analysis (Brazilian Pharmacopoeia 2001).

#### *Gas-liquid Chromatography/Mass Spectrometric Analysis (GC/MS)*

For GC/MS analysis, 10  $\mu\text{l}$  of oil were diluted in 90  $\mu\text{l}$  of acetone (1%). The samples were analyzed by GC/MS using a Chromatograph (Agilent 6890 Series) coupled to a mass spectrometry (MS) quadrupole system (Agilent 5973 Network Mass Selective Detector) with an electron impact ionization system at 70 eV of ionization energy. The injection temperature was  $230\text{ }^\circ\text{C}$ , the interface was  $250\text{ }^\circ\text{C}$ . We used helium as the carrier gas at a flow rate of 1 mL/min. The analyses were performed using a nonpolar 30 m  $\times$  0.25 mm HP-5MS column (0.25- $\mu\text{m}$  film thickness) (Supelco, Bellefonte, USA). We conducted the analysis using the following temperature program: 5 min of isothermal heating at  $70\text{ }^\circ\text{C}$ , followed by ramping at  $5\text{ }^\circ\text{C}/\text{min}$  to  $280\text{ }^\circ\text{C}$ , and 1 min at  $280\text{ }^\circ\text{C}$ . The constituents were identified by comparison of their mass spectra in the data bank (Wiley 275, Adams 2007 and NIST 2014). The Kovats retention indices were calculated for all volatile constituents using a homologous series of  $\text{C}_8$ – $\text{C}_{40}$  *n*-alkanes on the HP 5-MS column.

#### *Bioassays*

A stock solution (1% of essential oil) was prepared using 1 ml of essential oil emulsified with 1 ml of Tween 80® and dissolved in distilled water in a volumetric balloon, to obtain a total volume of 100 ml. We prepared two other solution concentrations, 0.1% and 0.01%, by dilution. Distilled water and a solution of Tween 80 (1%) were used as controls. We estimated the osmotic potential of solutions using a vapor pressure osmometer (WESCOR 5520, Logan, UT, USA). The bioassays were prepared in acrylic gerbox (11  $\times$  11  $\times$  4 cm) with two sterilized sheets of filter paper as substrate. We used 10 seeds of *A. macrocarpa* and *S. virgata*, 15 seeds of *P. dubium* and *M. bimucronata* in each gerbox, and five replicates of each treatment for each species. We added 8 ml of solution (essential oil concentrations, distilled water or Tween solution)

directly over the seeds. The experimental design was completely randomized, with five treatments (distilled water, Tween 80® 1%, essential oil 0.01%, 0.1%, 1%). The experiment was conducted on an acclimatized room at 25 °C ± 2 under constant light. We evaluated seeds germination every 24 h until register no new germination for three consecutive days. We considered germinated all seeds with at least 2 mm radicle protrusion. For *S. virgata* we evaluated the radicle length 7 days after germination.

### *Statistical Analyses*

We calculated the extraction yield by the ratio between weight of essential oil obtained and that of the dried rhizomes. The GSI was calculated according to Maguire (1962). Prior to the analyses, data were checked for normality and homoscedasticity. We used the analysis of variance (*ANOVA*) to test whether there were differences between treatments for *A. macrocarpa*, *P. dubium* and *M. bimucronata* (GSI and GP) and Kruskal-Wallis test for *S. virgata* (GSI, GP and radicle length). In case of difference between the treatments, we performed the *Tukey* and *Dunn post-hoc* tests for multiple comparisons. We considered significant values of  $P \leq 0.05$ . All analysis were performed in the R environment (R Development Core Team 2017). We used the package *ggplot2* (Wickham 2009) to create the graphs.

## RESULTS

The yield of essential oil obtained on extraction process was 0.32%, and 99.04% of the compounds were identified (Table III-1). The commonest oil compounds were the monoterpens 1.8-cineol (29.45%),  $\beta$ -pinene (24.09%) and  $\alpha$ -pinene (11.22%).



**Table III-1.** Chemical characterization of the essential oil of *Hedychium coronarium* rhizomes. KI=estimated kovats index, ki ref.= kovats index according (1) (Adams 2007), (2) (Wiley 2010 + Nist 2014).

Component	KI	KI Ref.	Essential oil (%)
<i>Monoterpene hydrocarbon</i>			40.24
α-thujene	927	930 (1)	0.50
α-pinene	936	939 (1)	11.22
Camphene	950	954 (1)	0.98
β-pinene	981	979 (1)	24.09
Myrcene	989	990 (1)	0.66
o-cymene	1027	1026 (1)	2.1
γ-terpinene	1059	1059 (1)	0.69
<i>Oxygenated monoterpene</i>			41.52
1,8-cineole	1037	1031 (1)	29.45
Borneol	1171	1169 (1)	1.12
terpinen-4-ol	1180	1177 (1)	3.34
α-terpineol	1195	1188 (1)	7.61
<i>Hydrocarbon</i>			12.23
n-eicosane	2088	2000 (1)	0.69
n-heneicosane	2122	2100 (1)	4.24
n-pentacosane	2548	2500 (1)	7.3
<i>Diterpene (%)</i>			5.05
coronararin A	2111	(2)	5.05
<i>Not Identified</i>			0.96
*M+ 136: 93(100) 71(59) 41(50) 69(41) 43(37)	1098		0.96
<i>Total identified</i>			99.04

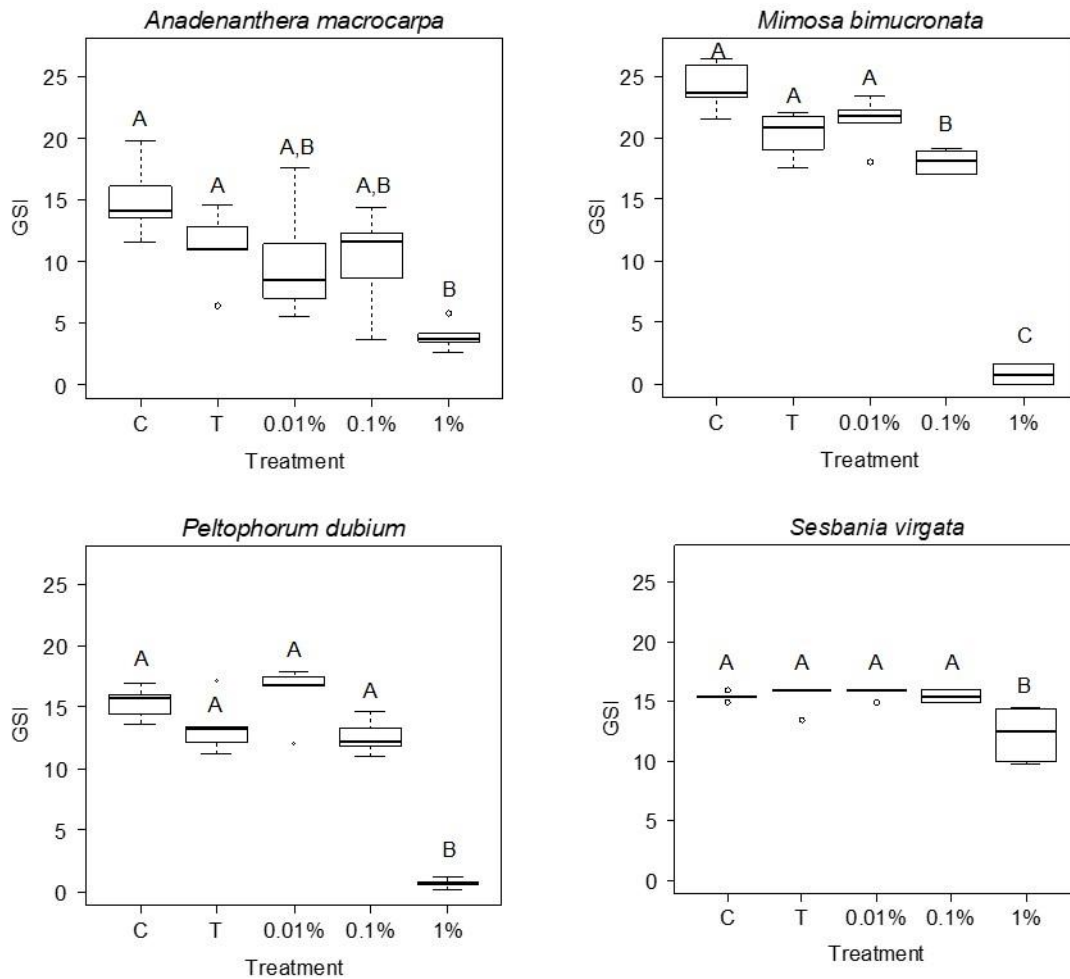
\*Molecular mass, main peaks of abundance followed by their relative percentage to the base peak between parentheses.

### Bioassays

The osmotic potential values of distilled water and the solutions of Tween, 0.01%, 0.1% and 1% were respectively -0.04902, -0.04988, -0.04816, -0.0516 and -0.05934 MPa. These values were similar to distilled water and they did not exceed 0.2 MPa, the maximum value indicated to germination of lettuce seeds (Gatti et al. 2004), excluding the possibility of osmotic effect on our results.

The GSI differed between treatments for all species (Table III-2). The GSI was reduced on the concentration 1% in relation to the controls for all species (Table III-3, Fig. III-2). The influence of essential oil treatments also differed from each other for *M.*

*bimucronata* (Table III-3), where the GSI was negatively related to the oil concentration (Fig. III-2). For the other species, the concentrations 0.01% and 0.1% did not differ from the controls (Fig. III- 2).



**Fig. III-2** Germination speed index (GSI) on different treatments for *Anadenanthera macrocarpa*, *Mimosa bimucronata*, *Peltophorum dubium* and *Sesbania virgata*. Treatments were distilled water (C), Tween 80 (T) and concentrations (0.01%, 0.1% and 1%) of the essential oil. Capitalized letters indicate significant differences.

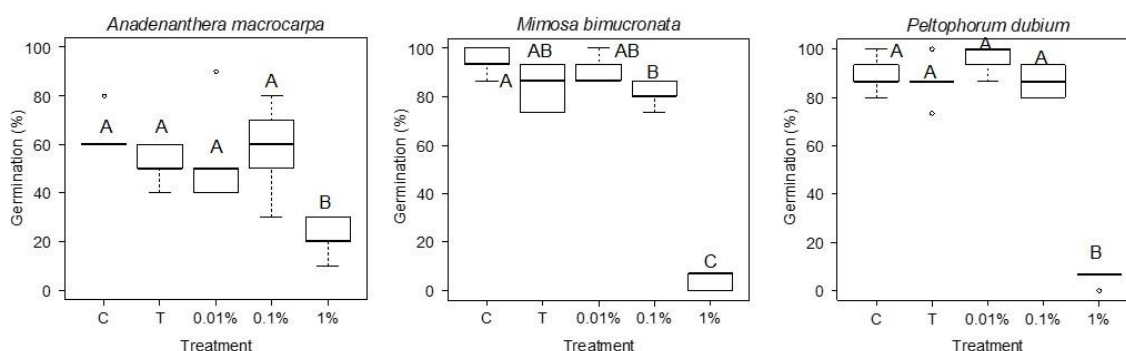
Most seeds germinated on the first three days. The GP differed between treatments for *A. macrocarpa*, *M. bimucronata* and *P. dubium* (Table III-2). For these species, the oil concentration 1% reduced significantly the GP, but lower concentrations did not differ from the controls (Fig. III-3a, b, c, Table III-4). The only exception was between 0.1% and the distilled water for *M. bimucronata* (Fig. III-3, Table III- 4). For *S. virgata*, the

radicle length differed between treatments (KW = 45.666, df = 4, P <0.001) (Table III-5). The radicle length was reduced on the concentration 1% in relation to the controls and the other concentrations (0.01% and 0.1%) (Table III-5, Fig. III-4).

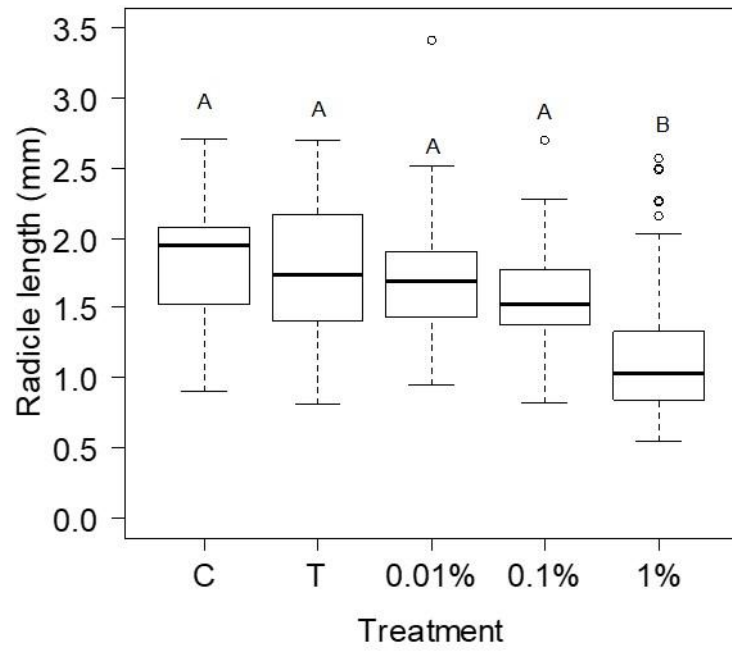
**Table III-2.** Results of the comparisons (ANOVA) among different concentrations of the essential oil, tween 80 and distilled water on the germination speed index (GSI) and germination percentage (GP) of five riparian tree species. DF = degrees of freedom, SS = sum of squares, MS = mean square.

Species	GSI					GP						
	DF	SS	MS	F	KW	P	DF	SS	MS	F	KW	P
<i>A. macrocarpa</i>	4	314.600	78.640	6.505		0.002*	4	5320	1330.000	6.52		<0.001*
<i>M. bimucronata</i>	4	1721.200	430.300	162.000		<0.001*	4	28558	7140.000	167.300		<0.001*
<i>P. dubium</i>	4	793.700	198.430	67.640		<0.001*	4	28740	7185.000	152.500		<0.001*
<i>S. virgata</i>	4				13.174	<0.010*	4				4.000	0.406

\*Significant values (P ≤ 0.05)



**Fig. III-3** Percentage of germination (GP) on different treatments for *Anadenanthera macrocarpa*, *Mimosa bimucronata* and *Peltophorum dubium*. Treatments were distilled water (C), Tween 80 (T) and concentrations (0.01%, 0.1% and 1%) of the essential oil. Capitalized letters indicate significant differences.



**Fig. III-4** Radicle length on different treatments for *Sesbania virgata*. Treatments were distilled water (C), Tween 80 (T) and concentrations (0.01%, 0.1% and 1%) of the essential oil. Capitalized letters indicate significant differences.

**Table III-3.** Comparison of germination speed index among distilled water (C), tween 80 (T) and different concentrations (0.01%, 0.1% and 1%) of the essential oil extracted from rhizomes of *Hedychium coronarium* for each native tree species

Treatment			<i>A. macrocarpa</i>				<i>M. bimucronata</i>				<i>P. dubium</i>				<i>S. virgata</i>	
comparisons			diff	lower	upper	adj. P	diff	lower	upper	adj. P	diff	lower	upper	adj. P	Z	adj. P
C	x	T	-3.938	-10.542	2.665	0.416	-3.706	-6.824	-0.588	0.082	-1.994	-5.214	1.226	0.399	-0.812	0.417
C	x	0.01%	4.819	-1.784	11.422	0.201	2.882	-0.236	6.000	0.633	-0.786	-4.006	2.434	0.938	1.015	0.310
C	x	0.1%	5.102	-1.501	11.706	0.214	6.132	3.014	9.250	<0.001*	2.700	-0.519	5.920	0.119	0.158	0.875
C	x	1%	10.956	4.353	17.560	0.001*	23.376	20.258	26.494	<0.001*	14.673	11.454	17.893	<0.001*	2.210	0.027*
T	x	0.01%	0.881	-5.722	7.484	0.988	-0.824	-3.942	2.294	0.554	-2.780	-6.000	0.440	0.115	0.203	0.839
T	x	0.1%	1.164	-5.439	7.768	0.991	2.426	-0.692	5.544	0.016*	0.707	-2.513	3.926	0.943	0.654	0.5132
T	x	1%	7.018	0.415	13.622	0.029*	19.670	16.552	22.788	<0.001*	12.680	9.460	15.899	<0.001*	3.022	0.003*
0.1%	x	0.01%	-0.283	-6.887	6.320	1.000	-3.250	-6.368	-0.132	0.033*	-3.487	-6.706	-0.267	0.026	0.857	0.392
1%	x	0.01%	-6.137	-12.741	0.466	0.078	-20.494	-23.612	-17.376	<0.001*	-15.460	-18.679	-12.240	<0.001*	3.225	0.001*
1%	x	0.1%	-5.854	-12.457	0.750	0.072	-17.244	-20.362	-14.126	<0.001*	-11.973	-15.193	-8.753	<0.001*	2.368	0.018*

\*Significant values ( $P \leq 0.05$ )

**Table III-4.** Comparison of germination percentage among distilled water (C), tween 80 (T) and different concentrations (0.01%, 0.1% and 1%) of the essential oil extracted from rhizomes of *Hedychium coronarium* for each native tree species

Treatment			<i>A. macrocarpa</i>				<i>M. bimucronata</i>				<i>P. dubium</i>			
comparisons			diff	lower	upper	adj. P	diff	lower	upper	adj. P	diff	lower	upper	adj. P
C	x	T	-12.000	-39.031	15.031	0.677	-10.667	-23.029	1.695	0.112	-2.666	-15.655	10.323	0.971
C	x	0.01%	10.000	-17.031	37.031	0.801	4.000	-8.362	16.362	0.866	-6.666	-19.655	6.323	0.553
C	x	0.1%	6.000	-21.031	33.031	0.962	13.333	0.971	25.695	0.031*	2.667	-10.323	15.657	0.971
C	x	1%	42.000	14.969	69.031	0.001*	90.664	78.301	103.027	<0.001*	84.000	71.010	96.990	<0.001*
T	x	0.01%	-2.000	-29.031	25.031	0.999	-6.666	-19.029	5.695	0.506	-9.333	-22.323	3.657	0.239
T	x	0.1%	-6.000	-33.031	21.031	0.962	2.666	-9.695	15.028	0.966	0.000	-12.990	12.990	1.000
T	x	1%	30.000	2.969	57.031	0.025*	80.000	67.638	92.362	<0.001*	81.333	68.343	94.323	<0.001*
0.1%	x	0.01%	4.000	-23.031	31.031	0.991	-9.333	-21.695	3.029	0.199	-9.333	-22.323	3.657	0.239
1%	x	0.01%	-32.000	-59.031	-4.969	0.015*	-86.666	-99.029	-74.305	<0.001*	-90.666	-103.657	-77.677	<0.001*
1%	x	0.1%	-36.000	-63.031	-8.969	0.005*	77.333	-89.695	-64.971	<0.001*	-81.333	-94.323	-68.343	<0.001*

\*Significant values ( $P \leq 0.05$ )

**Table III-5.** Comparison of radicle length of *Sesbania virgata* among distilled water (C), tween 80 (T) and different concentrations (0.01%, 0.1% and 1%) of the essential oil extracted from rhizomes of *Hedychium coronarium*.

Treatment comparisons			Dunn test	
			Z	adj. P
C	x	T	0.369	0.712
C	x	0.01%	-1.169	0.727
C	x	0.1%	-2.380	0.104
C	x	1%	-5.937	<0.001*
T	x	0.01%	-0.799	0.848
T	x	0.1%	-2.010	0.222
T	x	1%	-5.571	<0.001*
0.1%	x	0.01%	1.210	0.904
1%	x	0.01%	4.780	<0.001*
1%	x	0.1%	3.582	0.002*

## DISCUSSION

In this paper, we described the essential oil composition of *H. coronarium* rhizomes and verified their inhibitory effects on the speed and percentage of germination and on radicle growth of native riparian tree species. Although the invasive plants essential oil composition may change considerably among different regions (Prakash et al. 2010; Ayokun-nun and Moteetee 2017), our results indicated that the monoterpene 1.8-cineole is the most abundant compound, as described in other studies (Sabulal et al. 2007; Miranda et al. 2015). This compound can inhibit the germination, root growth and DNA synthesis of *Brassica campestris* (Koitabashi et al. 1997). The main oil compounds found in our samples (1.8-cineole,  $\beta$ -pinene and  $\alpha$ -pinene) are known by their allelopathic characteristics, and are produced by other invasive plants, such as *Artemisia vulgaris* (Barney et al. 2005), *Artemisia scoparia* (Kaur et al. 2010) and *Tithonia diversifolia* (Ayokun-nun and Moteetee 2017). Thus, the phytotoxic effect that we observed may be related to the allelopathic potential of these compounds obtained from *H. coronarium* rhizomes.

The germination speed and the percentage of seed germination of *Anadenanthera macrocarpa*, *Peltophorum dubium* and *Mimosa bimucronata* were reduced in 1% essential oil concentration. The lower concentrations of essential oil (i.e., 0.1% and

0.01%) did not affect the germination of tested species. However, the phytotoxicity effects may be pronounced in other non-studied germination aspects or following stages of seedling development (Koitabashi et al. 1997; Fujii and Hiradate 2007).

The seeds of *S. virgata*, although slightly delayed, presented high percentage of germination even under the highest oil concentration (1%). This species is dominant in wetlands and its seeds produce allelochemicals that may inhibit the establishment of other native (El Id et al. 2015) and invasive species (Mignoni et al. 2017). The low susceptibility of this species to the oil extracted from *H. coronarium* corroborate a recent study that showed the resistance of *S. virgata* seeds to allelochemicals of another global invasive species, *Leucaena leucocephala* (Mignoni et al. 2017). Nevertheless, the radicle growth of *S. virgata* seeds was lower in the concentration 1% of *H. coronarium* essential oil. This result suggests that the phytotoxicity of *H. coronarium* chemical compounds may have a role in reducing the performance of native competitors even after germination.

Besides allelopathy, other mechanisms may inhibit the germination of native species, as reported for the invasive *Solidago gigantea*, *Impatiens glandulifera* and *Erigeron annuus* (Del Fabbro et al. 2014). The germination success of native seeds can also be negatively affected by shading and high litter production (Facelli and Pickett 1991), which occur in areas invaded by *H. coronarium*. However, the presence of phytotoxic compounds gives to *H. coronarium* an additional competitive advantage in relation to native species, favoring its dominance. Furthermore, the clonal reproduction and continuous growth also contributes to its expansion towards the riparian forests decreasing the local diversity (De Castro et al. 2016). In invaded riparian areas, we rarely observe other species growing successfully even other invasive species such as grasses (*Brachiaria decumbens*, *Melinis minutiflora* and *Pennisetum purpureum*) or native dominant amphibious macrophytes, such as *Typha domingensis* and *Eichhornia crassipes*.

Our results revealed the potential phytotoxic effect of the oil obtained from *H. coronarium* rhizomes on pioneer tree species commonly found in riparian forests. Even native dominant species from riparian habitats were affected by the essential oil. Besides producing a great density of rhizomes to dominate recently invaded areas, we showed that the rhizomes contain phytotoxic substances that may contribute to increase the invasion success. This competitive pressure could result on ecological and evolutive changes, altering the ecosystems and their functioning (Gurevitch and Padilla 2004; Hoffmeister

et al. 2005). Further studies should evaluate the concentration of these chemical compounds *in situ*, comparing invaded and non-invaded areas, and investigate whether and how these compounds are naturally released. Our results provided insights on the invasiveness of *H. coronarium* and we strongly recommend that attention must be given to the expansion of this plant worldwide.

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# CAPÍTULO IV

TOXICITY OF THE INVASIVE *Hedychium coronarium* J. KÖNIG  
(ZINGIBERACEAE) ON AQUATIC SPECIES

ABSTRACT

The production and release of chemical compounds by invasive plants can affect competitors and other native species, destabilizing ecological interactions and harming ecosystem functioning. *Hedychium coronarium* is an invasive macrophyte common in Brazilian riparian areas. This species produces a wide variety of allelochemicals, but little is known about their effect on aquatic ecosystems. We identified the major chemical compounds of *H. coronarium* rhizomes and assessed the toxicity of different concentrations of aqueous extract, evaluating the growth inhibition of one algae (*Raphidocelis subcapitata*) and one macrophyte (*Lemna minor*) and the lethality of cladocerans (*Ceriodaphnia silvestrii* and *Daphnia similis*), Chironomidae larvae (*Chironomus sancticaroli*), and adult fish (*Danio rerio*). The majoritarian compounds of *H. coronarium* rhizomes were isolated and identified as Coronarin D and Coronarin D Ethyl Ether. The aqueous extract was toxic for all tested species. We observed population growth inhibition in *R. subcapitata* and biomass decrease on *L. minor*. *Danio rerio* was the most sensible, followed by *C. sancticaroli* and cladoceran species. The aqueous extract of *H. coronarium* was toxic on tested conditions, suggesting that the rhizome compounds may interfere on aquatic organisms and in the trophic web dynamics of aquatic ecosystems on invaded areas.

**Key Words-**Chironomidae, Cladocera, *Danio rerio*, ecological interactions, macrophyte

## INTRODUCTION

The presence of competitors, pathogens and herbivorous can limit the performance of invader species in a new environment (Levine et al. 2004). In this way, invaders evolved different strategies to overcome these biotic barriers, establishing new interactions that may increase their success and simultaneously reduce the fitness of native species (Mitchell et al. 2006). Alien species can alter the functioning of invaded communities reducing their biodiversity via releasing of chemical compounds, changes on microbiota and nutrients cycling (Callaway and Ridenour 2004; Reinhart and Callaway 2006; Ehrenfeld 2010). These deep changes may reduce the habitat resilience, increasing its vulnerability in an invasive-domino effect ('invasive-meltdown', Simberloff and Holle 1999).

The chemical compounds added by invasive plants on the environment usually have phytotoxic influence on the native competitors (Hierro and Callaway 2003), which are not adapted to these novel chemicals weapons (Callaway and Ridenour 2004). These compounds may have multiple actions to increase the fitness of invaders, for example reducing the germination of native seeds (Bais et al. 2003), repelling herbivorous and increasing alien resistance to pathogens (Hierro and Callaway 2003; Callaway and Ridenour 2004). Furthermore, invasive plants can affect animal populations indirectly by trophic effect, such as modifying the resource availability (Maerz et al. 2005), and changes on habitat structure, altering the habitat quality and reducing the biodiversity (Del-Rio et al. 2017).

*Hedychium coronarium* J. König (Zingiberaceae) is an invasive amphibian macrophyte common in Brazilian riparian areas (De Castro et al. 2016). In its native range, this species is considered a threatened species due to innumerable pharmacological applications (Pachurekar and Dixit 2017), including antifungal, antimicrobial, anti-inflammatory, cytotoxic, analgesic, antioxidant and other proprieties (Shrotriya et al. 2007; Pachurekar and Dixit 2017). This species produces allelochemicals in the leaves, flowers and rhizomes which are released to the environment (Miranda et al. 2015). Most of researches assess the effect of some of these toxins isolated (Chimnoi et al. 2009; Reuk-Ngam et al. 2014; Pachurekar and Dixit 2017), but investigations about their synergic action might be more similar to natural field conditions. The impact of toxic compounds released by invaders on aquatic communities is also poorly explored.



Here, we evaluated the toxicity of the invasive *H. coronarium* in aquatic organisms. More specifically, we evaluated the toxic effect of the aqueous extract of *H. coronarium* on the 1) growth of the algae *Raphidocelis subcapitata* (Korshikov) F. Hindák (Sphaeropleales, Selenastraceae) and the macrophyte *Lemna minor* L. (Alismatales, Lemnaceae), and 2) survival of cladoceran (*Ceriodaphnia silvestrii* Daday, 1902, and *Daphnia similis* Claus, 1876) (Crustacea, Cladocera), Chironomidae larvae (*Chironomus sancticaroli* Strixino & Strixino 1981) (Diptera, Chironomidae), and adult fish *Danio rerio* (Hamilton-Buchanan, 1822) (Cypriniformes, Cyprinidae). We expected that the production and release of secondary compounds from the terrestrial and aquatic environment, especially on riparian areas, negatively affect the aquatic organisms and increases the invasion success of *H. coronarium*.

## MATERIAL AND METHODS

### *Aqueous Extract*

*H. coronarium* rhizomes were collected in the Fazzary stream located in the São Carlos municipality (21°59'12''S, 47°52'23''W, 831 m a.s.l.). The regional climate is humid subtropical with hot summer and dry winter (Cwa), mean monthly temperature 19.9 °C and annual precipitation around 1450 mm (Alvares et al. 2013). Immediately after being collected, the rhizomes were washed to remove the adhered soil and chopped. The fragments of *H. coronarium* rhizomes were processed with distilled water in a 1:2 weight ratio on a commercial blender for 5 min. Thereafter, the material was filtered (0.1 mm mesh) to remove rhizome fragments and the filtrate was stored in a refrigerator (4–5 °C) for 24 h. This aqueous extract was considered the stock solution and all other dilutions were prepared from it. The dry weight of the extract was determined by gravimetry. Three porcelain crucibles were previously calcined in a muffle for 1 h (500±50 °C), cooled in a desiccator for 30 min, weighed and filled with 50 ml of the extract. The crucibles with extract were dried at 60 °C for 72 h, cooled in a desiccator and weighed on an analytical balance to the nearest 0.1 mg (SABESP 1997).

### *Chemical Characterization*

The chemical characterization of the aqueous extract of *H. coronarium* rhizomes was made by the research group from the Laboratory of Natural Products of Federal University of São Carlos, São Carlos - SP, Brazil. For this, after being washed, the rhizomes were dried in the oven at 60 °C for 7 days and crushed on a knife mill. The compounds were extracted on ethanol and concentrated using an evaporator. We used solid phase extraction to isolate and identify the compounds, using cartridges SPE – C18 (Phenomenex) of 500 mg and 6 ml.

The fraction obtained was analyzed on a high performance liquid chromatograph (HPLC) Prep (Shimadzu) coupled with a diode array detector. The samples were solubilized in Acetonitrile and adjusted to the concentration 200 mg ml<sup>-1</sup>. Posteriorly, we carried out the optimized chromatography using exploratory gradient elution in the C-18 column Shimpack prep-ODS 250 × 20mm. The injection volume was 200 µl and the elution used for separation was water Milli-Q and Acetonitrile, both acidified with 0.1% of formic acid.

### *Raphidocelis subcapitata Bioassay*

The toxicity of *H. coronarium* was evaluated by a population growth assay with the algae *R. subcapitata*, according to ABNT (2011a). We used an inoculum previously prepared from a culture maintained at the Laboratory of Ecotoxicology and Ecophysiology of Aquatic organisms on the Center for Water Resources and Environmental Studies of São Paulo University (hereafter “CRHEA-USP”). The L.C. Oligo medium autoclaved was used to prepare five concentrations of the *H. coronarium* aqueous extract (0.4%, 0.8%, 1.6%, 3.2% and 6.4%). We used an inoculum concentration 1.0 × 10<sup>5</sup> cells/ml and three replicates per concentration. The test was conducted on a climatized room (25 °C and constant light about 4.500 lux), with constant stirring (120 rpm) in a rotatory shaker for 96 h. The sensibility was attested by reference substance NaCl. We measured initial and final pH of the extract concentrations and of the control. In the end, we counted the cells in an Improved Neubauer Bright-Line hemocytometer under an optical microscope (Carl Zeiss).

### *Lemna minor* Bioassay

The toxicity test with the macrophyte *L. minor* was realized according to (OECD 2002). The plants were obtained from a laboratory culture maintained at the CHREA-USP. For the test, we used the Steinberg medium to prepare five concentrations of the aqueous extract (0.2%, 0.4%, 0.8%, 1.6%, and 3.2%) and the control. We used plastic pots with 50 ml of test solution covered with plastic film, with 12 fronds per replicate and three replicates per concentration. The plants were kept on a climatized room ( $24 \pm 2^\circ \text{C}$ , photoperiod 12:12 and light about 4.000 lux) for seven days. During the test, we exchanged the solutions on the second and fourth day and counted the number of fronds on the second, fourth and seventh day. The dry biomass of fronds of each replicate was estimated at the end of the test. The sensibility was attested by reference substance NaCl. We measured initial and final pH, temperature and dissolved oxygen of the solutions and the control.

### *Cladoceran* Bioassays

We performed bioassays with zooplanktonic organisms *D. similis* and *C. silvestrii*. The organisms were obtained from cultures maintained at the CRHEA-USP, according ABNT (2004, 2005). We used reconstituted water as control (pH 7.0–7.6, conductivity  $160 \mu\text{S cm}^{-1}$  and hardness between 40 and 48 mg  $\text{CaCO}_3 \text{ L}^{-1}$ ) and to prepare different concentrations of the *H. coronarium* aqueous extract by dilution (0.1%, 0.2%, 0.4%, 0.8% and 1.6%). Acute toxicity bioassays were conducted in plastic pots with 10 ml of each tested solution, with five organisms per replicate and four replicates per treatment (extract concentrations and control). The organisms were kept on climatized room ( $20^\circ \text{C}$  for *D. similis* and  $25^\circ \text{C}$  for *C. silvestrii*) with photoperiod of 16:8 h light/dark. The sensibility was attested by reference substance NaCl for *C. silvestrii* and KCl for *D. similis*. We measured initial and final pH, conductivity, dissolved oxygen and hardness of the extract concentrations and the control. After 48 h, we counted the immobile organisms.

### *Chironomus sancticaroli* Bioassay

We obtained four-instar larvae of *C. sancticaroli* from a culture maintained at the CRHEA-USP (OECD 2004). We used reconstituted water (pH 7.0–7.6, conductivity 56.9  $\mu\text{S cm}^{-1}$  and dissolved oxygen 7.33 mg/l) to prepare six concentrations of aqueous extract (0.0125%, 0.025%, 0.05%, 0.1%, 0.2% and 0.4%) and the control. Bioassays were performed in plastic pots with 60 g of sediment (special aquarium sand calcined to 500 °C for 1 h) and 240 ml of tested solution, with six larvae per replicate and three replicates per concentration. The test was conducted in a climatized room (25 °C and photoperiod of 12:12 h light/dark). We counted the surviving individuals in each replicate after 96 h. The sensibility was attested by the reference substance KCl. We made initial and final measures of pH, conductivity, dissolved oxygen and hardness of extract concentrations and the control.

### *Danio rerio* Bioassay

Adults zebrafish *D. rerio* obtained from a commercial breeder were acclimatized in reconstituted water (pH 7.0–7.6, conductivity 160  $\mu\text{S cm}^{-1}$  and hardness between 40 and 48 mg  $\text{CaCO}_3 \text{ L}^{-1}$ ) (ABNT 2011 b) for seven days, with constant oxygenation and fed daily with Tetramin®. We performed acute toxicity bioassays using different concentrations of aqueous extract of *H. coronarium* rhizomes (0.025%, 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6%) and reconstituted water as control. We used plastic pots with 1 L of test solution, with five fishes per replicate and two replicates per treatment. The test was conducted at the same conditions of the acclimation period (25 °C and photoperiod of 12:12 h light/dark). We assessed survival and removed dead individuals at 24 h and at the end of experiment (48 h). The sensibility of individuals was tested using NaCl as reference substance. We measured initial and final pH, dissolved oxygen and hardness of the solutions and control.

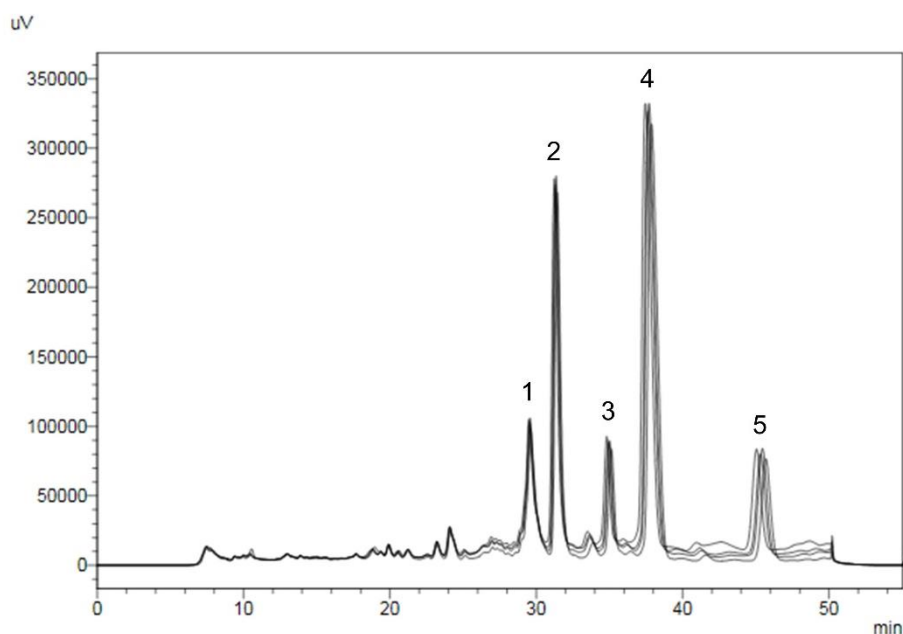
### Statistical Analyses

We estimated the median effective concentration that causes 50% of growth inhibition (IC<sub>50</sub>) for *R. subcapitata*, and 50% of immobility (EC<sub>50</sub>) on cladoceran, *C. santicarolli* larvae and zebrafish by nonlinear regression using the software Statistica

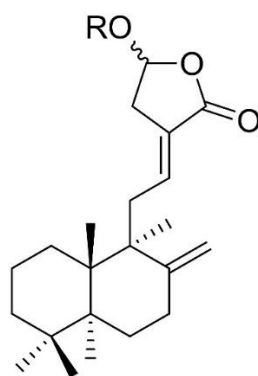
version 7 (StatSoft 2004). To evaluate the difference of the number and biomass of *L. minor* fronds (both normally distributed, Shapiro-Wilk test,  $P > 0.05$ ) between the extract concentrations, we used analysis of variance (ANOVA) followed by *Tukey's* test with Bonferroni's correction. The graphs were performed on the software Sigma Plot (Systat 2008) for *R. supcapitata* and *L. minor* and on software R (R Development Core Team 2017) for the others.

## RESULTS

The chromatogram of the exploratory gradient showed five chromatographic bands with significant absorption on wavelength 254–280 nm (Fig. IV-1). The major compounds (bands 2 and 4) were isolated and identified as Coronarin D and Coronarin D Ethyl Ether, respectively (Fig. IV-2).



**Fig. IV-1** Chromatogram superposition of exploratory gradient of the ethanolic extract of *Hedychium coronarium* rhizomes.



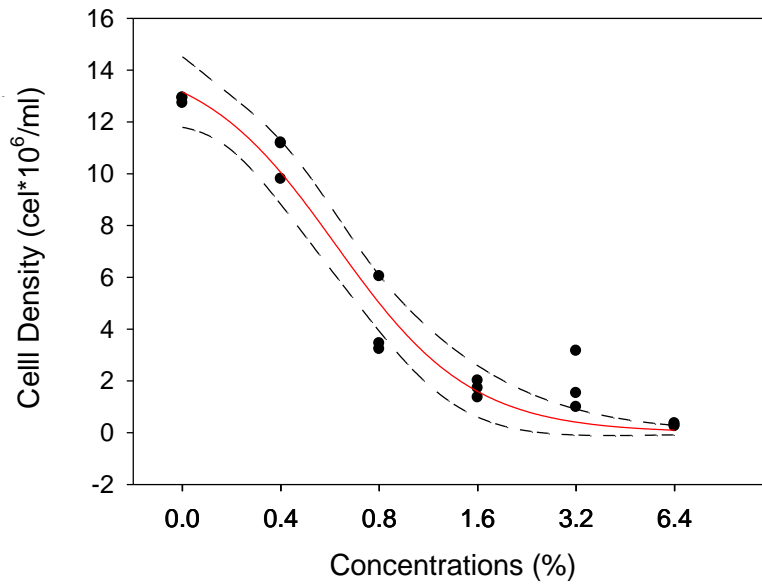
2- R: H  
4- R: CH<sub>2</sub>CH<sub>3</sub>

**Fig. IV-2** Major compounds identified on the ethanolic extract of *Hedychium coronarium* rhizomes: 2 (Coronarín D) and 4 (Coronarín D Ethyl Ether).

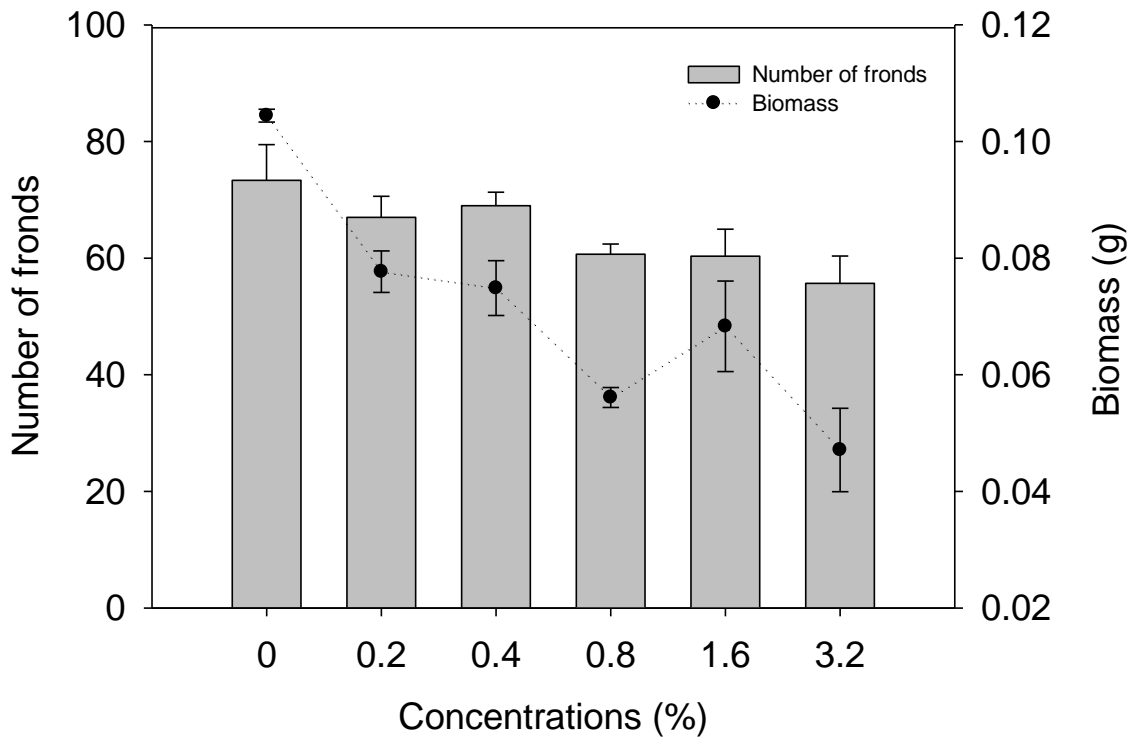
The dry weight of the aqueous extract was 11.37 mg/ml. The *H. coronarium* aqueous extract was toxic for all tested species (Table IV-1). We observed growth inhibition in *R. subcapitata* (Fig. IV-3) and biomass reduction in *L. minor* (Fig. IV-4). There was no difference on the number of fronds of *L. minor* between treatments (ANOVA SS = 643.333, df = 5, MS = 128.667, F = 2.512, P = 0.088), but the biomass of macrophytes differed between treatments (SS = 0.005, df = 5, MS = 0.001, F = 15.810, P <0.001) (Table IV-2).

**Table IV-1.** Effective concentration (EC 50) of aqueous extract *Hedychium coronarium* in percentage for tested species and their confidence interval.

Species	EC 50 (%)	Upper (%)	Lower (%)
<i>Raphidocelis subcapitata</i>	0.655	0.536	0.775
<i>Daphnia similis</i>	0.264	0.218	0.310
<i>Ceriodaphnia silvestrii</i>	0.266	0.224	0.308
<i>Chironomus sancticaroli</i>	0.225	0.183	0.265
<i>Danio rerio</i>	0.141	0.138	0.143



**Fig. IV-3** Population growth inhibition of *Raphidocelis subcapitata* by *Hedychium coronarium* aqueous extract expressed as cell density reached after 96h.



**Fig. IV-4** Effect of *Hedychium coronarium* aqueous extract on the number and biomass of *Lemna minor* fronds exposed during 168h.

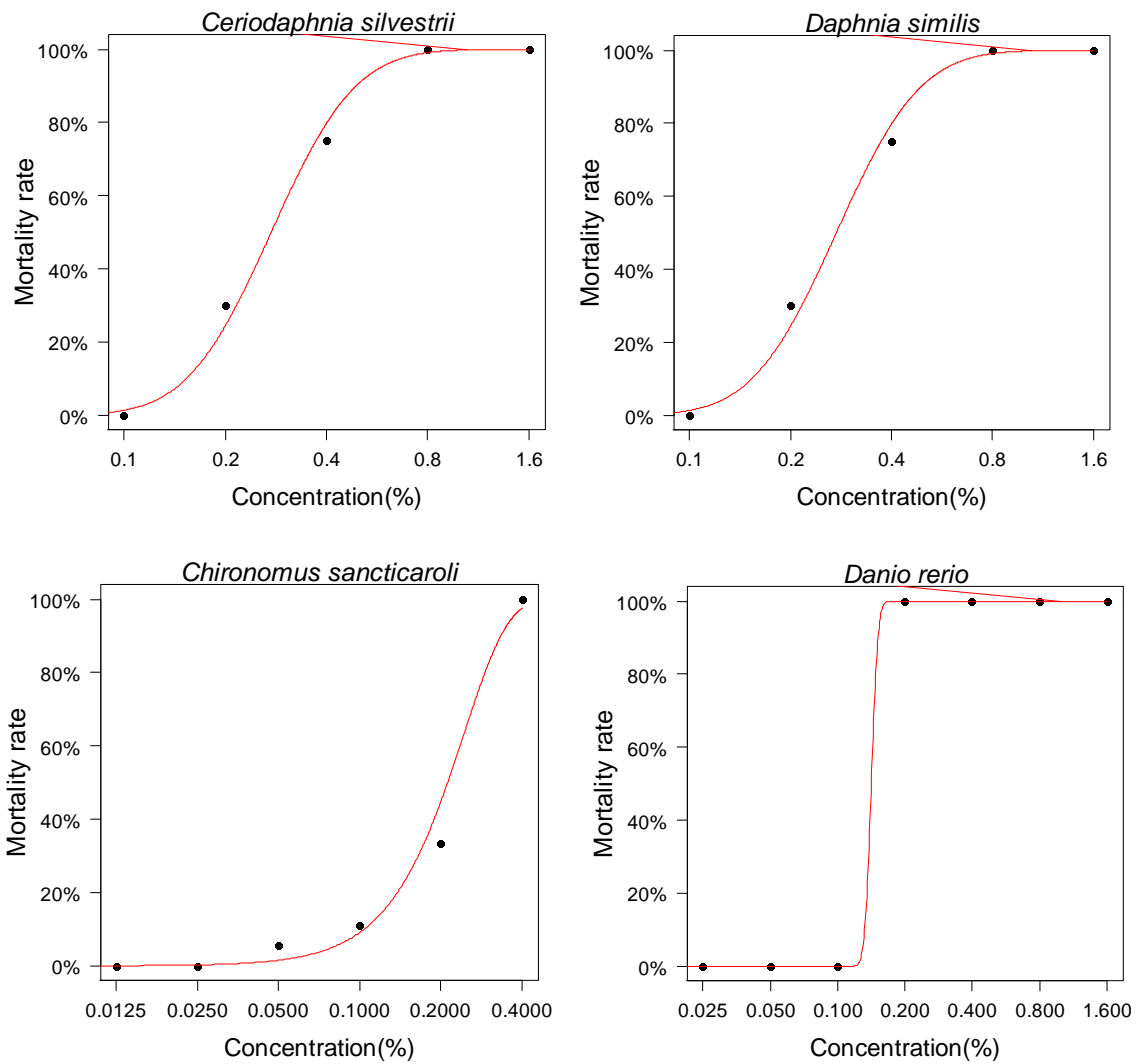
**Table IV-2.** Pairwise comparison between the biomass of *Lemna minor* fronds exposed to several concentrations of aqueous extract from *Hedychium coronarium* rhizomes during 168h.

Concentrations	0.0%	0.2%	0.4%	0.8%	1.6%
0.2%	0.251				
0.4%	0.194	0.878			
0.8%	0.004	0.079	0.108		
1.6%	0.092	0.592	0.702	0.221	
3.2%	0.001*	0.032	0.047	0.702	0.108

\*significant values ( $\alpha = 0.033$ )

*D. rerio* showed the lowest EC50, followed by *C. sancticaroli* and cladoceran species (Fig. IV-5). We observed early mortality of *Danio rerio* individuals. The individuals died on the first hour on highest concentrations (0.8% and 1.6%), and all deaths in other extract concentrations occurred in the first 24 h. No mortality or behavioral change was observed in the lower concentrations (0.2% and 0.4%) and in the control. *C. sancticaroli* was the second most sensible species (Fig. IV-5). *D. similis* and *C. silvestrii* exhibited the same EC 50, indicating similar sensibility to *H. coronarium* rhizome extract on tested conditions (Table IV-2, Fig. IV-5).





**Fig. IV-5** Effect of *Hedychium coronarium* aqueous extract on immobility rate of *Ceriodaphnia silvestrii*, *Daphnia similis*, *Chironomus sancticaroli* and *Danio rerio*.

## DISCUSSION

We evaluated the toxicity of *H. coronarium* aqueous extract on aquatic organisms. Corroborating our hypothesis, the extract caused mortality or grow inhibition in all studied species. Other studies showed the toxicity of *H. coronarium* rhizomes using essential oil (Gullo et al. 2016), aqueous (Rodrigues and Lopes 2006) and ethanolic extracts (Sowmya et al. 2016), suggesting that the toxic compounds are spread among different classes of chemical substances. Toxic effects have also been reported by other invasive plants, such as *Alliaria petiolata* (Brassicaceae) on arbuscular fungal mycorrhizal (Callaway et al. 2008), *Lythrum salicaria* (Lythraceae) on *Bufo americanus*

tadpoles (Maerz et al. 2005; Brown et al. 2006) and *Pteridium arachnoideum* (Dennstaedtiaceae) on seed germination and seedling morphology of pioneer and secondary trees (Silva Matos and Belinato 2010). Bioactive compounds are a remarkable trait of invasive plants and probably an important strategy along the invasion process.

In this study, the main compounds of *H. coronarium* were the diterpenes Coronarin D and Coronarin D Ethyl Ether. Coronarin D Ethyl Ether was related to activities against cancer cells (Zhan et al. 2012) and Coronarin D have antimicrobial (Reuk-Ngam et al. 2014), antifungal (Kaomongkolgit et al. 2012), anti-inflammatory and cytotoxicity activity (Matsuda et al. 2002). Kunnumakkara et al. (2008) showed that the cytotoxic and anti-inflammatory potential of Coronarin D is due to the suppression of NF- $\kappa$ B, a transcription factor involved in expression control of genes linked to cellular growth, differentiation, survival and immune response to inflammation.

Despite variations in the chemical composition of *H. coronarium* rhizomes among regions (Miranda et al. 2015; Ray et al. 2018), the terpenes are its main secondary compounds (Ray et al. 2018), being directly related with plant defense. They act as toxins against competitors, herbivorous (Pichersky and Gershenzon 2002), fungi (Morrissey and Osbourn 1999), bacteria (Joshi et al. 2008) and other microorganisms and animals (Langenheim 1994; Gershenzon and Dudareva 2007). Other toxic compound classes, such phenols, saponins, flavonoids and glycosides can also be found in *H. coronarium* rhizomes (Pachurekar and Dixit 2017). Furthermore, bioactive compounds can act synergistically, as demonstrated for both plant toxins (Leflaive and Ten-Hage 2007) and pesticides (Sanches et al. 2017). Therefore, despite the number of studies showing the toxicity of compounds isolated from *H. coronarium*, our results show that these toxic effects are even more dangerous to the native aquatic organisms than have been demonstrated.

The toxicity of *H. coronarium* against insects was previously reported, causing mortality in mosquito larvae (Phukerd and Soonwera 2013) and adult termites (Sowmya et al. 2016). Certain compounds of *H. coronarium* can act as insect repellent (Caballero-Gallardo et al. 2014), contributing to avoid herbivory. In fact, leaves of *H. coronarium* often have no signals of herbivory in the field (pers. comm.), even in invaded sites inhabited by capybaras, a large herbivorous mammal (Ferraz et al. 2007; De Castro et al. 2013). On the other hand, the toxicity of *H. coronarium* against zebrafishes that we observed apparently lacks a clear evolutive advantage, but similar toxic effects of plant

secondary compounds in fishes are reported by other studies. The aqueous extract of the climbing shrub *Millettia pachycarpa* (Fabaceae) showed toxicity on zebrafish embryos, causing oxidative stress, apoptosis cell, and embryonic lethality (Yumnamcha et al. 2015). Root extracts of *Polygonum multiflorum* (Polygonaceae) caused mortality and deformation on zebrafish embryos four days post fertilization (Thi et al. 2016). Therefore, we do not discard that the mortality of *C. sancticaroli* and *D. rerio* is related to the toxic compounds that are adaptive in reducing herbivory.

The releasing of “chemical weapons” is a defense mechanism against direct competitors (other plants), herbivorous and pathogens such as bacteria and fungi (Reinhart and Callaway 2006; Stinson et al. 2006). Invaders are usually more generalists in their associations, while native species are more specialist and more limited in their mutualistic relationships (Callaway et al. 2004). Rhizomes of *H. coronarium* have compounds that are known by their antifungal (Sabulal et al. 2007; Pandya et al. 2014; Santos et al. 2014; Gullo et al. 2016) and bactericidal properties (Sabulal et al. 2007; Prakash et al. 2010). Several families of plants that are potential competitors of *H. coronarium* have strong mutualistic relationships with fungal, which increase their competitive ability (Sprent and Parsons 2000). Thus, this invasive plant can modify the biota as a strategy to facilitate the invasion by reducing the fitness of native competitors.

Local biodiversity acts as a barrier to invasion mainly by the action of competitors, consumers and pathogens (Kennedy et al. 2002). Liberation of secondary compounds with wide toxicity may contribute to reduce the biotic resistance, creating favorable conditions to dominate invaded areas. The toxicity against organisms that are not direct natural enemies (non-target species) may cause indirect effects on target organisms, for instance controlling their populations via trophic cascade effect (Brown et al. 2006). Chemical compounds can also have indirect strategies to improve fitness of the invasive species, such as breaking mutualistic associations involving native competitors (Callaway et al. 2008) or acting against key species of trophic chain (Maerz et al. 2005), such as zooplankton (Zannatul 2009).

The *H. coronarium* aqueous extract was toxic for all studied species on tested conditions, suggesting that other organisms may also be affected. Our results indicate that toxic compounds of *H. coronarium* may interfere not only on direct competitors, but also on associated organisms and in the trophic web dynamics of invaded aquatic ecosystems.

Thus, it is important to further studies investigate the role of *H. coronarium* toxins and their implications for biodiversity.

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\*Supplementary material

Initial and final chemical parameters obtained from test-solutions of toxicity bioassays of *Hedychium coronarium* aqueous extract for *Raphidocelis subcapitata*, *Lemna minor*, *Chironomus sancti-caroli*, *Ceriodaphnia silvestrii*, *Daphnia similis* and *Danio rerio*. pH, dissolved oxygen (DO), conductivity (Cond), hardness, and temperature (Temp)

	Initial					Final				
	pH	DO (mg/L)	Cond ( $\mu\text{s}/\text{cm}^3$ )	Hardness (mg $\text{CaCO}_3/\text{L}$ )	Temp ( $^{\circ}\text{C}$ )	pH	DO (mg/L)	Cond ( $\mu\text{s}/\text{cm}$ )	Hardness (mg $\text{CaCO}_3/\text{L}$ )	Temp ( $^{\circ}\text{C}$ )
<i>R.subcapitata</i>										
0.0%	7.52					9.96				
0.40%	7.33					10.19				
0.8%	7.27					9.85				
1.6%	7.28					9.06				
3.2%	6.88					8.15				
6.40%	6.47					7.45				
<i>L. minor</i>										
0.0%	5.73	8.47			20.1	7.27	7.76			23.8
0.2%	5.86	7.22			21.9	7	7.79			23.4
0.4%	5.84	7.19			22.3	7.03	7.62			23.3
0.8%	5.82	7.17			22.5	7.15	7.55			23.2
1.6%	5.81	7.15			22.4	7.66	7.65			23.2
3.2%	5.82	7.14			22.6	7.82	7.03			23.3
<i>C. santicarolli</i>										
0.0%	7.51	7.33	56.9	16		7.42	7.38	77		18
0.0125%	7.35	7.03	57.6	16		7.38	7.38	72.8		16
0.025%	7.38	7.01	56.8	16		7.44	7.35	73.7		18
0.05%	7.38	7.01	58.7	16		7.39	7.32	75.3		20
0.1%	7.38	7	60	18		7.35	7.22	77.4		20
0.2%	7.29	6.99	64.3	20		7.44	7.02	82.1		22
0.4%	7.19	7.01	72.6	24		7.36	6.86	89.1		26
<i>C. silvestrii</i>										
0.0%	7.49	7.35	229	48.00		7.48	7.39			
0.1%	7.42	7.38	167.40	50.0		7.48	7.37			
0.2%	7.42	7.37	169.80	50.00		7.39	7.32			
0.4%	7.38	7.30	173.70	52.00		7.48	7.13			
0.8%	7.32	7.25	179.80	54.00		7.42	6.89			
1.6%	7.12	7.17	195.20	56.00		7.42	6.71			
<i>D. similis</i>										
0.0%	7.49	7.35	229	48.00		7.58	7.50			
0.1%	7.42	7.38	167.40	50.00		7.58	7.47			

0.2%	7.42	7.37	169.80	50.00	7.42	7.29
0.4%	7.38	7.30	173.70	52.00	7.23	6.96
0.8%	7.32	7.25	179.80	54.00	7.38	6.68
1.6%	7.12	7.17	195.20	56.00	7.52	6.92

*D. rerio*

0.0%	7.44	7.55	164.9	48	7.16	6.18	170.2
0.025%	7.59	7.41	164.7	48	7.13	4.53	171.3
0.05%	7.58	7.52	166.7	50	7.19	4.28	174.6
0.1%	7.55	7.52	167	52	7.11	3.74	174.6
0.2%	7.52	7.44	171.8	54	6.95	6.1	174.6
0.4%	7.47	7.44	176.9	56	7.14	6.15	174.6
0.8%	7.37	7.43	187.9	58	6.94	6.58	174.6
1.6%	7.23	7.34	214	64	6.79	6.95	174.6

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# CONSIDERAÇÕES FINAIS

Revelamos para *H. coronarium* a existência de um mecanismo de compensação de energia e investimento em diferentes condições ambientais, mostrando desempenho similar na capacidade de ocupação, tanto em ambientes abertos quanto sombreados. Em condições de alta luminosidade, o maior investimento em rebrotas mostrou sua habilidade de ocupação de clareiras e áreas abertas, enquanto em ambientes sombreados, o maior investimento em propagação subterrânea mostrou a capacidade de invadir áreas fechadas e ambientes de mata.

Apontamos que o efeito negativo da invasora *H. coronarium* no desenvolvimento de plântulas da nativa *A. macrocarpa* foi causado pela competição abaixo do solo e não pelo sombreamento provocado pelas partes aéreas. A competição por espaço e nutrientes, a ação de compostos aleloquímicos e as alterações na microbiota do solo causadas por essa invasora podem interferir diretamente no sucesso e estabelecimento de espécies nativas. Assim, esses mecanismos de competição podem funcionar como barreira ao estabelecimento de espécies importantes no processo de sucessão ecológica em áreas ripárias invadidas.

Os compostos secundários produzidos e liberados no meio por *H. coronarium* afetaram espécies nativas, sendo que o efeito observado não foi apenas sobre competidores diretos, mas também em organismos indiretamente associados. O efeito fitotóxico do óleo essencial obtido a partir dos rizomas da espécie ocasionou redução da germinação de espécies arbóreas, o que mostra seu alto poder de competição e de domínio em florestas ripárias. Constatamos também a toxicidade do extrato aquoso dos rizomas para organismos aquáticos (algas, macrófitas, cladóceros, insetos e peixes), indicando um possível efeito na dinâmica de cadeias tróficas de ecossistemas aquáticos em áreas invadidas.

Os resultados aqui apresentados apontaram diferentes estratégias e impactos da invasora *H. coronarium* sobre comunidades invadidas, reforçando sua plasticidade e seu poder de alteração do ambiente. Considerando a ampla distribuição e heterogeneidade de habitats em que essa espécie ocorre, é importante avaliar a adequação dos métodos de manejo e controle. Apesar do amplo uso medicinal da espécie, é importante avaliar as consequências da presença desses compostos no solo e na água, ressaltando o papel ecológico das toxinas de *H. coronarium* e suas implicações para sua biodiversidade.

Vários aspectos das estratégias de invasão por *H. coronarium* e os impactos ocasionados em nível ecossistêmico ainda devem ser esclarecidos. No processo de competição interespecífica, futuros estudos devem considerar os possíveis múltiplos efeitos da espessa serapilheira depositada por *H. coronarium*, avaliando o efeito da invasão em estágios posteriores de crescimento e reprodução de espécies nativas. Dada a complexidade de mecanismos de competição entre espécies invasoras e nativas, recomendamos que os aspectos relacionados à competição subterrânea sejam mais profundamente investigados. Considerando ainda a presença de compostos químicos em *H. coronarium* e o efeito tóxico a diferentes grupos de organismos, é preciso avaliar a disponibilidade dessas substâncias *in situ* e investigar os mecanismos de ação dos mesmos. Por fim, é necessário monitorar o processo de invasão e expansão da espécie, considerando seu poder de ocupação e potencial danoso aos ecossistemas.