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Toxic Peptides in Populations of Two Pergid Sawflies, Potential Biocontrol Agents of Brazilian Peppertree

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Abstract

Determination of the safety of agents prior to release is one of the most important research goals in biological control. In addition to concerns for the safety of non-target plants, determination of the potential toxic properties of new agents needs to be assessed. Numerous phytophagous insects are defended by chemicals against the attack of natural enemies. Some of these defensive compounds could pose an environmental risk if an agent is released. Here, larval populations of two pergid sawflies, *Heteroperreyia hubrichi* and *H. jorgenseni*, were analyzed by LC-MS/MS to investigate whether they contain alleged toxic peptides. The first species is a potential candidate for biological control of the invasive weed Brazilian peppertree in Florida and Hawaii. The chemical analyses revealed the presence of the peptides pergidin (Perg), 4-valinepergidin (VPerg), dephosphorylated pergidin (dpPerg), lophyrotomin (LGln and LGlu). The effect of sawfly population for each species was significantly influencing peptide concentration. All peptides occurred at lower concentrations compared with purportedly toxic species of this sawfly family. However, the concentrations of the peptides are of concern for the welfare of wildlife and livestock that would be exposed to these species. These results demonstrate that release of this biological control agent in the invaded range may pose an environmental threat.

Keywords Sawfly larvae · *Heteroperreyia* · Biological control agent · Florida Everglades · LC-MS/MS · Pergidin

Introduction

The Brazilian peppertree, *Schinus terebinthifolia* Raddi (Sapindales: Anacardiaceae) is a South American woody plant that invades many subtropical regions, including Florida, Texas and Hawaii (USA) (Schmitz et al. 1997; Yoshioka and Markin 1991). Brazilian peppertree is one of the most aggressive invasive weeds of agricultural and environmental habitats (Ewel 1986; Rodgers et al. 2014). In Florida this weed has invaded much of the peninsula, covering more than 280,000 ha with monospecific stands that eliminate understory-plant growth (Schmitz et al. 1997). Thus, Brazilian peppertree decreases biodiversity in coastal and upland habitats (Gann et al. 2018; Mytinger and Williamson 1987).

Biological control research against Brazilian peppertree has been conducted since the 1950s (Wheeler et al. 2016). The sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae) was considered a safe and effective biocontrol candidate (Hight et al. 2003; Medal et al. 1999) (Fig. 1a). It is a narrow specialist according to host specificity tests of plant species from Florida and Hawaii (Hight et al. 2003; Medal et al. 1999). Moreover, it is very damaging as it can completely defoliate Brazilian peppertree trees in its native range (Cuda et al. 2005; Medal et al. 1999). However, other members of the Pergidae are known to contain peptides that are toxic to livestock in Australia and South America (Dutra et al. 1997; Oelrichs et al. 2001). The presence and concentrations of these peptides have not been documented in *H. hubrichi* or its

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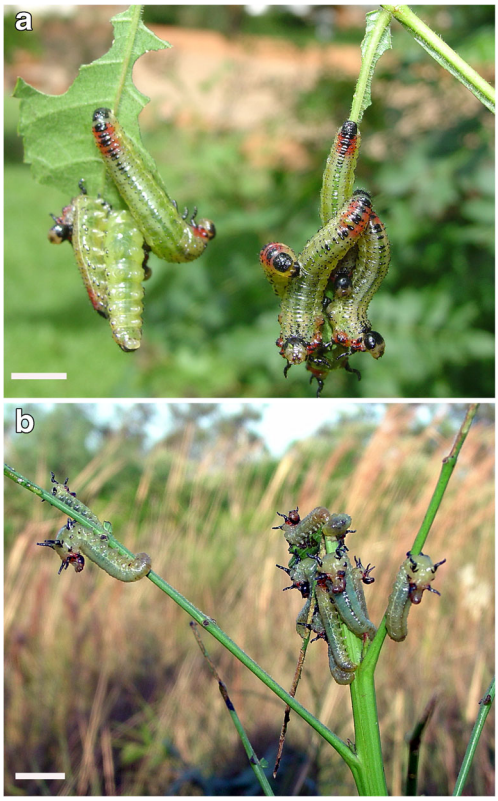

Fig. 1 Larvae of two sawfly species, *Heteroperreyia hubrichi* (**a**) and *H. jorgenseni* (**b**), feeding on *Schinus* spp. in northeastern Argentina. White scale bars = 1 cm. Pictures by Fernando Mc Kay

congeneric, *H. jorgenseni* (Jörgensen) (Fig. 1b), known to feed on other *Schinus* species in its native range (Mc Kay et al. 2009). Understandably, there is concern that field releases of these Pergidae sawflies for biological control could pose a toxicological hazard to native and domesticated fauna in the invaded range (Cuda et al. 2004; Oelrichs et al. 2001).

There are eight described species of the Pergidae genus *Heteroperreyia* and all are from the Neotropics (Schmidt and Smith 2018). Larvae of several species of Pergidae and its sister sawfly family, the Argidae, contain three heptapeptides, pergidin (Perg, MW = 864), 4-valinepergidin (VPerg, MW = 850) and dephosphorylated pergidin (dpPerg, MW = 784), as well as two closely related octapeptides both called lophyrotomin (LGln, MW = 1039, and LGlu, MW = 1040) (Boevé et al. 2014). Following their identification and quantification in the Pergidae species *Lophyrotoma interrupta* (Klug), *Lophyrotoma zonalis* (Rohwer) and *Perreyia flavipes* Konow (Oelrichs et al. 1999), updated extraction and analytical procedures allowed (re)assessing peptide concentrations in larvae of these, and other species including *Lophyrotoma analis* (Costa), *Pterygophorus insignis* Kirby, *Pterygophorus* nr *turneri* Rohwer and *Philomastix macleaii* (Westwood) (Boevé et al. 2014). These Pergidae larvae generally contain Perg and VPerg as major peptide components. The species-specific concentrations of each peptide typically range from 0.1 to 0.4% larval fresh weight (FW), although VPerg reaches ca. 0.6% FW in *P.* nr *turneri*.

Here, the primary goal was to determine if and at what concentrations these peptides occur in field-collected larvae of the potential biological control agent *H. hubrichi* and the related species *H. jorgenseni*. We used the extraction methodology that allows the analysis of a single larva, thus enabling screening of individuals from several populations in order to detect possible qualitative and quantitative variations in the corresponding chemical profiles. This allowed detailed examination of the variation among both individuals and populations.

Materials and Methods

Insects Larvae of the two sawfly species, *H. hubrichi* and *H. jorgenseni,* were collected from localities in Corrientes and Misiones provinces of northeastern Argentina (Fig. 2). The sawfly species were identified following Smith (1990).

All specimens of *H. hubrichi* were collected on *S. terebinthifolia*. Most *H. jorgenseni* larvae were collected on *S. molle* L. except those that were collected on *Schinus longifolia* (Lindl.) Speg. (one larva from Apóstoles), *Schinus lentiscifolia* Marchand (three from Apóstoles), *S. lentiscifolia* (one from Santa Ana), and *Schinus weinmannifolia* Engl. (two larvae and one prepupa from Santa Ana). As these four individuals from Santa Ana were collected on *S. lentiscifolia* and *S. weinmannifolia,* they were considered a different population compared to the eight larvae from the same locality collected on *S. molle*.

Extraction of Larvae Freshly collected larvae, generally eight per population, were weighed individually at 0.1 mg precision and placed singly in microtubes containing 1 ml ethanol (100%). All samples were stored at −25 or − 80 °C until use. The ethanol was transferred to a 12 ml tube. The larva was thoroughly crushed in 1 ml 50% ethanol. The sample was then vortexed, sonicated for 15 min at 50 ± 5 °C, and centrifuged 5 min at 8000 rpm. This process was repeated (with 5 min sonication) and the supernatants of each extraction were combined by adding them to the 12 ml tube. The number of extractions depended on the FW of each larva: 2 extractions for larvae <75 mg, 3 extractions for larvae between 76 and 150 mg, 4 extractions for larvae between 151 and 300 mg, and 5 extractions for larvae >300 mg. Ethanol (50%) was then added to these pooled extracts to obtain 3, 4, 5, and 6 ml of solution, respectively. The pooled extracts were stored at −80 °C until further dilution. Three aliquots from each pooled extract were prepared by a dilution 1:20, 1:30, 1:40, or 1:50, again depending on the larval weight category.

Fig. 2 Localities in northeastern Argentina where larvae of *Heteroperreyia hubrichi* and *H. jorgenseni* were collected for peptide analysis. Collections included 4–8 larvae per population (see Table 3)

LC-MS Analyses Larval extracts were analyzed with a LC system (ThermoFisher, San Jose, California, USA) equipped with an Accela 1250 pump and Accela autosampler. Separation of peptides was performed on a C18-HL Alltima column (150 × 2.0 mm i.d., 3 μm; Grace, Deerfield, Illinois, USA) using a linear gradient from 90% H_2O (with 1% CH_3CN and 0.1% HCOOH)/10% CH_3CN to 10% H_2O in 31 min; the flow rate was 0.2 ml min^{-1}. The column was maintained at 30 °C and the autosampler at 7 °C. Mass spectra were acquired with a Q-Exactive mass spectrometer (ThermoFisher) equipped with an electrospray ionization (ESI) source in the positive mode. ESI inlet conditions were: spray voltage 4 kV, capillary temperature 320 °C, sheath gas 35 PSI, and auxiliary gas 5 PSI. MS^2 spectra were taken at relative collision energy of 25%.

The elution peaks of four peptides, dpPerg, VPerg, LGln, and LGlu overlapped. Since integrating more than three peaks (per millisecond) is not possible, we discarded LGlu in the quantifications. Previous analysis of Pergidae species indicated this compound is either not detected, or detected in trace amounts (Boevé et al. 2014). Nevertheless, a few selected samples were analyzed a second time but with MS^2 set at $m/z = 1041$ $(M+H]^+$, to specifically search for this peptide. It was not detected in *H. hubrichi* and in very minute amounts in *H. jorgenseni*.

Peptide Quantification Standards of the peptides Perg, VPerg, dpPerg, LGln and LGlu were purchased from Biosyntan GmbH (Berlin, Germany) at >95% purity. They were analyzed by full MS^2 on the $[M+H]^+$ ions. Calibration curves were constructed over four concentrations in the range of 1–1000 ng/ml, and proved to be linear, with r^2 values >0.990. Peptide concentrations in the samples were determined by comparing their ratios of peak areas to calibration curves. These concentrations (in ng/ml) were multiplied by one of the factors 60, 120, 200 or 300, depending on the dilutions (i.e., number of extractions leading to a pooled extract, and subsequent dilution). The final concentrations were averaged over the three replicates (aliquots) and expressed in μg/larva and % FW.

Statistical Analyses To determine if peptide concentrations were influenced by sawfly species and larval FWs, ANCOVA was performed using a mixed model with SAS (SAS Institute version 9.3, 2010). Larval FWs were used as a covariate and sawfly species were considered a fixed effect. To compare peptide concentrations for the two species, the species x larval FW interaction was examined for each peptide. To examine the influence of sawfly populations on peptide concentration, each species and peptide were analyzed separately with one-way ANOVAs and means were compared with a Tukey's HSD test ($P = 0.05$).

Results

The four peptides Perg, VPerg, dpPerg and LGln were detected in each specimen of both *Heteroperreyia* species. In both species, Perg was the major peptide component in all populations (Fig. 3). In *H. hubrichi,* the total peptide content was made up, in mean ± SE, by 78.7 ± 1.6% Perg, 17.1 ± 1.1% LGln, 2.8 ± 0.4% VPerg, and 1.9 ± 0.3% dpPerg. For *H. jorgenseni* it was made up by 84.0 ± 1.9% Perg, 7.5 ±

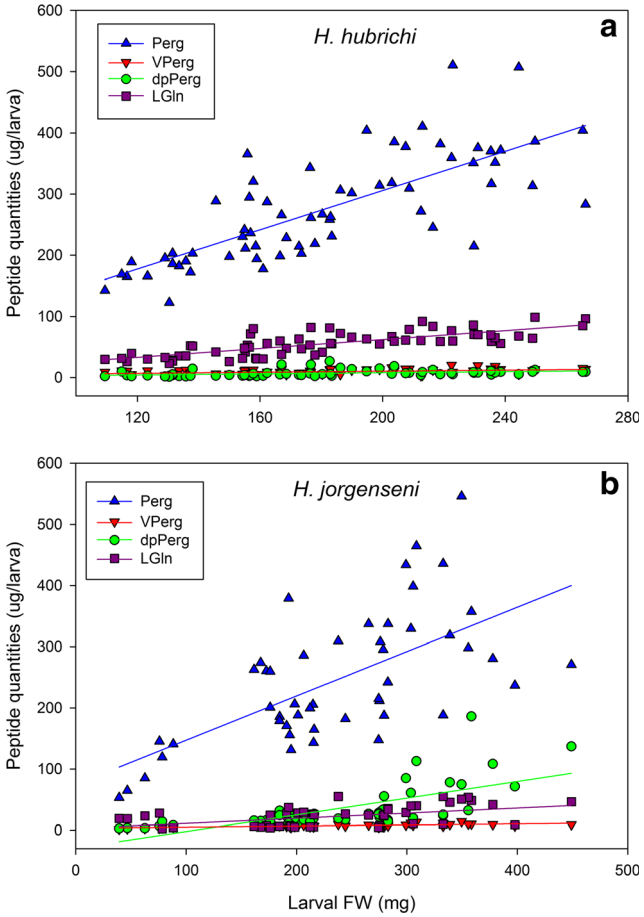

Fig. 3 Quantities of four peptides in larvae of the sawfly species *Heteroperreyia hubrichi* (**a**) and *H. jorgenseni* (**b**) as a function of larval FW. Peptides in both species include Perg (= pergidin), VPerg (= 4-valinepergidin), dpPerg (= dephosphorylated pergidin), and LGln (= lophyrotomin)

1.9% dpPerg, 7.2 ± 1.8% LGln, and 2.3 ± 0.2% VPerg. The summed amount of the four peptides in each *Heteroperreyia* species was not significantly different ($F_{1,110} = 0.1$; $P > 0.9$) and represented, in mean ± SE, 331 ± 12 μg/larva for *H. hubrichi* and 334 ± 22 μg/larva for *H. jorgenseni*. The concentration of all peptides for both sawfly species increased significantly with increased larval FW (Tables 1 and 2). Moreover, the slope of this increase was significantly greater for *H. hubrichi* for the peptides Perg, VPerg, and LGln compared with *H. jorgenseni* larvae (Table 1; Fig. 3). Only the peptide dpPerg increased more with larval FW in *H. jorgenseni* compared with *H. hubrichi*.

The effect of species on peptide concentrations was only significant for Perg and dpPerg (Table 2). Larval FW had a significant influence on all peptides for both species (Table 2). The significant species x FW interaction indicated that the effect of larval weight influenced the quantity of each peptide differently for each species (Fig. 3).

The populations from which the sawflies were collected significantly influenced concentrations of all four peptides

Table 1 Test statistics, parameter estimates (β) and their standard errors for the regression coefficients predicting four peptide concentrations in the larvae of the sawfly species *Heteroperreyia hubrichi* and *H. jorgenseni* at different larval fresh weights

Peptide	Species	df	*F*	*P*	β	SE	r^2
Perg	*H. hubrichi*	1,62	70.55	<0.001	1.4724	0.1753	0.53
	H. jorgenseni	1,46	27.84	<0.001	0.8222	0.1558	0.36
VPerg	*H. hubrichi*	1,62	12.85	<0.001	0.0389	0.0109	0.16
	H. jorgenseni	1,46	11.04	0.0028	0.0163	0.0049	0.18
dpPerg	*H. hubrichi*	1,62	7.24	0.0091	0.0399	0.0148	0.09
	H. jorgenseni	1,46	34.92	<0.001	0.2652	0.0449	0.43
LGln	*H. hubrichi*	1,62	89.90	<0.001	0.3677	0.0388	0.59
	H. jorgenseni	1,46	9.07	0.0042	0.0458	0.0152	0.15

Peptides in both species include Perg (= pergidin), VPerg (= 4-valinepergidin), dpPerg (= dephosphorylated pergidin), and LGln (= lophyrotomin)

(Table 2; Fig. 4). For *H. hubrichi*, the larvae from Apóstoles had the highest Perg levels (Table 3). The San Pedro and Garuhapé-mi populations had the greatest VPerg levels (Table 3). The highest LGln levels were found in the Oberá and General Roca populations. Only the dpPerg levels of *H. hubrichi* were not influenced by the population effect. For *H. jorgenseni*, the highest Perg and VPerg levels were found in larvae from Apóstoles, Santa Ana (#4), and San Jose. The highest dpPerg levels were found in larvae from the Santo Tomé, San Jose, and Santa Ana populations. Finally, the highest LGln levels were found in larvae from the Apóstoles population. The *H. jorgenseni* larvae collected from different host species, *S. lentiscifolia* and *S. weinmannifolia* at the Santa Ana site, had higher levels of only LGln compared with the larvae collected from the normal host, *S. molle* (Table 3). Populations of the two sawfly species only co-occurred at Apóstoles. The summed concentrations of all four peptides were greatest for both *H. jorgenseni* and *H. hubrichi* larvae from the Apóstoles populations. The summed concentrations for larvae collected from each population ranged from 0.10 to 0.22% of the larval FW (Table 3).

Discussion

Analysis of the sawfly species *H. hubrichi* and *H. jorgenseni* found similar chemical profiles with both having Perg as the major peptide. Compared with other Pergidae genera, the peptide profile of the two *Heteroperreyia* species resembled that of *L. analis* as these three species have Perg as the major constituent (Boevé et al. 2014). The amount of the Perg in each *Heteroperreyia* species ranged from ca. 0.1 to 0.2% of larval FW. The next major *Heteroperreyia* peptide, LGln, ranged from 0.01 to 0.04% FW. For comparison, species of the genera *Lophyrotoma* and *Pterygophorus*

Table 2 Results of statistical analyses to determine the effect of sawfly species, larval weights, and populations on peptide concentrations in the sawfly species *Heteroperreyia hubrichi* and *H. jorgenseni*

Peptide	Source	df	*F*	*P*
Perg	Species	1,95	6.87	0.0102
	Fresh weight (FW)	1,95	46.17	<0.001
	Species * FW	1,95	11.26	<0.001
	Population	14,95	4.19	<0.001
VPerg	Species	1,95	1.01	>0.3
	Fresh weight (FW)	1,95	16.47	<0.001
	Species * FW	1,95	4.97	0.0281
	Population	14,95	13.02	<0.001
dpPerg	Species	1,95	4.30	0.0409
	Fresh weight (FW)	1,95	13.37	<0.001
	Species * FW	1,95	9.88	0.002
	Population	14,95	3.19	<0.001
LGln	Species	1,95	0.07	>0.7
	Fresh weight (FW)	1,95	59.96	<0.001
	Species * FW	1,95	36.81	<0.001
	Population	14,95	70.47	<0.001

The effect of species and larval weight were determined by ANCOVA, whereas the effect of population was determined by ANOVA. Peptides in both species include Perg (= pergidin), VPerg (= 4-valinepergidin), dpPerg (= dephosphorylated pergidin), and LGln (= lophyrotomin)

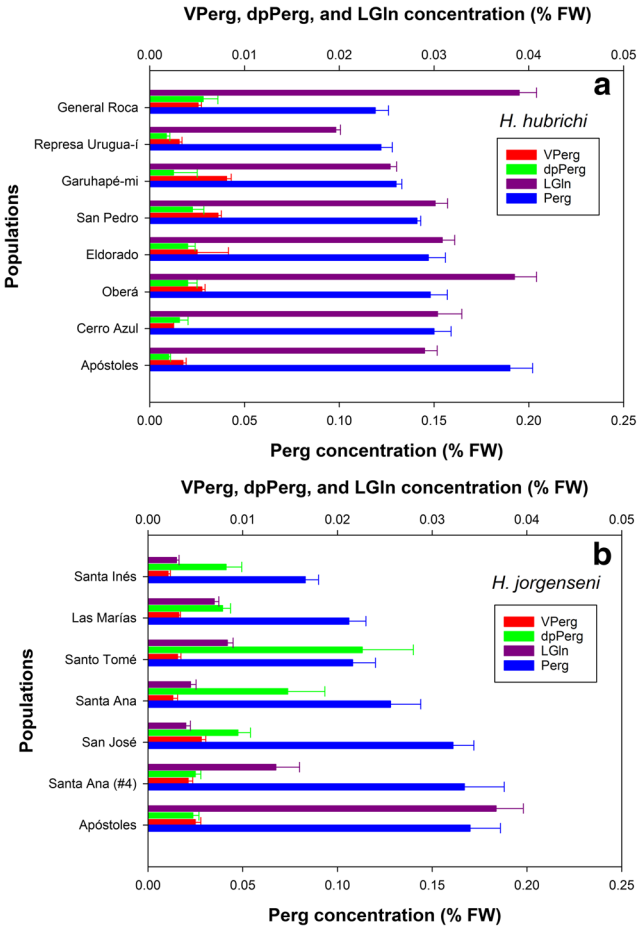

Fig. 4 Concentrations of four peptides in larvae of the sawfly species *Heteroperreyia hubrichi* (**a**) and *H. jorgenseni* (**b**). Peptides in both species include Perg (= pergidin), VPerg (= 4-valinepergidin), dpPerg (= dephosphorylated pergidin), and LGln (= lophyrotomin). Values are shown as means and SE (*error bars*) of % larval FW

contain primarily VPerg, but Perg is also a major component (Boevé et al. 2014). At their highest levels, both peptides range from ca. 0.2% FW in *Lophyrotoma* to 0.7% FW in *Pterygophorus* species (Boevé et al. 2014). Thus, the two *Heteroperreyia* spp. analyzed here have peptide levels that are ca. 20% that of reportedly toxic species. However, the peptide profiles are different for *Heteroperreyia* vs. *Lophyrotoma*/*Pterygophorus* and little is known about the relative toxicity of each peptide.

The populations from which sawfly larvae were collected influenced peptide levels for both species. The summed amounts of the four peptides were greatest for the Apóstoles populations, where both species co-occurred. This was largely due to the consistently higher concentration of Perg for both species from this population. However, several other populations had similar levels of this peptide. The remaining individual peptides were variable for larvae from the different populations and no clear patterns were discernable.

The poisoning of cattle and sheep by ingestion of the larvae of an Australian sawfly has repeatedly been reported as a serious problem (Oelrichs et al. 1999). This poisoning during episodic outbreaks of the sawfly *L. interrupta* was associated with livestock grazing in areas where larvae of this sawfly had fed on leaves of *Eucalyptus melanophloia* F. Muell. (Oelrichs et al. 1999). Additional examples of livestock poisoning in sheep and goats in Denmark were from the ingestion of *Arge pullata* (Zaddach) (Hymenoptera: Argidae) larvae that had fed on *Betula* spp. tree leaves (Thamsborg et al. 1987). Furthermore, sheep, cattle and pigs were poisoned in Uruguay and Brazil from ingestion of *P. flavipes* larvae that had fed on leaves of grasses and shrubs (Dutra et al. 1997; Raymundo et al. 2008). Despite the occurrence of *Heteroperreyia* species in southern Brazil and northern Argentina, they have not been implicated in livestock poisoning. This is not for a lack of opportunity as it is conceivable that grass-feeding livestock could be exposed to the larvae when they travel from Brazilian peppertree foliage to pupate in the soil (Hight et al. 2003).

In another biological control project, a potentially toxic sawfly was released, apparently without negative environmental effects. The Argidae sawfly species *Cibdela janthina* Klug is native to Sumatra, was released in Reunion Island to control the invasive giant bramble, *Rubus alceifolius* Poiret (Rosaceae), and efficiently controlled the weed within several years (Le Bourgeois et al. 2011; Mathieu et al. 2014). Although this sawfly species contains substantial quantities of Perg and LGln (Boevé et al. 2014), to the best of our

Table 3 Larval fresh weights and peptide concentrations of two species of sawflies, *Heteroperreyia hubrichi* and *H. jorgenseni*

		Fresh weight		Perg			VPerg			dpPerg			LGln			Sum		
Species/Population	N	mg	SE	Mean	SE	1	Mean	SE	1	Mean	SE	1	Mean	SE	1	Mean	SE	1
H. hubrichi																		
Apóstoles	8	169.1	15.7	0.190	0.012	a	0.004	0.000	c	0.002	0.000	a	0.029	0.001	b	0.22	0.01	a
Oberá	8	160.6	6.3	0.148	0.009	b	0.006	0.000	b	0.004	0.001	a	0.039	0.002	a	0.20	0.01	ab
Eldorado	8	219.8	10.7	0.147	0.009	b	0.005	0.003	b	0.004	0.001	a	0.031	0.001	b	0.19	0.01	abc
Cerro Azul	8	199.5	8.3	0.150	0.009	b	0.003	0.000	c	0.003	0.001	a	0.030	0.003	b	0.18	0.01	abc
San Pedro	8	199.7	10.6	0.141	0.002	b	0.007	0.000	a	0.005	0.001	a	0.030	0.001	b	0.18	0.01	bc
Garuhapé-mi	8	140.9	14.9	0.130	0.003	b	0.008	0.001	a	0.003	0.003	a	0.025	0.001	c	0.17	0.01	bc
General Roca	8	212.9	12.2	0.119	0.007	b	0.005	0.000	b	0.006	0.002	a	0.039	0.002	a	0.17	0.01	bc
Represa Urugua-í	8	147.0	4.8	0.122	0.006	b	0.003	0.000	c	0.002	0.000	a	0.020	0.001	c	0.15	0.01	c
H. jorgenseni																		
Apóstoles	4	56.1	8.2	0.170	0.016	a	0.005	0.001	a	0.005	0.001	b	0.037	0.003	a	0.22	0.01	a
Santa Ana (#4)[2]	4	261.6	30.1	0.167	0.021	a	0.004	0.001	ab	0.005	0.001	b	0.014	0.003	b	0.19	0.02	ab
San José	8	151.7	15.2	0.161	0.011	a	0.006	0.001	a	0.010	0.001	ab	0.004	0.001	d	0.18	0.01	abc
Santa Ana	8	308.0	14.9	0.128	0.016	ab	0.003	0.001	bc	0.015	0.004	ab	0.005	0.001	cd	0.15	0.02	bcd
Santo Tomé	8	352.0	17.7	0.108	0.012	b	0.003	0.000	bc	0.023	0.005	a	0.008	0.001	c	0.14	0.01	bcd
Las Marías	8	205.5	7.2	0.106	0.009	b	0.003	0.000	bc	0.008	0.001	b	0.007	0.001	cd	0.12	0.01	cd
Santa Inés	8	240.5	13.6	0.083	0.007	b	0.002	0.000	c	0.008	0.002	b	0.003	0.000	d	0.10	0.01	d

Populations were from Corrientes and Misiones provinces, Argentina. Peptides in both species include Perg (= pergidin), VPerg (= 4-valinepergidin), dpPerg (= dephosphorylated pergidin), and LGln (= lophyrotomin). Number of larvae analyzed (N). Values of peptide concentrations are given as means ± SE percentages of larval FW. ([1]) Means followed by the same letter within a species were not significantly different according to a Tukey's HSD (P = 0.05). ([2]) Santa Ana (#4) sawfly larvae were collected from *S. lentiscifolia* and *S. weinmannifolia*

knowledge there has been no report of toxic effects on fauna in the release range.

Our results show that, compared with other species of toxic Pergidae, both *Heteroperreyia* species contained significant, but lower peptide concentrations. Previous research showed no toxicity when calves were fed three doses, 900 (126 g), 450 (42 g), or 225 *H. hubrichi* (36 g) larvae (Dittrich et al. 2004). Although the weights of the calves were not provided by Dittrich et al. (2004), a similar study with *P. flavipes* larvae found that 40 g/kg were lethal to a 70 kg calf (Dutra et al. 1997). The major peptide in larvae of *P. flavipes,* Perg, was the same as that of *H. hubrichi* larvae (MacLeod et al. 2000). If Dittrich et al. (2004) used similar size calves, the *H. hubrichi* oral dose may have been too low to show a toxic response, ca. 0.3–1.8 g/kg. Moreover, our results showed significant peptide variability among *H. hubrichi* populations and larval FWs. To obtain an accurate prediction of toxicity of these *H. hubrichi* larvae studies need to be conducted with higher sawfly doses from known peptide profiles.

There are approximately 330 species of Argidae and 270 of Pergidae that occur in the Neotropics (Taeger et al. 2010), and from this Region we are only aware of the aforementioned toxicity reports associated with *P. flavipes*. However, considering the peptide concentrations reported here in *H. hubrichi* larvae, we recommend caution regarding plans to field release this potentially toxic biological control agent. Release of this potentially toxic sawfly poses unknown risks to the fauna of the invaded range. Before such a biological control release is considered quantifiable toxicology studies are needed with several vertebrate models.

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References

Boevé J-L, Rozenberg R, Shinohara A, Schmidt S (2014) Toxic peptides occur frequently in pergid and argid sawfly larvae. PLoS One 9(8): e105301. https://doi.org/10.1371/journal.pone.0105301

Cuda JP, Habeck DH, Hight SD, et al (2004) Brazilian Peppertree. In: Combs EM, Clark JK, Piper GL, Cofrancesco jr. AF (eds) Biological Control of Invasive Plants in the United States. Oregon State University Press, Corvallis, Oregon, pp 439–441

Cuda JP, Medal JC, Vitorino MD, Habeck DH (2005) Supplementary host specificity testing of the sawfly *Heteroperryia hubrichi*, a

candidate for classical biological control of Brazilian peppertree, *Schinus terebinthifolius*, in the USA. BioControl 50:195–201. https://doi.org/10.1007/s10526-004-0459-3

Dittrich RL, Macedo JHP, Cuda J, Biondo AW (2004) Brazilian peppertree sawfly larvae toxicity in bovines. Vet Clin Pathol 33:191. https://doi.org/10.1111/j.1939-165X.2001.tb00424.x

Dutra F, Riet-Correa F, Mendez MC, Paiva N (1997) Poisoning of cattle and sheep in Uruguay by sawfly (*Perreyia flavipes*) larvae. Vet Hum Toxicol 39:281–286

Ewel J (1986) Invasibility: lessons from South Florida. In: Mooney HA, Drake JA (eds) Ecology of biological invasions of North America and Hawaii, Mooney. H. Springer-Verlag, New York, pp 214–230

Gann GD, Bradley K, Woodmansee SW (2018) Floristic inventory of South Florida database online. In: Inst. Reg. Conserv. Delray Beach, Florida. http://regionalconservation.org/ircs/database/database.asp

Hight SD, Horiuchi I, Vitorino MD, Wikler C, Pedrosa-Macedo JH (2003) Biology, host specificity tests, and risk assessment of the sawfly *Heteroperreyia hubrichi*, a potential biological control agent of *Schinus terebinthifolius* in Hawaii. Biocontrol Sci Tech 48:461–476

Le Bourgeois T, Baret S, de Chenon RD (2011) Biological control of *Rubus alceifolius* (Rosaceae) in La Réunion Island (Indian Ocean): from investigations on the plant to the release of the biological control agent *Cibdela janthina* (Argidae). In: XIII International Symposium on Biological Control of Weeds - 2011. pp 153–160

MacLeod JK, Braybrook C, Simmonds PM, Oelrichs PB (2000) A unique toxic peptide from the larvae of the south American sawfly, *Perreyia flavipes*. Aust J Chem 53:293–297

Mathieu A, Dumont Y, Chiroleu F et al (2014) Predicting the altitudinal distribution of an introduced phytophagous insect against an invasive alien plant from laboratory controlled experiments: case of *Cibdela janthina* (Hymenoptera: Argidae) and *Rubus alceifolius* (Rosaceae) in La Réunion. BioControl 59:461–471. https://doi.org/10.1007/s10526-014-9574-y

Mc Kay F, Oleiro M, Cabrera Walsh G et al (2009) Natural enemies of Brazilian peppertree (Sapindales: Anacardiaceae) from Argentina: their possible use for biological control in the USA. Fla Entomol 92:292–303. https://doi.org/10.1653/024.092.0213

Medal JC, Vitorino MD, Habeck DH et al (1999) Host specificity of *Heteroperreyia hubrichi* malaise (Hymenoptera: Pergidae), a potential biological control agent of Brazilian peppertree (*Schinus terebinthifolius* Raddi). Biol Control 14:60–65. https://doi.org/10.1006/bcon.1998.0670

Mytinger L, Williamson GB (1987) The invasion of *Schinus* into saline communities of Everglades National Park. Florida Scientist 50:7–12

Oelrichs PB, MacLeod JK, Seawright AA et al (1999) Unique toxic peptides isolated from sawfly larvae in three continents. Toxicon 37:537–544

Oelrichs PB, MacLeod JK, Seawright AA, Grace PB (2001) Isolation and identification of the toxic peptides from *Lophyrotoma zonalis* (Pergidae) sawfly larvae. Toxicon 39:1933–1936

Raymundo DL, Bezerra PS, Bandarra PM et al (2008) Spontaneous poisoning by larvae of *Perreyia flavipes* (Pergidae) in sheep. Pesqui Vet Bras 28:19–22. https://doi.org/10.1590/S0100-736X2008000100003

Rodgers L, Pernas T, Hill SD (2014) Mapping invasive plant distributions in the Florida everglades using the digital aerial sketch mapping technique. Invasive Plant Science and Management 7:360–374

Schmidt S, Smith DR (2018) Pergidae of the world – an online catalogue of the sawfly family Pergidae (Insecta, Hymenoptera, Symphyta). In: World Wide Web Electron. Publ. http://pergidae.snsb-zsm.de. Accessed 25 Jul 2016

Schmitz DC, Simberloff D, Hofstetter RL et al (1997) The ecological impact of nonindigenous plants. In: Simberloff D, Schmitz DC, Brown TC (eds) Strangers in paradise: impact and management of nonindigenous species in Florida. Island Press, Washington, DC, pp 39–61

Smith DR (1990) A synopsis of the sawflies (Hymenoptera, Symphyta) of America south of the United States: Pergidae. Revista Brasileira de Entomologia 34:7–200

Taeger A, Blank SM, Liston AD (2010) World catalog of Symphyta (Hymenoptera). Zootaxa 2580:1–1064

Thamsborg SM, Jørgensen RJ, Brummerstedt E (1987) Sawfly poisoning in sheep and goats. Vet Rec 121:253–255

Wheeler G, Mc Kay F, Vitorino M et al (2016) Biological control of the invasive weed, Brazilian peppertree, *Schinus terebinthifolia*: a review of the project with an update on the proposed agents. Southeast Nat 15:15–34

Yoshioka ER, Markin GP (1991) Efforts of biological control of Christmas berry *Schinus terebinthifolius* in Hawaii. In: Center TD, Doren RF, Hofstetter RL, et al. (eds) Proceedings of the Symposium on Exotic Pest Plants. pp 377–385