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Diclidophlebia smithi (Hemiptera: Psyllidae), a potential biocontrol agent for *Miconia calvescens* in the Pacific: Population dynamics, climate-match, host-specificity, host-damage and natural enemies

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HIGHLIGHTS

- We studied the potential of *D. smithi* as a biological control agent for *M. calvescens*.
- We verified *D. smithi* has host-specificity and causes severe damage to *M. calvescens*.
- Our studies suggest that *D. smithi* has potential to establish in Hawaii.
- We suggested that *D. smithi* is a good biocontrol agent for *M. calvescens*.
- *D. smithi* should be introduced in places where *M. calvescens* causes problems.

GRAPHICAL ABSTRACT

Damages caused for *Diclidophlebia smithi* to the leaf tissue of *Miconia calvescens.*

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ABSTRACT

Diclidophlebia smithi (Hemiptera: Psyllidae) has been proposed as a candidate biological control agent for *Miconia calvescens* (Melastomataceae), a neotropical tree regarded as one of the worst threats to the rainforest ecosystems of several Pacific islands. Populations of *D. smithi* monitored over three years at three sites in the state of Minas Gerais, Brazil were found to peak during the cooler, drier months from April to July, with air temperatures as the factor most strongly correlated (negatively) with population density. A biologically significant match of climate conditions (+0.7 CMI, using CLIMEX regional matching) was found between the study region in Brazil and 3 of 9 weather stations in the potential release area of Hawaii. This result coupled with the wide variation in climatic conditions under which *D. smithi* populations have been found in Brazil suggest that *D. smithi* has good potential to establish in at least some regions of Hawaii. No significant predator, parasitoids or entomopathogens were found attacking *D. smithi* in its native range. No nymphs or adults of *D. smithi* were found on any plant belonging to a list of 102 species of 58 plant families growing in the vicinity of colonized *M. calvescens* individuals. Conversely, potted sentinel plants of the Hawaiian biotype of *M. calvescens* were readily colonized by *D. smithi* during an experimental six-month field exposure. Greenhouse no-choice tests with nine species of Melastomataceae also demonstrated that *D. smithi* has a host-range restricted to *M. calvescens.* A study of the morphological changes caused on tissues of *M. calvescens* by *D. smithi* showed that attack on leaves caused a collapse in the epidermis, cell disorganization and degeneration of the vascular system. This study confirms that *D. smithi* has significant potential as a biocontrol agent for *M. calvescens.*

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1. Introduction

Invasions by *Miconia calvescens* DC. (Melastomataceae) in rainforests of French Polynesia, Hawaii, New Caledonia and Australia are a serious threat to these ecosystems (Csurhes, 1997; Medeiros et al., 1997; Meyer, 1996; Meyer and Loope, 2011). *M. calvescens* is a small tree native to Central and South America, which was introduced into these regions as an ornamental plant. Competition for water, light and nutrients with native plants is the main problem caused by *M. calvescens* (Meyer et al., 2003; Meyer et al., 2007), and in Hawaii and Tahiti, this weed already dominates tracts of land that were originally occupied by the native forests (Medeiros et al., 1997; Meyer, 1996). In its native range in Brazil, *M. calvescens* is usually found in disturbed habitats, in secondary forests at advanced succession stages after clear cutting or fire have destroyed the primary forest or as an understory forest plant. It occurs at low population densities and is often severely affected by a suite of pathogens and phytophagous arthropods (Hanson et al., 2010; Picanço et al., 2005). It is widely accepted that the only sustainable method of control for exotic weeds invading natural ecosystems is classical biological control by means of natural enemies introduced from the native range (Barreto et al., 2012). In some areas where *M. calvescens* has emerged as a more recent ecosystem invader, management efforts have been concentrated on the chemical and mechanical eradication of plant populations (Brooks and Jeffery, 2010; Goarant and Meyer, 2010; Meyer and Goarant, 2010) However it is doubtful whether these approaches are sustainable over the long term.

Preliminary surveys of natural enemies attacking *M. calvescens* that were conducted in Brazil, Costa Rica and Trinidad from 1993 to 1995 by exploratory entomologist Robert Burkhart (Hawaii Department of Agriculture 1998) stimulated later studies on the classical biocontrol of *M. calvescens*. In 1995, a search for *M. calvescens* pathogens native to the neotropics was initiated and involved survey of areas in Brazil, Costa Rica and Ecuador yielding numerous pathogens (Alves et al., 2010; Santin, 2008; Seixas et al., 2007, 2004, 2002). Arthropods attacking *M. calvescens* were also surveyed in Brazil (Burckhardt et al., 2006; Picanço et al., 2005), Costa Rica (Burckhardt et al., 2005; Hanson et al., 2010) and Mexico (Hanson et al., 2010). To date only one natural enemy has been released for classical biological control of *M. calvescens*; the fungus *Colletotrichum gloeosporioides* f. sp. *miconiae* which was introduced into Hawaii in 1997 (Killgore et al., 1997) and into French Polynesia in 2000 (Meyer et al., 2008). Although it has become established in both archipelagoes and is contributing to the control of miconia, particularly in French Polynesia (Meyer et al., 2012), it is now clear that additional agents will be required for effective control of this weed (Killgore et al., 1997).

One potential biocontrol agent that was identified in surveys in Brazil is the newly described psyllid *Diclidophlebia smithi* Burckhardt, Morais and Picanço (Hemiptera: Psyllidae) (Burckhardt et al., 2006). Field observations and results of studies on *D. smithi* (Burckhardt et al., 2006; Morais et al., 2007; Picanço et al., 2005) combined with the favorable track record in terms of host-specificity with the use of psyllids in weed biocontrol (Center et al., 2007; Hollis, 2004; Shaw et al., 2009) suggest, that *D. smithi* may be a useful biocontrol agent for *M. calvescens*. This psyllid is a member of the possibly monophyletic species group (Burckhardt et al., 2006) associated with Melastomataceae and appears to be widely distributed in south-eastern Brazil. A second psyllid species, *Diclidophlebia lucens* Burckhardt, Hanson, and Madrigal, was also collected and described by a separate team involved in the miconia biocontrol program, based in Costa Rica (Hanson et al., 2010). This species was also independently chosen and investigated as potential agent for control of *M. calvescens* (Burckhardt et al., 2005).

To further the evaluation of *D. smithi* as a biocontrol agent of *M. calvescens*, we report on the following biological studies in its native Brazil: (1) population dynamics of *D. smithi* in the field; (2) climate-matching to infer its potential distribution in Hawaii; (3) survey of its natural enemies; (4) initial evaluation of host-specificity; and (5) observations on damage to miconia tissues.

2. Materials and methods

2.1. Population dynamics

Independent populations of *D. smithi* were monitored at three well-separated sites in the municipalities Dionísio, Guaraciaba, and Viçosa in the state of Minas Gerais (Brazil) (Table 1). At each site, reproductive *M. calvescens* plants ranging in height from 1–7 m and having a basal diameter varying between 6–15 cm, were a common part of the plant community.

Evaluations were made during three different periods of time, first, from June 2001 to June 2002, second, from February 2004 to February 2005 and third, from March 2005 to February 2006. During the first period the plants were evaluated at three-week intervals in Viçosa and Dionísio. During the second period evaluations were made at 15-day intervals in Viçosa and monthly in Dionísio. In the third period evaluations were only made in Guaraciaba and Viçosa and at three-week intervals at both places (Table 1).

On each visit to a site, ten *M. calvescens* plants were selected without regard to the presence or absence of visible psyllid colonies. For each selected plant, all aerial plant parts were examined, i.e. leaves, leaf buds, flowers and fruits, and the total number of adults and nymphs of *D. smithi* on each plant part were counted. Predatory arthropods associated with *D. smithi*, as well as any seemingly parasitized individuals, were counted and collected for further examination.

Climate data was obtained from the following climate stations: for Viçosa – Universidade Federal de Viçosa station, Guaraciaba – Ponte Nova station, Dionísio – Ponte Alta station (belonging to

Table 1
Information about localities included in population dynamics field studies of *Diclidophlebia smithi*.

Municipalities (coordinates)	Periods covered during survey	Vegetation	Location
Viçosa (20°46′37″S, 42°50′36″W)	Jun 2001–Jun 2002, Feb 2004–Feb 2005, Mar 2005–Feb 2006	Secondary forest fragment (semi-deciduous seasonal forest)	Cristais, next to abandoned rail track
Dionísio (19°50′34″S, 42°46′36″W)	Jun 2001–Jun 2002, Feb 2004–Feb 2005, Mar 2005–Feb 2006	Understory in eucalyptus plantation	Balsa Nova, CAF Santa Bárbara Ltda, next to the margins of the Rio Doce
Guaraciaba (20°34′36″S, 43°02′26″W)	Mar 2005–Feb 2006	Abandoned pasture with secondary forest overgrowth (early stages of succession)	Vicinities of Rio Piranga

the private company CAF Santa Bárbara Ltda). Canonical correlations (PROC CANCORR statement) were carried out using the SAS program in order to verify if there was an interrelation between the population density of nymphs and adults and climatic factors (average air temperature, relative humidity, rainfall, photoperiod, insolation and wind speed).

2.2. Climate matching

The CLIMEX (version 3.00.009) Regional Climate Matching algorithm was used to compare the Hawaiian Islands and a rectangular region in Brazil located between latitude 17.8–22.8° S and longitude 42.8–43.8° W. This area in Brazil encompasses our three population study sites (Table 1) as well as known *D. smithi* populations in Rio de Janeiro and Minas Gerais. Nine Hawaiian weather stations, included in the standard meterological database provided with CLIMEX, were selected for climate matching. This provided more points on the islands than using the regional grid generated from the CLIMEX 0.5° world grid database, and thus a better representation of climate across the islands. Comparisons were made using the composite match index (CMI) with the standard set of four variables, maximum and minimum temperature, total rainfall and the weekly distribution of rainfall (Sutherst et al., 2007). CMI values greater than 0.7 for the climatic variables has been suggested as useful biological threshold to evaluate risk of species invasion (Sutherst et al., 2007; Kriticos, 2011).

2.3. Host specificity in the field and use of sentinel plants

Indirect assessment of the host-specificity of *D. smithi* was made using a method previously applied to pathogens attacking *M. calvescens* (Seixas et al., 2004; Santim, 2008). During the population monitoring surveys, all vegetation within a 2 m radius of each sampled *M. calvescens* individual was inspected to check whether *D. smithi* could be found attacking them. Whenever necessary, botanical samples of individual plants occurring in the vicinity of miconia plants were taken, dried in a plant press and brought to the botanists at the Departamento de Biologia Vegetal of the Universidade Federal de Viçosa for identification. Particular care was taken for examination of plants belonging to the Melastomataceae. Members of the Melastomataceae were identified by the Brazilian expert in the group (R. Goldenberg, Universidade Federal do Paraná). Colonies of *D. smithi* are easily spotted by abundant white waxy filaments secreted by the nymphs. The following three localities where natural populations of *D. smithi* occurred on *M. calvescens* were surveyed in order to indirectly assess its host-specificity: Cristais – municipality of Viçosa (20°46′37″S and 42°52′27″W), MG; Dendrologia, Campus of Universidade Federal de Viçosa, municipality of Viçosa, MG (20°46′30″S and 42°50′36″W); municipality of Guaraciaba, MG (20°34′36″S and 43°02′26″W). The complete list of plant species that were examined for the presence of psyllid colonies is given in Table 2. These observations were collected at the Viçosa sites on February 15 and September 4, 2001 and at Guaraciaba (March 3 and September 18, 2001).

A "sentinel-plant strategy" was also used in order to assess the suitability of *M. calvescens* populations from the Pacific as hosts for *D. smithi* under natural conditions. Seeds of *M. calvescens* originating from Hawaiian populations were planted and when the plants reached a height of 30–40 cm, ten vigorous individuals were chosen and planted at the Guaraciaba study site (Sept 2005) in a row with 2 m intervals between plants. Observations were made on a regular basis until February 2006 and occurrence of colonies of *D. smithi* was recorded. On the last visit all remaining sentinel plants were removed from the field site and destroyed.

Table 2
Plant species examined for the presence of *Diclidophlebia smithi* at field sites in Brazil 2001–2006.

Division Magnoliophyta
Class Magnoliopsida

Order	Family	Species	Locality[*]
Myrtales	Melastomataceae	*Leandra* sp.	C
		Clidemia hirta D. Don	C/D/G
		Tibouchina granulosa (Desr.) Cogn.	C/D
		Tibouchina herbacea (DC). Cogn.	G
		Miconia calvescens DC.	C/G
		Miconia sp. 1	G
		Miconia sp. 2	C
		Miconia albicans Steud.	C/D/G
		Miconia cinnamomifolia Triana	C/D
		Miconia ibaguensis Triana	C/D/G
		Miconia latecrenata Naudin	C/D
	Myrtaceae	*Psidium guajava L.*	C/G
		Eugenia uniflora L.	C
		Eucalyptus sp.	D
	Lythraceae	Not identified	C
Malpighiales	Malpighiaceae	*Lophanthera lactescens* Ducke	C
	Clusiaceae	*Vismia brasiliensis* Choisy	C/G
	Phyllanthaceae	*Phyllanthus* sp.	D
	Passifloraceae	*Passiflora* sp.	G
		Passiflora edulis Sims	C
Fabales	Fabaceae	*Desmodium* sp.	C/D
		Piptadenia sp.	G
	Polygalaceae	*Diclidanthera* sp.	C
Euphorbiales	Euphorbiaceae	*Joaneria* sp.	G
		Manihot esculenta Crantz	C
		Croton floribundus Spreng	D/G
		Croton sp.	C
		Mabea sp.	C/G
Linales	Erythroxylaceae	*Erythroxylum* sp.	G
		Erythroxylum pelleterianum A. St.-Hil.	C
Sapindales	Meliaceae	*Trichilia pallida* Sw.	D
		Toona sp.	D
		Guarea macrophylla Vahl	G
	Rutaceae	*Citrus* sp.	C
		Zanthoxylum sp.	D
	Anacardiaceae	*Schinus terebinthifolius* Raddi	C/G
	Sapindacea	*Paullinia* sp.	D
		Cardiospermum sp.	C
Geraniales	Balsaminaceae	*Impatiens walleriana* Hook.f.	D
Rosales	Rosaceae	*Eriobotrya japonica* (Thunb.) Lindl.	C
		Rubus rosifolius Stokes	C
Apiales	Umbelliferae	*Centella* sp.	G
		Centella asiatica (L.) Urb.	C
Laurales	Lauraceae	*Nectandra rigida* (Kunth) Nees	D
		Persea americana Mill.	C
	Monitiniaceae	*Siparuna guianensis* Aubl.	G
Ranunculales	Menispermaceae	Not identified	D
Magnoliales	Annonaceae	*Malmea* sp.	C
Piperales	Piperaceae	*Piper* sp.	D
		Piper aduncum L.	C/G
		Pothomorphe umbellata (L.) Miq.	C
Urticales	Moraceae	*Ficus* sp.	D
	Urticaceae	*Urtica* sp.	D
	Cecropiaceae	*Cecropia* sp.	C/D/G
Malvales	Malvaceae	*Pavonia communis* A. St.-Hil.	C
		Sida sp.	D/G
	Tiliaceae	*Luehea grandiflora* Mart.	C
		Luehea sp.	G
Violales	Curcubitaceae	*Cucurbita* sp.	C
Solanales	Solanaceae	*Solanum* sp.	C
		Solanum cernuum Vell.	G

(*continued on next page*)

Table 2 (*continued*)

Division Magnoliophyta			
Class Magnoliopsida			
Order	Family	Species	Locality*
	Convolvulaceae	*Ipomoea* sp.	C
		Ipomoea cairica (L.) Sweet	C
Gentianales	Apocynaceae	*Peschiera laeta* Miers	C
Lamiales	Verbenaceae	*Lantana camara* L.	C/G
	Boraginaceae	*Borago* sp.	G
	Gesneriaceae	*Sinningia* sp.	C
	Lamiaceae	*Schizonepeta* sp.	C
	Plantaginaceae	*Plantago* sp.	C
Rubiales	Rubiaceae	*Coffea arabica* L.	C/D
		Spermacoce latifólia Aubl.	C
		Psychotria sp.	G
		Richardia brasiliensis Gomes	C
Scrophulariales	Acanthaceae	*Thunbergia alata Bojer* ex Sims	G
		Hypoestes sanguinolenta Hook.	D
	Bignoneaceae	*Tabebuia* sp.(1)	G
		Tabebuia sp.(2)	D
		Macfadyena unguiscati (L.)	D
Asterales	Asteraceae	*Mikania* sp.	C
		Chromolaena odorata L.	C
		Baccharis sp.	G
		Vernonia sp.	C
Caryophyllales	Amaranthaceae	*Gomphrena* sp.	C
Class Liliopsida			
Alismatales	Araceae	*Xanthosoma sagittifolium* K. Koch	C
Commelinales	Commelinaceae	*Commelina benghalensis* Wall	G/C
Arecales	Arecaceae	*Euterpe* sp.	C
Poales	Bromeliaceae	*Tillandsia* sp.	D
	Cyperaceae	*Fimbristylis* sp.	D
	Poaceae	*Panicum maximum Nees*	G
		Melinis minutiflora P. Beauv.	C
		Imperata brasiliensis Trin.	G
Zingiberales	Maranthaceae	*Calathea* sp.	D
	Heliconiaceae	*Heliconia* sp.	C
	Musaceae	*Musa* sp.	C
Orchidales	Orchidaceae	*Oeceoclades maculata* L.	D
Liliales	Smilacaceae	*Smilax sp.*	D
Asparagales	Hypoxidaceae	*Hypoxis decumbens* L.	C
	Ruscaceae	*Sansevieria* sp.	D
Division Cycadophyta			
Class Cycadopsida			
Order	Famíly	Species	Locality*
Cycadales	Cycadaceae	*Cycas* sp.	D
Division Pteridophyta			
Class Pteridopsida			
Filicales	Gleicheniaceae	*Gleichenia* sp.	C
	Schizaeaceae	*Anemia phyllitidis* (L.) Sw	C
Division Lycopodiophyta			
Class Selaginellopsida			
Lycopodiales	Selaginellaceae	*Selaginella* sp.	G

* Survey locality: C = Cristais – municipality of Viçosa, MG; D = Dendrologia, Campus of Universidade Federal de Viçosa, municipality of Viçosa, MG; G = municipality of Guaraciaba, MG.

2.4. Host specificity in the greenhouse

Host specificity of *D. smithi* was evaluated using direct no-choice tests performed in a greenhouse. Testing was conducted on nine plant species, all belonging to the Melastomataceae: *Clidemia capitellata* (Bonpl.) D. Don., *Clidemia hirta* (L.) Don, *Leandra lacunosa* Cogn, *Miconia albicans* (Sw.) Triana, *Miconia mendoncaei* Cogn., *Miconia ibaguensis* (Bonpl.) Triana, *Ossaea confertiflora* (DC.), *Tibouchina granulosa* (Desr.) Cogn. and *Tibouchina moricandiana* (DC) Baill. In addition plants belonging to the Hawaiian biotype of *M. calvescens* were also included in the test.

For each melastome species, four plants (70–100 cm tall in 5-L pots) were placed in a wooden cage enclosed in fine mesh organza (0.5 × 1.0 × 1.2 m) in a greenhouse. Twenty *D. smithi* nymphs, between the third and fifth instars, from laboratory mass rearing were transferred to each plant (total 80 psyllids per cage). Survival, developmental stages of the nymphs, and damage to plants were recorded daily for a month.

2.5. Natural enemies of *Diclidophlebia smithi*

The occurrence of natural enemies of *D. smithi* was investigated at two-month intervals during the period of evaluation of *D. smithi* populations in the field. Psyllid colonies were carefully examined in order to determine the presence of predatory arthropods feeding on the colonies. Any such occurrence was recorded and specimens were collected and maintained in alcohol (70%) for later identification. At each site visit, up to two leaf buds, inflorescences and fruits bunches of *M. calvescens* bearing psyllid colonies were collected and brought to the laboratory, where they were left with their petioles or stem bases immersed in a water-filled container. These plant parts were left within wooden cages (0.5 × 0.5 × 0.5 m), enclosed in organza and maintained in the laboratory for three weeks under a light regime of 12:12 h at 26 ± 2 °C and 70 ± 10% relative humidity. Samples were checked at two-day intervals for the potential emergence of adult parasitoids and for individuals infected by entomopathogens.

2.6. Damage to leaves of *Miconia calvescens*

Branches of *M. calvescens* plants that were heavily damaged by *D. smithi* as well as undamaged branches (controls) were collected in the field and brought to the laboratory for anatomical examination of selected leaves. Median parts of the leaf lamina of second node leaves of healthy and severely attacked leaves were immersed in FAA$_{50}$ (Formal-Acetic-Alcohol), for 48 h and stored in 70% ethanol (Johansen, 1940). Later, the samples were dehydrated in an increasing ethanol series and embedded in methacrylate resin (Historesin, Leica Instruments, Heidelberg, Germany). Cross sections were prepared with an automatic advanced rotary microtome (RM2155 model, Leica Microsystems Inc.), fitted with disposable glass razors. Sections (4–7 μm) were stained with Toluidine blue pH 4.0 (O'Brien and McCully, 1981) and mounted in synthetic resin (Permount).

Pictures of healthy and damaged leaf parts were taken with a light microscope (AX70TRF, Olympus Optical) equipped with the U-Photo system allowing for a comparison and assessment of the consequences of psyllid attack to the integrity of the tissues.

3. Results

3.1. Population dynamics

At Guaraciaba, populations peaked from May to August 2005 and again during the last week of September 2005. However, nymphs of *D. smithi* were found throughout the evaluation period (Fig. 1A). Guaraciaba had the lowest occurrence of *D. smithi* as compared with Dionísio and Viçosa (Fig. 1).

At Dionísio, the peak nymphal population was observed in July 2004 and the peak adult population at Dionísio was observed in May 2002 (Fig. 1B). At Viçosa peak nymphal populations were observed between April 2000 to June 2002 and the adult population peak was in July 2005 (Fig. 1C). At Dionísio, in the second period evaluations, and at Viçosa, both nymphs and adults of *D smithi* were found throughout the year (Fig. 1B and C).

There was a significant canonical correlation between the density of nymphs and adults of *D. smithi* and climatic elements (Wilks' Lambda = 0.51; $F = 4.26$; gl$_{num/den}$ = 12/130; $p < 0.0001$).

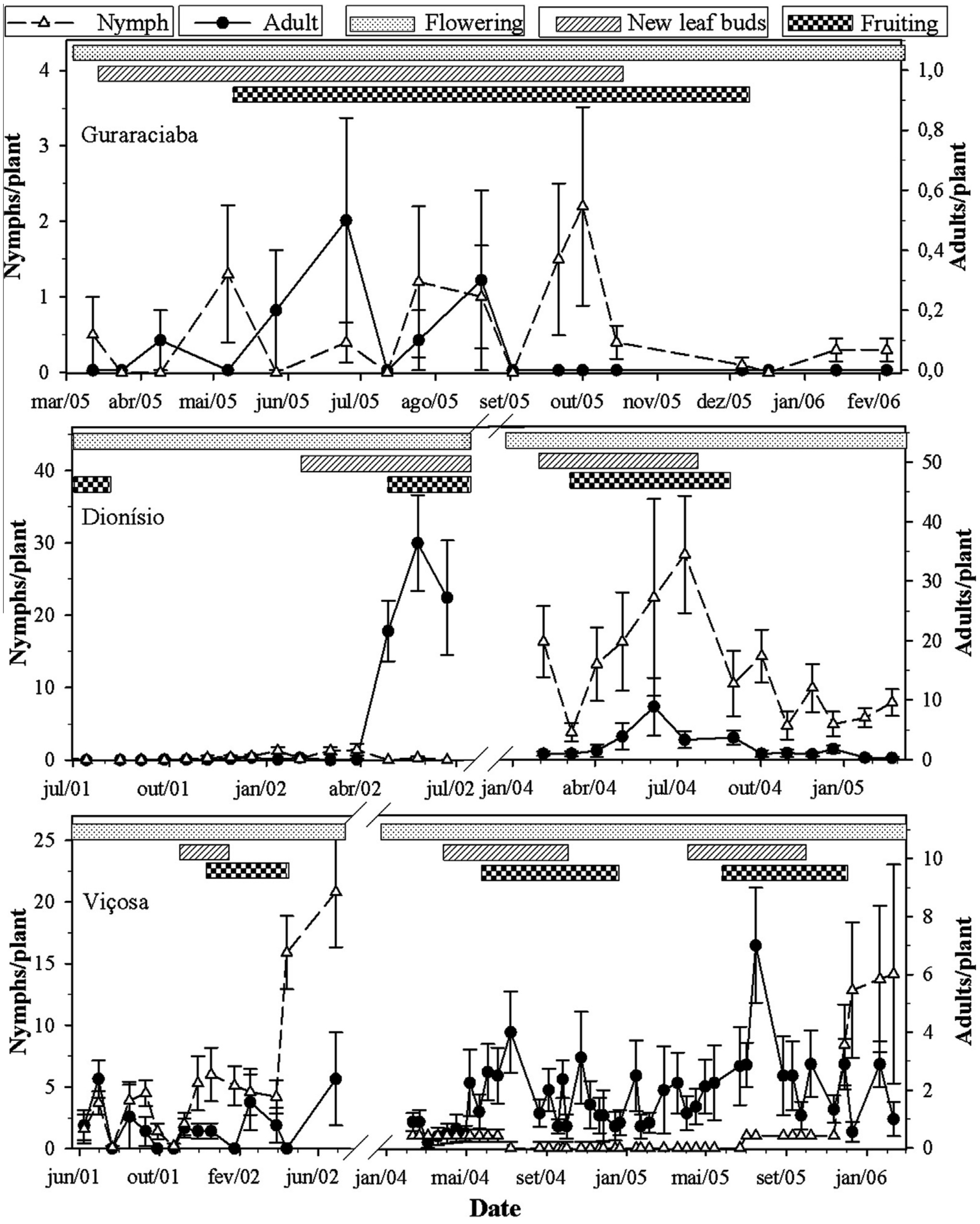

Fig. 1. Phenology of *Miconia calvescens* and *Diclidophlebia smithi* in the municipalities of (A) Guaraciaba, (B) Dionísio and (C) Viçosa, Minas Gerais, Brazil, between 2001–2006. Vertical bars = standard error (note differences in scale on *y*-axis). Formation of new leaf buds; flowering; fruiting.

Two canonical axes were generated, but only the first was significant at 5% probability ($p = 0.0001$) and it explained 78% of the correlation (Table 3). Based in this axis, the adult phase is the most influenced by climate elements ($r = 0.99$). The climatic factors that most negatively affected the density of adult *D. smithi* was the average air temperature ($r = -0.78$), followed by the day length ($r = -0.66$) and the rainfall levels ($r = -0.42$), which also had a negative influence on psyllid density. Relative humidity ($r = 0.50$), had a significantly beneficial influence for *D. smithi* populations (Table 3).

3.2. Climate matching

The total extent of the distribution of *D. smithi* in Brazil is not known. The insect is found however in the contrasting climates of relatively dry Minas Gerais and wet Rio de Janeiro and sites in between (R.W. Barreto, personal observation). Using a CMI value of 0.7 for the four standard variables as a minimum requirement for establishment of *D. smithi*, the climate match between the known potential source region of *D. smithi* in Brazil was greater than 0.7 for 3 of 9 Hawaiian locations tested (Table 4). Thus there are locations within the current Hawaiian distribution of *M. calvescens*, on the islands of Hawaii, Maui, Oahu and Kauai (Medeiros et al., 1997), where climate will probably not limit establishment of *D. smithi*.

3.3. Host specificity in the field and use of sentinel plants

A total of 102 species belonging to 58 plant families were searched in the field on dates encompassing the warm-wet season (Summer) and dry-cool season (Winter) for the presence of colonies of *D. smithi* (Fig. 1). No nymph or adult *D. smithi* were

Table 3
Canonical correlations and canonical pairs between abundance of nymphs and adults of *Diclidophlebia smithi* and climatic factors in Guaraciaba, Dionísio and Viçosa, state of Minas Gerais, Brazil.

Variables	First canonical pair		
	Coefficient	r	r^2
Diclidophlebia smithi			
Nymphs	0.01	0.33	0.00
Adults	0.78	0.99	0.98
Climatic elements			
Average air temperature (°C)	−0.31	−0.78	0.61
Relative humidity (%)	0.10	0.50	0.25
Rainfall (mm)	−0.002	−0.42	0.18
Wind speed (m/s)	0.45	0.28	0.08
Insolation (hours)	0.09	−0.09	0.01
Photoperiod (hours)	0.12	−0.66	0.44
r	0.78		
F	4.26		
df (numerator/denominator)	12/130		
p	<0.0001		

Table 4
Climate Match Index (CMI) between Brazilian native range[1] of *Diclidophlebia smithi* populations and 9 sites in Hawaii.

Island	Climate station	Altitude (m)	CMI
Oahu	Wheeler AFB	255	0.78
Kauai	Lihue	46	0.76
Oahu	Kaneohe Bay	49	0.72
Oahu	Honolulu	3	0.66
Hawaii	Kamuela	316	0.63
Oahu	Barbers Point	9	0.62
Maui	Kahului	0	0.62
Hawaii	Hilo	59	0.49
Hawaii	Bradshaw AAF	1886.7	0.48

[1] Brazilian native range for comparison is rectangle with known *D. smithi* populations located between: latitude 17.8° S to 22.8° S and longitude 42.8° W to 43.8° W. Area encompasses Rio de Janeiro and Minas Gerais.

found on any plant species other than *M. calvescens*. On some dates another white wax producing psyllid was observed on *M. albicans* Steud., at Guaraciaba and Viçosa, however upon examination this insect was found to belong to a different (unidentified) genus of Psyllidae.

One month after *M. calvescens* seedlings of the Hawaiian biotype were left in the field at Guaraciaba, new colonies with nymphs of *D. smithi* were found on the plants, demonstrating their susceptibility to attack by the psyllid. During subsequent visits, established colonies, with nymphs, eggs and adults were present on leaf buds of the plants.

3.4. Host specificity in the greenhouse

In the host specificity tests in greenhouse, *D. smithi* nymphs were not able to develop through to adult on any of the Melastomataceae species tested other than *M. calvescens*. All nymphs that were transferred to non-target plants died within two days of being transferred. Whereas *D. smithi* was able to complete its life cycle on *M. calvescens*, forming dense colonies on shoot tips which became completely covered with white wax produced by nymphs. The apical leaves soon became shriveled and, approximately six months after colonization the tips of the plant had died. The plant responded by producing axillary branches (which were also colonized by the psyllid.

3.5. Natural enemies of Diclidophlebia smithi

Two predators were found in low numbers associated with *D. smithi* when psyllid populations were peaking at our monitoring sites. Unidentified Syrphidae larvae were observed at Viçosa in November 2005 (less than one larva per plant on average) and at Guaraciaba in October and December 2005 (one adult on only one plant per sample period). No parasitoids emerged from psyllids brought from the field, and no entomopathogens were observed in the field or in samples held in the laboratory.

3.6. Damage to leaves

Significant anatopical changes were observed in leaves of *M. calvescens* attacked by *D. smithi*. Young non-attacked leaves have an epidermis consisting of a single layer of cells and a lower mesophyll, formed by two layers of palisade parenchyma and five to six layers of spongy parenchyma (Fig. 2). When attacked, the upper epidermis collapses (particularly in the midrib region) in areas with intense feeding by *D. smithi*. In these regions, the leaf tissue is depressed, cells become disorganized and the vascular system degenerates (Fig. 2B). Psyllid attack on leaves involves insertion of the stylet through the upper epidermis, reaching the mesophyll and the vascular tissue of the smaller leaf veins and the midrib (Fig. 2C and D). In the smaller leaf veins, the stylet of *D. smithi* reaches the tissues of the phloem (Fig. 2D) leading to mesophyll thickening due to the abnormal cell divisions (Fig. 2C and D).

4. Discussion

Diclidophlebia smithi populations peaked between April and June, which coincided with flowering and fruiting of *M. calvescens* in the field. This appears to be the major factor governing population size for *D. smithi*. For psyllids such as *D. smithi* and other sap-feeding insects population growth is highly dependent on the timing of the flushing of soft tissues, such as the inflorescences, infrutescences and leaf buds (Hodkinson, 2009).

Diclidophlebia smithi population densities reached their highest levels during the periods of cool temperatures, shorter days and drier conditions, typical of autumn–winter months in the regions of the state of Minas Gerais (Fig. 1). A previous study under controlled laboratory conditions (Morais et. al., 2010) examining in the showed that *D. smithi* has as high reproductive capacity and short life cycle under cool temperatures (17–21 °C). Air temperature was the climatic factor having the greatest influence on the population density of *D. smithi* in the field ($r^2 = 61$). Hawaii therefore has ideal temperature conditions for *D. smithi* with the islands having an average temperature range of 20–25 °C.

Air temperature is the factor that most affects the establishment, proliferation, dispersal and impact of arthropods in new areas of distribution (Baker, 2002). High air temperatures may kill nymphs and reduce oviposition rate in psyllids, because of abnormal development of the ovaries (Liu and Tsai, 2000; Mehrnejad and Copland, 2005; Stratopoulou and Kapatos, 1995). Reduction in ovary development and egg-laying, as well as reduced survival and longevity is also known for other psyllid species under very high temperatures (Mehrnejad and Copland, 2005; Liu and Tsai, 2000; Nava et al., 2007).

The population density of *D. smithi* is also lower during rainy periods, which in Guaraciaba, Dionísio and Viçosa occur from November to March. It is possible that heavy rain kills individual insects through mechanical impact of large drops which dislodge individuals from their host-plant. Water can also remove the wax cover that serves as a protection for nymphs, exposing them to adverse environmental conditions or predators. Such a deleterious effect of rain is also known for other psyllids such as for *Boreioglycaspis melaleucae* Moore which is used as a biological control agent against the invasive tree *Melaleuca quinquenervia* (Cav.) Blake (Myrtaceae) in Florida (Center et al., 2006). Although

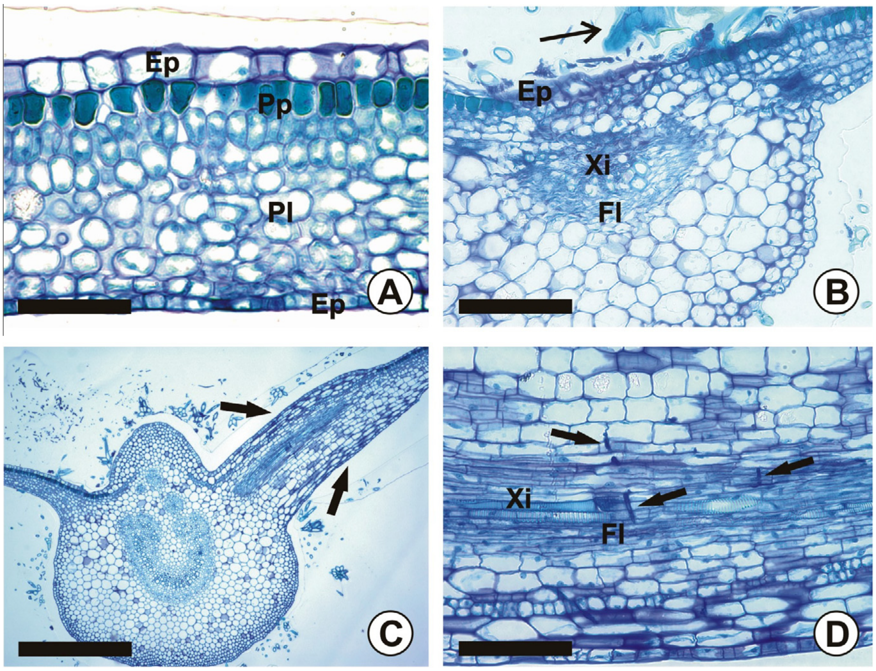

Fig. 2. Anatomical sections of *Miconia calvescens* leaves*:* (A) cross section of non-attacked young leaf (Ep = epidermis; Fl = phloem, Pp = palisade parenchyma; Pl = spongy parenchyma Xi = xylem); (B) details of the midrib of the young leaf attacked by *Diclidophlebia smithi* (note remains of nymphs on the leaf surface – arrowed); (C) midvein and lamina of an attacked leaf showing swelling of mesophyll (note areas with cell distortions - arrowed); (D) detail of the 3C (Fl = phloem, Xi = xylem), note stylets of *D. smithi* inserted in the vascular system. Bars: (A) = 50 µm, (B and D) = 100 µm, (C) = 500 µm.

populations of *D. smithi* can be adversely affected by high air temperatures and by heavy and frequent rainfall, a rapid recovery of populations occurs after such unfavorable periods and colonies of the psyllid were observed in the field at the study sites throughout the year.

Knowledge about the effects of climatic factors on potential biological control agents is useful for estimation of their chances of success and to determine the correct time for introductions in the field. In the case of *D. smithi*, our results, here and elsewhere (Morais et al., 2010), indicate that introduction should be timed during periods of low rainfall and cool temperatures. Releases should also be timed to allow for overlap of these climatic conditions with the ideal phenology of *M. calvescens* (peak flowering and fruiting) to maximize the chances for populations establishment and growth.

Based on observation of *D. smithi* over multiple field seasons and at three locations in Brazil no parasitoids or pathogens were observed attacking this species. This was a somewhat surprising result because many psyllid species are known to be often severely parasitized by Encyrtidae and Eulophidae (Hymenoptera) (Dietrich, 2009) which if present could limit the efficacy of *D. smithi* as a weed biocontrol agent. Predation by generalist predators was observed during the population peaks of *D. smithi*, but it appears to have a limited potential to affect populations of *D. smithi* at the study sites because predator counts were generally low.

During the sampling, the leaf buds and young leaves of *M. calvescens* with high densities we observed of *D. smithi* became distorted, chlorotic and stunted when compared with neighboring plants with no insects. These symptoms were also observed on plants belonging to the Hawaiian biotype of *M. calvescens* during the host specificity tests and on sentinel plants naturally colonized by *D. smithi* at Guaraciaba or in samples held in the laboratory. The attack by *D. smithi* on ‘‘sentinels plants’’ demonstrated the susceptibility of the Hawaiian biotype of *M. calvescens*, despite the clear morphological differences (primarily the color of the abaxial surface of leaves, leaf size and leaf texture) between plants occurring in Hawaii and native populations in Brazil. While these morphological differences between Brazilian and Hawaiian miconia are not regarded by botanists as having major taxonomic significance they might have an ecological significance for the psyllid.

The results of both direct and indirect host-range evaluations showed that *D. smithi* is host specificity on *M. calvescens*. No adult or nymph of this psyllid was ever found attacking any other plant species in the vicinity of colonized *M. calvescens* individuals. The host specificity tests involving the nine Melastomataceae species also demonstrated that *D. smithi* is highly specialized on *M. calvescens*. Potential non-target effects are less likely to be an issue because there are no native Melastomataceae or economically relevant crops belonging to this family in Hawaii.

The observations of anatomical changes on leaf tissues resulting from the attack of *D. smithi* indicate that there is direct damage to tissues resulting from psyllid feeding. Direct damage has also been observed to leaf tissues of *Eucalyptus camaldulensis* Dehnh by the psyllid *Cardiaspina rotator* (Crawford and Wilkens, 1996). In contrast for the leaves of *Citrus sinensis* attacked by *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), there was no visible damage from the stylet feeding (Bonani et al., 2010). In addition to these direct impacts of feeding, the removal of sap from an attacked plant also leads to a reduction of plant reserves that likely contributes towards a reduction on plant growth, flower and fruit setting. Psyllids may inject toxic salivary secretions into plants causing systemic effects and premature leaf senescence (Crawford and Wilkens, 1996). During the experiments in the field and in the laboratory, chlorosis and premature leaf abscission resulting from attack by *D. smithi* were observed (Morais, personal observation), suggesting that feeding toxins may also be present for this species.

Although efficacy of biocontrol agents is difficult to predict before releases are made, results of this work and previous studies on *D. smithi* (Burckhardt et al., 2006; Morais et al., 2010) confirm that this species has significant potential for biological control of *M. calvescens*. Characteristics that suggest *D. smithi* could be an effective biological control agent include: (1) its high level of host specificity on *M. calvescens*; (2) ease of rearing and short life cycle that make mass-production feasible; (3) membership in a taxon (Hemiptera:

Psyllidae) that has yielded several effective and safe weed biocontrol agents; (4) its broad climatic tolerance and compatibility with areas of intended release in Hawaii; (5) the apparent absence of specialized parasitoid or pathogens in its native range; and (6) the damage it causes on *M. calvescens*, including plants of ''Hawaiian biotype.''

Pre-release impact of biocontrol agents in the field is difficult to predict however results of this work, combined with previous studies on *D. smithi* (Burckhardt et al., 2006; Morais et al., 2010) confirm that this species has significant potential as a classical biocontrol agent for *M. calvescens*.

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References

Alves, J.L., Barreto, R.W., Pereira, O.L., Soares, D.J., 2010. Additions to the mycobiota of the invasive weed *Miconia calvescens* (Melastomataceae). Mycologia 102, 69–82.

Baker, R.H.A., 2002. Predicting the Limits to the potential distribution of alien crop pests. In: Hallman, G.J., Schwalbe, C.P. (Eds.), Invasive Arthropods in Agriculture Problems and Solutions. Science Publishers, Enfield, pp. 207–241.

Barreto, R.W, Ellison, Carol, A., Seier, M.K., Evans, H., 2012. Biological control of weeds with plant pathogens: four decades on. In: Abrol, Dharam P., Uma Shankar (Eds.), Integrated Pest Management Principles and Practice. CABI, Wallingford, pp. 299–350.

Bonani, J.P., Fereres, A., Garzo, E., Miranda, M.P., Appezzato-da-Gloria, B., Lopes, J.R.S., 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. Entomol. Exp. Appl. 134, 35–49.

Brooks, S., Jeffery, M., 2010. Status of *Miconia calvescens* and the eradication program in Australia. In: Loope, L.L., Meyer, J.-Y., Hardesty, B.D., Smith, C.W. (Eds.), Proceedings of the International Miconia Conference, Keanae, Maui, Hawaii, 2009, Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. (http://www.issg.org/pdf/publications/Island_invasives/IslandInvasives.pdf).

Burckhardt, D., Hanson, P., Madrigal, L., 2005. *Diclidophlebia lucens*, n. sp. (Hemiptera: Psyllidae) from Costa Rica, a potential control agent of *Miconia calvescens* (Melastomataceae) in Hawaii. Proc. Entomol. Soc. Wash. 107, 741–749.

Burckhardt, D., Morais, E.G.F., Picanço, M.C., 2006. *Diclidophlebia smithi* sp. n., a new species of jumping plant-lice (Hemiptera, Psylloidea) from Brazil associated with *Miconia calvescens* (Melastomataceae). Mitt. Schweiz. Entomol. Ges. 79, 241–250.

Center, T.D., Pratt, P.D., Tipping, P.W., Rayamajhi, M.B., Van, T.K., Wineriter, S.A., Dray, F.A., 2007. Initial impacts and field validation of host range for *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae), a biological control agent of the invasive tree *Melaleuca quinquenervia* (Cav.) Blake (Myrtales: Myrtaceae: Leptospermoideae). Environ. Entomol. 36, 569–576.

Center, T.D., Pratt, P.D., Tipping, P.W., Rayamajhi, M.B., Van, T.K., Wineriter, S.A., Dray, F.A., Purcell, M., 2006. Field colonization, population growth, and dispersal of *Boreioglycaspis melaleucae* Moore, a biological control agent of the invasive tree *Melaleuca quinquenervia* (Cav.) Blake. Biol. Control. 39, 363–374.

Crawford, S.A., Wilkens, S., 1996. Ultrastructural aspects of damage to leaves of *Eucalyptus camaldulensis* by the Psyllid *Cardiaspina rotator*. Micron 27, 359–366.

Csurhes, S.M., 1997. Miconia calvescens, a potentially invasive plant in Australia's tropical and sub-tropical rain forests. First Regional Conference on Miconia Control, Papeete, pp. 72–77.

Dietrich, C.H., 2009. Auchenorrhyncha. In: Resh, V.H., Cardé, R.T. (Eds.), Encyclopedia of Insects, 2nd ed. Academic Press, San Diego, pp. 56–63.

Goarant, A.C., Meyer, J.Y., 2010. Attempting the eradication of *Miconia calvescens* in a comprehensive strategy to control invasive species in New Caledonia. In: Loope, L.L., Meyer, J.-Y., Hardesty, B.D., Smith, C.W. (Eds.), Proceedings of the International Miconia Conference, Keanae, Maui, Hawaii, 2009, Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. (http://www.issg.org/pdf/publications/Island_invasives/IslandInvasives.pdf).

Hanson, P., Nishida, K., Allen, P., Chacón, E., Reichert, B., Castillo, A., Alfaro, M., Madrigal, L., Rojas, E., Badenes-Perez, F. and Johnson, T., 2010. Insects that feed on *Miconia calvescens* in Costa Rica. In: Loope, L.L., Meyer, J.-Y., Hardesty, B.D., Smith, C.W. (Eds.), Proceedings of the International Miconia Conference, Keanae, Maui, Hawaii, 2009, Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. (http://www.issg.org/pdf/publications/Island_invasives/IslandInvasives.pdf).

Hodkinson, Ian.D., 2009. Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis'. J. Nat. Hist. 43 (1), 65–179.

Hollis, D.A., 2004. Australian Psylloidea: Jumping Plant Lice and Lerp Insects. Canberra Australian Biological Resources Study, Canberra.

Johansen, D.A., 1940. Plant Micro Technique. McGraw-Hill, New York.

Killgore, E.M., Sugiyama, L.S., Barreto R., 1997. Prospective biological control of *Miconia calvescpens* in Hawaii with a non-indigenous fungus *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *miconiae*. In: Proceedings of the First Regional Conference on Miconia, Control, pp. 26–29.

Kriticos, D.J., 2011. Regional climate-matching to estimate current and future sources of biosecurity threats. Biol. Invas. 14, 1533–1544.

Liu, Y.H., Tsai, J.H., 2000. Effects of temperature on biology and life table parameters of the Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Ann. Appl. Biol. 137, 201–206.

Medeiros, A.C., Loope, L.L., Conant, P., Mcelvaney, S., 1997. Status, ecology, and management of the invasive plant, *Miconia calvescens* DC (Melastomataceae) in the Hawaiian Islands. Bishop Mus. Occas. Pap. 48, 23–36.

Mehrnejad, M.R., Copland, M.J.W., 2005. The seasonal forms and reproductive potential of the common Pistachio Psylla, *Agonoscena pistaciae* (Hem., Psylloidea). J. Appl. Entomol. 129, 342–346.

Meyer, J.Y., 1996. Status of *Miconia calvescens* (Melastomataceae), a dominant invasive tree in the society Islands (French Polynesia). Pac. Sci. 50, 66–76.

Meyer, J.-Y., Loope, L.L., Goarant, A.-C., 2011. Strategy to control the invasive alien tree *Miconia calvescens* in Pacific islands: eradication, containment or something else? In: Veitch, C.R., Clout, M.N., Towns, D.R. (Eds.), Island Invasives: Eradication and Management. UICN, Gland, pp. 91–96 (http://www.issg.org/pdf/publications/Island_invasives/IslandInvasives.pdf).

Meyer, J.-Y., Fourdrigniez, M., Taputuarai, R., 2012. Restoring habitat for native and endemic plants through the introduction of a fungal pathogen to control the alien invasive tree *Miconia calvescens* in the island of Tahiti. BioControl 57, 191–198.

Meyer, J.Y., Taputuarai, R., Killgore, E., 2008. Dissemination and impacts of the fungal pathogen *Colletotrichum gloeosporioides* f.sp. *miconiae* (Deuteromycetinae) on the invasive alien tree *Miconia calvescens* (Melastomataceae) in the rainforests of Tahiti (French Polynesia, South Pacific). In: Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L., Rector, B.G. (Eds.) Proceedings of the XII International Symposium on Biological Control of Weeds, CAB International, Wallingford.

Morais, E.G.F., Picanco, M.C., Barreto, R.W., Silva, N.R., Campos, M.R., 2010. Biological performance of *Diclidophlebia smithi* (Hemiptera: Psyllidae), a potential biocontrol agent for the invasive weed *Miconia calvescens*. Biocontrol Sci. Techn. 20, 107–116.

Nava, D.E., Torres, M.L.G., Rodrigues, M.D.L., Bento, J.M.S., Parra, J.R.P., 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. J. Appl. Entomol. 131, 709–715.

O'Brien, T.P., McCully, M.E., 1981. The study of plant structure: principles and methods. Thermacarphi Pty Ltd., Melbourne.

Picanço, M.C., Barreto, R.W., Fidelis, E.G., Semeão, A.A., Rosado, J.F., Moreno, S.C., Barros, E.C., Silva, G.A., Johnson, T., 2005. Biological control of *Miconia calvescens* by Phytophagous Arthropods. Pacific Cooperative Studies Unit, University of Hawaii. Tech. Rep. 134, 30.

Santin, A.M. 2008. Controle biológico da planta invasora *Miconia calvescens* por uma nova espécie de *Ditylenchus*. MSc. dissertation presented to the Departamento de Fitopatologia of the Universidade Federal de Viçosa (Brazil), UFV, Viçosa.

Seixas, C.D.S., Barreto, R.W., Freitas, L.G., Maffia, L.A., Monteiro, F.T., 2004. *Ditylenchus drepanocercus* (Nematoda), a potential biological control agent for *Miconia calvescens* (Melastomataceae): host-specificity and epidemiology. Biol. Control. 31, 29–37.

Seixas, C.D.S., Barreto, R.W., Matsuoka, K., 2002. First report of a phytoplasm-associated witches' broom disease of *Miconia calvescens*. Plant Pathol. 6, 801.

Seixas, C.D.S., Barreto, R.W., Killgore, E., 2007. Fungal pathogens of *Miconia calvescens* (Melastomataceae) from Brazil, with reference to classical biological control. Mycologia 99, 99–111.

Shaw, R.H., Bryner, S., Tanner, R., 2009. The life history and host range of the Japanese Knotweed Psyllid, *Aphalara itadori* Shinji: potentially the first classical biological weed control agent for the European Union. Biol. Control. 49, 105–113.

Sutherst, R.W., Maywald, G.F., Kriticos, D.J., 2007. CLIMEX Version 3: User's Guide. Available from: http://www.hearne.com.au, Hearne Scientific Software Pty Ltd.

Stratopoulou, E.T., Kapatos, E.T., 1995. The Dynamics of the adult population of Pear Psylla, *Cacopsylla pyri* L. (Hom., Psyllidae) in the region of Magnesia (Greece). J. Appl. Entomol. 119, 97–101.