

Host specificity of *Ischnodemus variegatus*, an herbivore of West Indian marsh grass (*Hymenachne amplexicaulis*)

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Abstract West Indian marsh grass, *Hymenachne amplexicaulis* Rudge (Nees) (Poaceae), is an emergent wetland plant that is native to South and Central America as well as portions of the Caribbean, but is considered invasive in Florida USA. The neotropical bug, *Ischnodemus variegatus* (Signoret) (Hemiptera: Lygaeoidea: Blissidae) was observed feeding on *H. amplexicaulis* in Florida in 2000. To assess whether this insect could be considered as a specialist biological control agent or potential threat to native and cultivated grasses, the host specificity of *I. variegatus* was studied under laboratory and field conditions. Developmental host range was examined on 57 plant species across seven plant families. Complete development was obtained on *H. amplexicaulis* (23.4% survivorship), *Paspalum repens* (0.4%), *Panicum anceps* (2.2%) and *Thalia geniculata* (0.3%). Adults survived 1.6 times longer and laid 6.6 times more eggs on *H. amplexicaulis* than the other species. Oviposition on suboptimal host species was positively related to *I. variegatus* density under multiple choice conditions. Results from field experiments indicated that *H. amplexicaulis* had higher densities of *I. variegatus* than other species. Spill-over

to suboptimal hosts occurred in an area where *H. amplexicaulis* was growing in poor conditions and there was a high density of *I. variegatus*. Thus, laboratory and field studies demonstrate that *I. variegatus* had higher performance on *H. amplexicaulis* compared to any other host, and that suboptimal hosts could be colonized temporarily.

Keywords Blissidae · Hemiptera · Herbivore performance · Host quality · Poaceae

Introduction

West Indian marsh grass, *Hymenachne amplexicaulis* Rudge (Nees) (Poaceae), is a perennial emergent weed in wetlands of Florida USA and northeastern Australia. It is considered native to the neotropics and was introduced into Florida and Australia prior to 1954 and the 1970s, respectively (University of Florida Herbarium 2007; Csurhes et al. 1999). There are four species of *Hymenachne* in the neotropics with overlapping distributions in South America (Soreng et al. 2007). This grass is considered a valuable forage in its native range (Tejos 1978; Enriquez-Quiroz et al. 2006) and its potential as a ponded pasture forage grass stimulated studies in South Florida in the late 1990s (Kalmbacher et al. 1998). However, aggressive growth during long hydroperiods in Florida allowed *H. amplexicaulis* to outcompete native vegetation and create large

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monotypic stands in natural wetlands. Due to the severity of its impact to native ecosystems, the Florida Exotic Pest Plant Council designated *H. amplexicaulis* as a Category I invasive species which are invasive exotics that alter native plant communities by displacing native species, changing community structure or ecological functions, or hybridizing with natives (Florida: Florida Exotic Pest Plant Council 2007). Similarly, Houston and Duivenvoorden (2002) found that Australian wetlands invaded by *H. amplexicaulis* had lower plant species richness, greater relative abundance of exotic fish and altered macroinvertebrate community structure.

The seasonal cycle of *H. amplexicaulis* in Florida can be described as follows: seeds germinate and shoots begin growing from underwater stolons during the spring. As the water level, day-length and temperature increase, the plant grows aggressively and reaches maximum biomass during late summer (R. Diaz unpublished data). Flowering occurs in late fall (September–November) and is triggered by short days (Tropical Weeds Research Centre 2007). Aerial parts of the plant senesce during the winter, but stolons and seeds remain dormant underwater until more favorable conditions appear. While most of local spread is thought to be through stolons (R. Diaz unpublished data), long distance dispersal may occur by either seeds and/or broken stolons (Csurhes et al. 1999). Difficult access to infested wetlands limits the ability to contain and control *H. amplexicaulis* invasions. Where possible, management of *H. amplexicaulis* can be accomplished through several applications of registered herbicides or changes in wetland hydroperiod and shading (Csurhes et al. 1999). Currently, herbicides constitute the major tool for managing emerged parts of the plant, but control of underwater stolons is more difficult. Exploration for pathogens of *H. amplexicaulis* in Brazil has not resulted in the identification of promising biological control agents (Soares and Barreto 2006).

Ischnodemus variegatus (Signoret) (Hemiptera: Lygaeoidea: Blissidae) was found feeding on *H. amplexicaulis* in Sarasota Co., Florida, USA in 2000 (Halbert 2000). This species is native to South America (Baranowski 1979; Slater 1987) and the timing and method of the herbivore's arrival into Florida are unknown. The insect has been collected in several wetlands in Central and South Florida and its distribution appears to coincide with that of

H. amplexicaulis. *Ischnodemus variegatus* has five nymphal stages. Females lay eggs in tight spaces within the leaf sheath and, upon hatching, nymphs form aggregations near the site of eclosion (Diaz et al. 2008). This sap-sucking bug is commonly found between the leaf sheath and the stem which is the most suitable place for feeding due to the absence of the silicon dioxide, a feeding deterrent, in actively growing meristematic areas (Slater 1976). Damage can be recognized by the accumulation of anthocyanins in the leaves which appear dark red, and later turn brown and die if the infestation persists. Overholt et al. (2004) found that feeding damage of *I. variegatus* reduced the photosynthetic capacity and growth rate of *H. amplexicaulis* under greenhouse conditions. Temperature dependent studies indicated that the lower threshold for development of *I. variegatus* was between 14 and 16°C and 588 degree-days were needed to complete development (Diaz et al. 2008). This information allowed the development of prediction models which indicated that *I. variegatus* could complete from 2.3 to 4.8 generations per year across Florida and its most northern distribution may extend to Leon and Wakulla counties in Northern Florida (Diaz et al. 2008). Field sampling in Florida indicated that *I. variegatus* outbreaks occur from August to November, but the herbivore did not appear to have a major effect on *H. amplexicaulis* density (R. Diaz unpublished data).

Host associations of species in the genus *Ischnodemus* have been described by Slater (1976, 1987), Slater and Wilcox (1973) and Baranowski (1979). *Ischnodemus* spp. feed and reproduce on monocotyledonous plants and some species show a remarkable degree of host specificity, including several marsh grass specialists (Harrington 1972; Slater 1976; Slater and Wilcox 1973). Table 1 shows the host associations of *Ischnodemus* spp. found in Florida.

Due to the ecological and economical importance of grasses and the potential of *I. variegatus* as a biological control agent, we conducted an extensive host specificity investigation. We hypothesized that because of the longer association of *I. variegatus* and *H. amplexicaulis* in their native range, native plants in Florida would be unsuitable hosts for development, and oviposition. The suitability of plant species for development of *I. variegatus* was determined using first instar nymphs and adults. We conducted several tests which examined the preference of ovipositing

Table 1 Host associations of *Ischnodemus* spp. in Florida

Species	Plant host (breeding)	Geographic distribution	Source
<i>I. badius</i>	<i>Spartina bakeri</i> , <i>S. alterniflora</i>	Primary coastal species	Slater and Baranowski (1990)
<i>I. brunipennis</i>	<i>Panicum hemitomon</i>	Across peninsular Florida	Slater and Baranowski (1990)
<i>I. conicus</i>	<i>Spartina bakeri</i>	North-West Florida	Slater and Baranowski (1990)
<i>I. fulvipes</i>	<i>Thalia geniculata</i> , <i>Canna indica</i> , <i>Musa</i> spp.	Central and South Florida	Slater and Baranowski (1990)
<i>I. lobatus</i>	Unknown	South-West Florida	Slater and Baranowski (1990)
<i>I. praecultus</i>	<i>Cyperus aggregatus</i> , <i>Cyperus ligularis</i>	Coastal South Florida	Slater and Baranowski (1990)
<i>I. robustus</i>	<i>Echinochloa</i> sp., <i>Paspalidium geminatum</i>	Central and South Florida	Slater and Baranowski (1990)
<i>I. rufipes</i>	<i>Cyperus odoratus</i>	Central and South Florida	Slater and Baranowski (1990)
<i>I. sallei</i>	<i>Thalia geniculata</i>	Central and South Florida	Slater and Baranowski (1990)
<i>I. slossoane</i>	<i>Sacciolepis striata</i> , <i>Panicum agrostoides</i>	Coastal Florida	Slater and Baranowski (1990)
<i>I. variegatus</i>	<i>Hymenachne amplexicaulis</i>	Central and South Florida	Baranowski (1979)

females for *H. amplexicaulis* and native species. Finally, the presence of *I. variegatus* in several wetlands in Florida allowed investigation of the herbivore's realized host range.

Materials and methods

Origin and maintenance of organisms

Laboratory experiments were conducted at the Biological Control Research and Containment Laboratory (BCRCL), Fort Pierce, Florida. *Ischnodemus variegatus* and *H. amplexicaulis* material were collected in Myakka River State Park, Sarasota Co., Florida (27.2° N, 82.2° W), and Fisheating Creek (26.5° N, 81.7° W). The genetic variability of the insect colony was maintained by adding field collected individuals at least three times per year. Potted plants were placed in small, mesh screened cages (0.90 m × 0.90 m × 0.90 m) located within a walk-in rearing room maintained at 25–30°C, 50–80% RH and a 14:10 L:D photoperiod. Field collected individuals were released in these cages and monitored every other day for nymphal survival and colonization. When fresh neonates were needed, adults were held in small cages containing *H. amplexicaulis* stems. Eggs were removed every other day and placed in Petri dishes until eclosion. Adults were handled using an aspirator and nymphs by using a small brush. Development and oviposition studies were conducted in a walk-in rearing room set at 28°C, 70–80% RH and 14:10 L:D photoperiod. We used

28°C since a previous study indicated a high survival of individuals at this temperature (Diaz et al. 2008). Voucher specimens of *I. variegatus* are maintained in the Florida State Collection of Arthropods (FSCA) accession # E2002-6139 and the Australian National Insect Collection.

Test plants

Plant species were selected following guidelines of the Technical Advisory Group (TAG) of the United States Department of Agriculture (USDA), Animal and Plant Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) (2000). These guidelines follow the centrifugal phylogenetic method developed by Wapshere (1989) and include species related to the target plant and those which have economical or ecological importance. Plant selection was biased towards species in the subtribes of Paniceae as well as wetland species that grow in the same habitat as *H. amplexicaulis*. Plants tested also included species which are known hosts of other *Ischnodemus* spp. Additionally, *Hymenachne acutigluma* (Steudel) Guilliland, a plant native to Australia, was included to examine the host specificity of *I. variegatus* at the generic level. Plants were either collected from the field or purchased at native plant nurseries. Seeds of *H. acutigluma* were provided by Tim Heard at CSIRO-Queensland. Test plants were maintained in the greenhouses at the BCRCL in potting soil and received one tablespoon of Osmocote® (18N:7P:12K; Scotts, Marysville, Ohio, USA) after transplanting and weekly

applications of Miracle-Grow® water soluble fertilizer (15N:30P:15K; Scotts, Marysville, Ohio, USA).

No-choice nymphal development

The fundamental host range was estimated using neonate nymphs because this insect stage is characterized by poor dispersal capabilities, absence of habituation, sensitization, and associative learning (Heard 2000). Eggs were collected every other day from the colonies and placed in Petri dishes until hatching. Five neonate nymphs were transferred using a fine brush to plastic vials (45 mm diameter × 102 mm tall) containing a piece of stem covered by a leaf sheath of each test plant species. The end of each vial was covered with a fine mesh for air circulation. An 8-cm plant piece was placed in moist sand contained at the bottom of the vial (Fig. 1a) and replaced every other day. The number of nymphs tested per plant species was 250, with a few exceptions (see Table 2). Nymphal survival and development was monitored every 48 h.

Fig. 1 Laboratory set-up for *I. variegatus* host range tests. (a) no-choice development, (b) no-choice oviposition, (c) multiple-choice oviposition, and (d) female laying eggs

No-choice and choice oviposition

Plant species that supported complete development in no-choice nymphal development studies were used in no-choice oviposition trials. Species included *Panicum anceps* Michx., *Panicum hemitomon* Schult, *Phanopyrum gymnocarpon* (Elliot) Nash, *Thalia geniculata* L. and *H. amplexicaulis* as a control. Despite not finding development to adult on *P. hemitomon* and *P. gymnocarpon*, these species were included because they occur in the same habitat as the known host in Florida. Fifth instars were collected from the colonies and placed in Petri dishes containing *H. amplexicaulis*. Newly emerged males and females were placed in a Petri dish containing *H. amplexicaulis* and allowed to mature sexually for one week. One individual of each sex (pair hereafter) was transferred to a cylindrical cage (5 cm diameter × 20 cm tall) containing a single 20 cm plant stem in wet sand (Fig. 1b). Since *I. variegatus* females actively search for leaf sheaths to lay eggs and hide (Fig. 1d) (Diaz et al. 2008), all cuttings

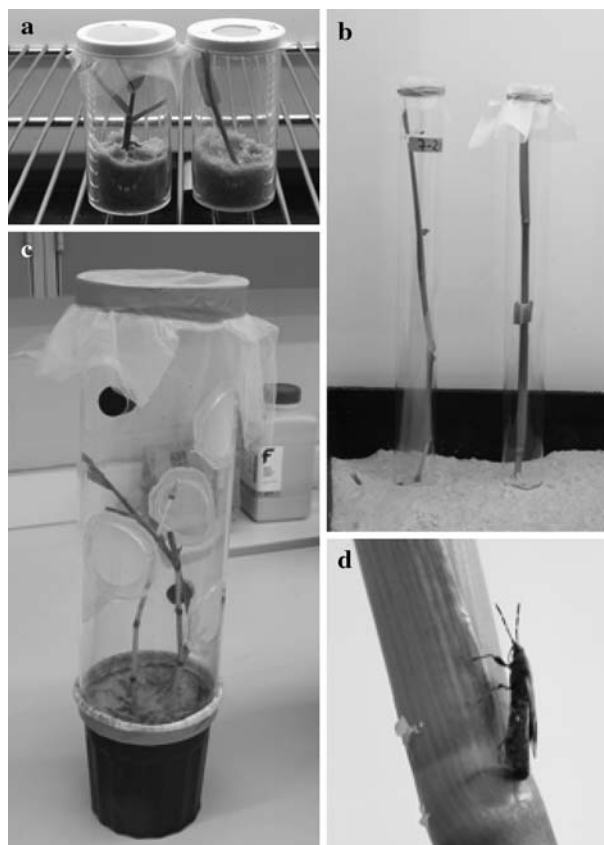


Table 2 No-choice nymphal survival of *I. variegatus* on test plants^a

Phylogenetic classification	Origin ^b	Habitat/ Uses ^c	1st instar		Percent survival to					
			Initial	Survived	Second	Third	Fourth	Fifth	Adult (n, days)	
Family Poaceae										
Tribe Paniceae										
Subtribe Paspalinae										
<i>Amphicarpum muhlenbergianum</i> (Schult.) Hitchc	N	Wetlands	250	0.0	0	0	0	0	0	0
<i>Axonopus fissifolius</i> (Raddi) Kuhl	N	Wetlands	250	0.0	0	0	0	0	0	0
<i>Axonopus furcatus</i> (Flüggé) Hitchc	N	Wetlands	250	0.0	0	0	0	0	0	0
<i>Hymenachne amplexicaulis</i> (Rugde) Nees	I	Wetlands	188	79.8	61.7	42.6	29.8	23.4	23.4	(44, 45.3)
<i>Hymenachne acutigluma</i> (Steud.) Guilliland	Q	Wetlands	250	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Paspalum repens</i> P.J. Bergius	N	Wetlands	258	12.8	9.7	8.1	4.3	0.4	0.4	(1, 40)
<i>Paspalum vaginatum</i> Sw.	N	Salt marsh	14	0.0	0	0	0	0	0	0
<i>Paspalum floridanum</i> Michx.	N	Wetlands	250	0.0	0	0	0	0	0	0
<i>Paspalum blodgettii</i> Chapm	N	Rocky pinelands	30	0.0	0	0	0	0	0	0
<i>Paspalum conjugatum</i> P.J. Bergius	N	Wetlands	225	0.0	0	0	0	0	0	0
<i>Paspalum urvillei</i> Steud.	I	Wetlands	190	0.0	0	0	0	0	0	0
<i>Paspalum notatum</i> Flüggé	I	Pastures	250	0.0	0	0	0	0	0	0
<i>Phanopyrum gymnocarpon</i> (Elliott) Nash	N	Wetlands	250	0.0	0	0	0	0	0	0
Subtribe Cenchrinae										
<i>Pennisetum purpureum</i> Schumach.	I	Wetlands	212	3.8	0.5	0	0	0	0	0
<i>Pennisetum setaceum</i> (Forssk.) Chiov.	I	Disturbed sites	300	0.0	0	0	0	0	0	0
Subtribe Digitariinae										
<i>Digitaria ciliaris</i> (Retz.) Koeler			250	0.0	0	0	0	0	0	0
Subtribe Melinidinae										
<i>Melinis minutiflora</i> P. Beauv	I	Dry/Disturbed sites	299	0.0	0	0	0	0	0	0
<i>Urochloa mutica</i> (Forssk.) T.Q. Nguyen	I	Wetlands	246	1.6	0	0	0	0	0	0
Subtribe Panicinae										
<i>Echinochloa walteri</i> (Pursh) A. Heller	N	Wetlands	200	0.0	0	0	0	0	0	0
<i>Panicum amarum</i> Elliott	N	Salt marsh	110	0.0	0	0	0	0	0	0
<i>Panicum anceps</i> Michx.	N	Wetlands	225	6.7	4.0	4.0	4.0	2.2	2.2	(5, 45)
<i>Dichantheium ensifolium</i> (Baldwin ex Elliott) Gould	N	Wetlands	245	0.0	0	0	0	0	0	0
<i>Panicum hemitomom</i> Schult.	N	Wetlands	345	3.2	2.0	1.4	0	0	0	0

Table 2 continued

Phylogenetic classification	Origin ^b	Habitat/ Uses ^c	1st instar		Percent survival to					
			Initial	Survived	Second	Third	Fourth	Fifth	Adult (n, days)	
<i>Panicum maximum</i> Jacq.	I	Old fields	250	0.4	0	0	0	0	0	
<i>Panicum repens</i> L.	I	Wetlands	80	0.0	0	0	0	0	0	
<i>Panicum virgatum</i> L.	N	Salt marsh	250	0.0	0	0	0	0	0	
<i>Sacciolepis striata</i> (L.) Nash	N	Wetlands	247	4.5	1.2	1.2	0.8	0	0	
Subtribe Setariinae										
<i>Paspalidium geminatum</i> (Forssk.) Stapf	N	Wetlands	250	5.2	0.8	0.8	0	0	0	
<i>Setaria sphacelata</i> (Schumach.) M.B. Moss ex Stapf & C.E. Hubb	I	Disturbed sites	250	0.0	0	0	0	0	0	
<i>Stenotaphrum secundatum</i> (Walter) Kuntze	N	Wetlands	250	0.0	0	0	0	0	0	
Tribe Andropogoneae										
<i>Andropogon brachystachyus</i> Chapm	N	Wetlands	190	0.0	0	0	0	0	0	
<i>Andropogon glomeratus</i> (Walter) Britton et al.	N	Wetlands	226	0.4	0	0	0	0	0	
<i>Andropogon virginicus</i> L.	N	Wetlands	241	3.3	2.5	1.2	0	0	0	
<i>Andropogon virginicus</i> L. var. glaucus Hack	N	Scrub/Dry pinelands	250	0.0	0	0	0	0	0	
<i>Hemarthria altissima</i> (Poir.) Stapf & C.E. Hubb.	I	Cultivated pasture	241	0.0	0	0	0	0	0	
<i>Imperata cylindrica</i> (L.) P. Beauv.	I	Disturbed sites	250	0.0	0	0	0	0	0	
<i>Saccharum officinarum</i> L.	I	Cultivated	250	0.0	0	0	0	0	0	
<i>Sorghum bicolor</i> Moench	I	Cultivated	36	5.6	2.8	2.8	0	0	0	
<i>Tripsacum dactyloides</i> (L.) L.	N	Wetlands	250	0.0	0	0	0	0	0	
<i>Tripsacum floridanum</i> Porter ex Vasey	N	Rocky pinelands	250	0.0	0	0	0	0	0	
<i>Zea mays</i> L.	I		285	0.0	0	0	0	0	0	
Tribe Arundineae										
<i>Arundo donax</i> L.	I	Wet to dry sites	250	0.0	0	0	0	0	0	
Tribe Cynodonteae										
<i>Cynodon dactylon</i> (L.) Pers.	I	Disturbed sites	192	6.3	1.0	0	0	0	0	
<i>Muhlenbergia capillaris</i> (Lam.) Trin.	N	Wetlands	250	0.0	0	0	0	0	0	
Tribe Eragrostideae										
<i>Eragrostis elliottii</i> S.Watson	N	Disturbed sites	248	0.8	0.4	0	0	0	0	
<i>Eragrostis spectabilis</i> (Pursh) Steud.	N	Wetlands	226	3.5	3.1	0.9	0	0	0	
Tribe Oryzeae										
<i>Oryza sativa</i> L.	I	Wetlands	807	1.1	0.1	0	0	0	0	
<i>Luziola fluitans</i> (Michx.) Terrell & H. Rob.	N	Wetlands	50	0.0	0	0	0	0	0	

Table 2 continued

Phylogenetic classification	Origin ^b	Habitat/ Uses ^c	1st instar		Percent survival to					
			Initial	Survived	Second	Third	Fourth	Fifth	Adult (n, days)	
Tribe Zoysieae										
<i>Spartina bakeri</i> Merr.										
	I	Dry hammocks	250	0.0	0	0	0	0	0	0
	I	Disturbed sites	250	0.0	0	0	0	0	0	0
Family Alismataceae										
	N	Wetlands	250	0.0	0	0	0	0	0	0
Family Cyperaceae										
	N	Disturbed sites	65	3.1	1.5	0	0	0	0	0
Family Haemodoraceae										
	N	Wetlands	90	0.0	0	0	0	0	0	0
Family Marantaceae										
	N	Wetlands	304	1.0	0.3	0.3	0.3	0.3	0.3	0.3 (1, 41)
Family Pontederiaceae										
	N	Wetlands	250	0.0	0	0	0	0	0	0
Family Typhaceae										
	N	Wetlands	165	1.2	0	0	0	0	0	0

^a Higher classification based on the Catalogue of new world grasses (Poaceae) (Soreng et al. 2007) (<http://mobot.mobot.org/W3T/Search/nwgc.html>)

^b N, Native to Florida; I, introduced; Q, not present in Florida

^c Habitat data from Wunderlin and Hansen (2004)

were selected to provide these conditions. Adult survival and number of eggs laid were recorded every two days as cuttings were replaced. At least 15 pairs (range: 15–25) were tested per plant species. If a male died, it was replaced by a young male from the colony; when a female died, that replicate was terminated.

Plant species used for the choice experiment included *P. anceps*, *P. hemitomon*, *T. geniculata*, *Spartina bakeri*, *Hymenachne acutigluma* and *H. amplexicaulis* as a control. Despite no nymphal development on *S. bakeri*, *H. acutigluma* and *P. hemitomon*, these species were included to provide a wider range of potential hosts for oviposition. Stems (20 cm) of each plant species were placed randomly in a circular fashion in wet sand. A plastic cylindrical cage (30 cm tall × 15 cm diameter) was used to cover the stems (Fig. 1c). The effect of adult density on oviposition was tested by placing 1, 5 or 10 pairs per cylinder and each density was replicated six times. Adults were collected from the colony and

placed in the middle of the arena in a 45 mm tall × 15 mm diameter vial. Stems were replaced every three days and the number of eggs was counted. If adults died, they were replaced by individuals from the colony to maintain the same density throughout the experiment. After two weeks the experiment was terminated.

Field colonization of potted plants

Two sites were established in Sarasota Co. The first site (27.2106° N, 82.2554° W) was selected in 2005 and consisted of a 100 m long by 3 m wide roadside canal. The approximate water depth and *H. amplexicaulis* height were 10 and 80 cm, respectively. One side of the canal had tall trees which provided partial shade. The *H. amplexicaulis* stand was considered poor since the plants were short, with small leaves and thin stems. The second site (27.2611° N, 82.2859° W), used in 2006, had a large infestation of *H. amplexicaulis* in an open marsh in Myakka River State Park. The

approximate water depth and *H. amplexicaulis* height were 1 and 2 m, respectively. The marsh was flooded periodically during the summer. The *H. amplexicaulis* stand was considered healthy since the plants were tall, with large leaves and thick stems. The initial population of *I. variegatus* was estimated by destructive sampling of two contiguous plants at 1 m intervals along a 20 m transect at each site. Then, 1-2 pots containing a single uninfested species of *T. geniculata*, *P. hemitomon*, *P. anceps*, *S. bakeri* or *H. amplexicaulis* were randomly placed along the transect and separated by 1 m. Each plant species had six replicates. To ensure direct contact, two *H. amplexicaulis* stems were joined by a cord to each potted plant. After two weeks, the potted plants were recovered from each site and examined in the laboratory for presence of *I. variegatus*.

Spill-over to co-occurring species

Four marshes were selected according to the presence of *I. variegatus*, *H. amplexicaulis* and other potential hosts during 2005 and 2006. Three marshes separated by at least 50 m were located in Myakka River State Park in the summer and fall of 2006. The fourth marsh was located along a roadside ditch in Sarasota Co. in the fall of 2005. The first three marshes were in floodplains located on the east side of the Myakka River and had a depth of >30 cm. Depending of the marsh shape, one or two longitudinal transects were established per site. These marshes in general had low plant species diversity because of the dominance of *H. amplexicaulis*. Marsh 1 had *P. hemitomon*, marsh 2 had *P. hemitomon* and *Sacciolepis striata* (L.) Nash, and marsh 3 had an unidentified “panicoid” grass. Along each transect, 17 to 20 stations were selected containing a native plant and *H. amplexicaulis* in its immediate surroundings. At each station, a stem of the native plant and three surrounding stems of *H. amplexicaulis* were clipped just above the water level and stored separately in plastic bags. Samples of native plants and *H. amplexicaulis* were dissected in the laboratory and the number of *I. variegatus* or other *Ischnodemus* spp. was recorded. Feeding damage on *H. amplexicaulis* was evaluated by using the top three leaves of each plant. The amount of anthocyanins (red color) per leaf was estimated using a scale (0 = leaf completely green, 1 = 10% red, 2 = 20% red to 10 = 100% red). Plant samples were oven dried for

one week and dry weight was measured on a Ohaus Navigator™ balance (precision 0.01 g) (Bradford, Massachusetts, USA).

The fourth wetland (27.2452° N, 82.3524° W) contained *H. amplexicaulis* and *T. geniculata*. Twenty plant samples of both species, separated at least by 1 m, were collected along a longitudinal transect and the presence of *Ischnodemus sallei* (Signoret) or *I. variegatus* was assessed in the laboratory. Variables collected from samples included number and stage of insects per stem and presence of egg parasitism (evident from a black coloration of eggs). All samples were collected once at each marsh during the course of the study.

Statistical analysis

The effect of plant species on variables was compared with the general linear model procedure (PROC GLM; SAS Institute 1999). Whenever significant ($P < 0.05$) *F*-values were obtained, means were separated using the Student-Neuman-Keuls (SNK) test.

Results

No-choice nymphal development trials

A total of 57 plant species, including 34 natives, in seven families were tested (Table 2). Nymphs molted to the third instar on *H. amplexicaulis*, *Paspalum repens* P. J. Bergius, *P. anceps*, *P. hemitomon*, *S. striata*, *Andropogon virginicus* L., *Sorghum bicolor* Moench, *Eragrostis spectabilis* (Pursh) Steud. and *T. geniculata*. Nymphal development to adult was completed on the known host *H. amplexicaulis* and the Florida natives *P. repens* (subtribe Paspalinae) and *P. anceps* (subtribe Paniceae), both members of the same tribe, Paniceae, as *H. amplexicaulis*; and *T. geniculata*, a member of the monocot family Marantaceae (Table 2). Survival to adult on the native *P. repens* (0.4%), *P. anceps* (2.2%) and *T. geniculata* (0.3%), was much lower compared to survival on *H. amplexicaulis* (23.4%), suggesting inferior host quality. There was 100% mortality of first instar nymphs on the Australian native *Hymenachne acutigluma*. Nymphs reared on hosts other than *H. amplexicaulis* were often observed wandering on the vial walls, and later were found dead on the sand, which suggests a total rejection of cut foliage offered.

No development occurred on economically important grasses except the partial development on *S. bicolor*.

No-choice and choice oviposition trials

Females laid significantly more eggs (296.5 ± 34.7 , mean \pm SE) during their lifetime and survived longer (74.1 ± 9.5 days) on *H. amplexicaulis* compared with other test species (eggs: $F = 20.7$; d.f. = 4, 87; $P < 0.0001$; longevity: $F = 67.4$; d.f. = 4, 86; $P < 0.0001$) (Fig. 2). Females laid an average of 44.9 ± 14.56 eggs and lived 30.6 ± 4.3 days on *T. geniculata*; no eggs were found on the other species tested (Fig. 2).

Results from the multiple-choice test indicated that females laid significantly more eggs on

H. amplexicaulis compared to other plant species (Fig. 3). There was an increase in the number of eggs laid on suboptimal species with an increase in adult density (Fig. 3).

Field colonization of potted plants

Initial observations of *H. amplexicaulis* plants at site 1 indicated the presence of severe feeding damage by *I. variegatus* which was reflected by the accumulation of anthocyanins and necrosis in the leaves. The initial infestation was 13.8 adults and nymphs combined, and 51.8 eggs per stem (Fig. 4). Results from potted plants showed that *H. amplexicaulis*, and to a lesser degree the other species, were colonized by *I. variegatus* during the 2-week study (Fig. 4). Adults,

Fig. 2 Mean (+SE) adult longevity and number of eggs laid under non-choice conditions. Plant species with different letters within a variable are significantly different ($P < 0.05$)

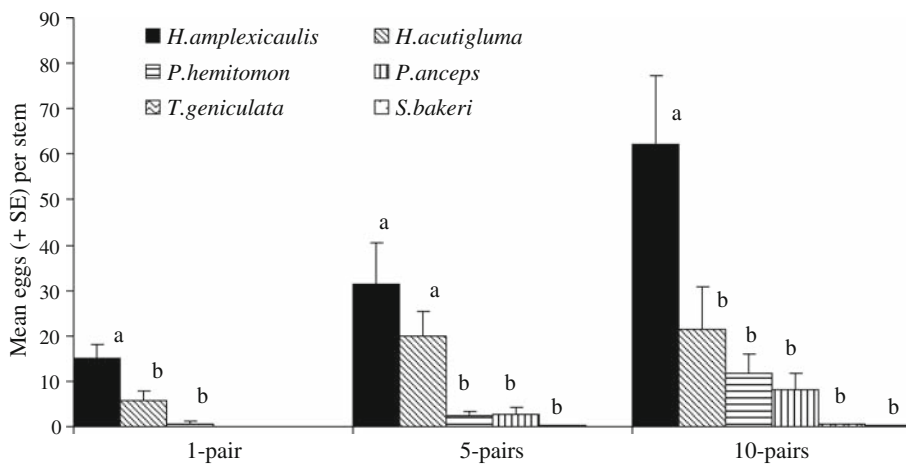
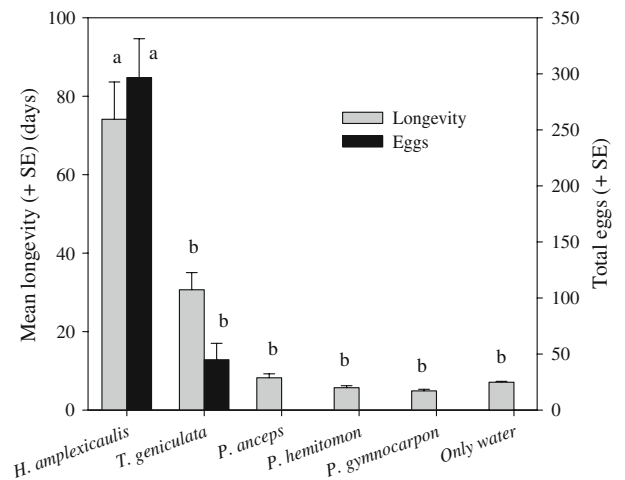


Fig. 3 Mean (+SE) number of eggs laid at three different adult densities. Plant species in the same treatment with different letters are significantly different ($P < 0.05$). 1 pair

($F = 15.23$; d.f. = 5,138; $P < 0.0001$), 5 pairs ($F = 9.34$, d.f. = 5, 138; $P < 0.0001$), 10 pairs ($F = 9.89$; d.f. = 5, 138; $P < 0.0001$)

Fig. 4 Open field test results at site 1. Adult and nymphs: ($F = 13.52$; d.f. = 3, 19; $P < 0.001$); eggs ($F = 5.28$; d.f. = 3, 19; $P < 0.001$). Plant species with different letters within a variable are significantly different ($P < 0.05$)

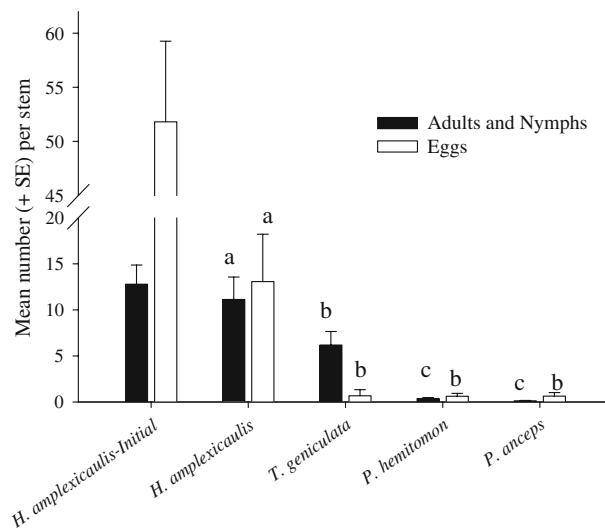
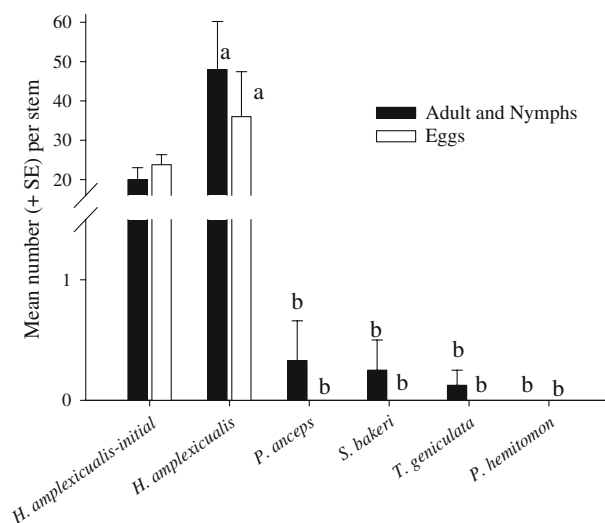


Fig. 5 Open field test results at site 2. Adult and nymphs: ($F = 14.0$; d.f. = 3, 26; $P < 0.001$); eggs: ($F = 8.99$, d.f. = 3, 26; $P < 0.001$). Plant species with different letters within a variable are significantly different ($P < 0.05$)



nymphs and eggs of *I. variegatus* were found on all test plants, but in lower numbers than on *H. amplexicaulis* (Fig. 4).

Hymenachne amplexicaulis plants at site 2 were healthier than those at site 1 and showed less feeding damage despite a high initial density of insects. The initial infestation was 20.0 adults and nymphs, and 23.8 eggs per *H. amplexicaulis* stem (Fig. 5). Lower average numbers of adults and nymphs of *I. variegatus* were found on test species (<1) compared to *H. amplexicaulis* (48) (Fig. 5). While a high number of eggs (mean per stem = 36) were recorded on *H. amplexicaulis*, no eggs were found on the other species.

Spill-over to co-occurring species

The presence of native grasses and the density of *I. variegatus* were different among sampled marshes (Table 3). There were higher numbers of adults and nymphs on *H. amplexicaulis* compared to native plants in all four marshes (Table 3). More *I. variegatus* were found on the panicoid grass, followed by *P. hemitomom* and *S. striata* (Table 3). A few individuals of *Ischnodemus brunnipennis* were found on *P. hemitomom* (data not presented). There was no indication of spill-over of *I. variegatus* to *T. geniculata* or *I. sallei* to *H. amplexicaulis* in the site where both species co-occurred. The number of

Table 3 Mean number of *I. variegatus* per stem and plant parameters (\pm SE) collected from spill-over studies in Florida during 2006

Location/plant species	Mean <i>I. variegatus</i> per stem ^a	Damage scale ^b (0 to 10)	Stations	Dry weight ^c (g tiller ⁻¹)	Water level (cm)
Marsh 1					
<i>H. amplexicaulis</i>	10.43 \pm 1.78	4.13 \pm 0.32 a	20	7.36 \pm 0.25 a	40
<i>P. hemitomom</i>	0.50 \pm 0.30 a	–		–	
Marsh 2 ^d					
<i>H. amplexicaulis</i>	1.20 \pm 0.18	0.80 \pm 0.15 c	18	7.18 \pm 0.29 a	30
<i>S. striata</i>	0.11 \pm 0.08 a	–		–	
<i>H. amplexicaulis</i>	1.55 \pm 0.42	0.62 \pm 0.13 c	20	–	40
<i>P. hemitomom</i>	0.10 \pm 0.06 a	–		–	
Marsh 3					
<i>H. amplexicaulis</i>	7.55 \pm 1.39	2.90 \pm 0.41 b	17	10.93 \pm 0.77 b	50
Panicoid	3.7 \pm 0.75 b	–		17.33 \pm 1.31 c	

^a Adult and nymphs, means were significantly different when comparing *H. amplexicaulis* versus native plant (1-way ANOVA, $F > 5.9$, d.f. = 1, 33; $P < 0.05$), different letters indicate comparisons among native plants only ($F = 19.69$, d.f. = 3, 74; $P < 0.0001$); ^b Average of top three leaves ($F = 39.8$, d.f. = 3,74; $P < 0.0001$); ^c Above water biomass ($F = 40.5$, d.f. = 3, 72; $P < 0.0001$); ^d Marsh 2 contains two transects

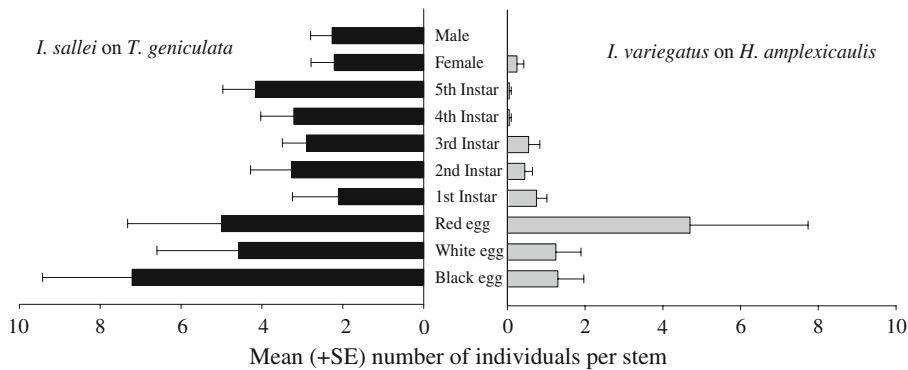


Fig. 6 Age distribution as mean (+SE) of all the stages of *I. variegatus* and *I. sallei* on their hosts found at marsh 4

individuals of *I. sallei* per stem of *T. geniculata* was higher than the number of *I. variegatus* on *H. amplexicaulis* (Fig. 6).

Discussion

Host range testing is an essential step for quantifying risks posed by exotic herbivores to native and economically important plant species. The arrival of *I. variegatus* to Florida wetlands triggered several questions regarding its specificity and potential impacts to the invasive grass *H. amplexicaulis* as well to native species within the wetland

communities and to cultivated grasses. Therefore, we quantified the fundamental host range of *I. variegatus* under controlled conditions and, through field experiments, monitored spill-over to suboptimal hosts.

Results of the no-choice developmental study showed that *I. variegatus* nymphs were highly specific to *H. amplexicaulis*. Under restrictive conditions, very few nymphs reached the second instar and even fewer reached the adult stage on plants other than *H. amplexicaulis*, which indicates a narrow fundamental host range. Survival to the third stage was partially related to the taxonomic relatedness of test plants to the invasive weed (Table 2). Survival to

adult was 23% on *H. amplexicaulis* while less than 3% survival was found on *P. anceps*, *P. repens* and *T. geniculata*. Despite the different soil moisture requirements of *Sorghum bicolor* and *H. amplexicaulis*, the partial nymphal development found on *S. bicolor* warrants further attention. Except for *T. geniculata*, survival to adult was restricted to species in the tribe Paniceae. Most plant species did not support complete development of *I. variegatus*, despite some survival of early nymphal instars. Further laboratory studies on the suitability of suboptimal hosts could include insect population growth parameters, developmental times and fitness of emerging adults (van Klinken 2000).

Studies under no-choice conditions revealed that *I. variegatus* females survived longer and laid more eggs on *H. amplexicaulis* compared to other species. Females lived an average of 74 days on *H. amplexicaulis*, 31 days on *T. geniculata* and less than eight days on *P. anceps*, *P. hemitomon* and *P. gymnocarpon*. Longevity of adults provided only water was similar to longevity on *P. anceps*, *P. hemitomon*, *P. gymnocarpon* which may suggest that adults did not feed on these plants. Only 9 out of 18 females laid eggs on *T. geniculata* while all 18 females laid eggs on *H. amplexicaulis*. Oviposition choice experiments indicated that at high densities of adults, females may lay eggs on species that provide suitable oviposition sites (Fig. 3). Females preferred to lay eggs on *H. amplexicaulis* at 1-pair and 10 pairs per cylinder compared to other plants, and there was no preference for a particular location along the stem (R. Diaz unpublished data). Eggs were found on species in which stems were morphologically similar to *H. amplexicaulis*, such as *H. acutigluma* and to a lesser degree, on *P. hemitomon*. Field observations indicated that some *I. variegatus* females preferred to oviposit slightly above nodes (R. Diaz unpublished data) but under high insect densities, this preference was not observed. Lack of unoccupied suitable locations for oviposition on *H. amplexicaulis* could have forced females to relax discrimination for oviposition sites and move to suboptimal hosts (Schoonhoven et al. 2005).

Field experiments during 2005 and 2006 demonstrated that *H. amplexicaulis* supported higher densities of *I. variegatus* compared to other plant species. Spill-over to native plants differed between sites 1 and 2 (Figs. 4, 5). The quality of

H. amplexicaulis plants at site 1 was inferior to those at site 2, probably due to partial shading and low water level, and this was reflected in greater spill over to native plants. Eggs, nymphs and adults of *I. variegatus* were found on *T. geniculata*, *P. hemitomon* and *P. anceps* at site 1, but in fewer numbers than on *H. amplexicaulis* (Fig. 4). Despite having a higher density of insects per stem than at site 1, spill over of *I. variegatus* to native plants occurred to a lesser degree than at site 2. *Hymenachne amplexicaulis* plants at site 2 were taller, with a much higher biomass than at site 1, and were able to support ~20 adults and nymphs per stem with little visible damage (R. Diaz unpublished data). We suspect that the quality of *H. amplexicaulis* influences the risk of spill over. Where quality of the primary host is low, *I. variegatus* may exhibit a greater tendency to move to alternative hosts.

Field studies revealed that *H. amplexicaulis* supported a higher density of *I. variegatus* and experienced greater feeding damage compared to co-occurring native species. Morphological features of the “panicoid” grass, such as the gap size between the leaf sheath and the stem, as well as the stem diameter, may explain the spill-over observed to this plant. Under the local field conditions, we could not detect spill-over of *I. sallei* to *H. amplexicaulis* nor of *I. variegatus* to *T. geniculata* (Fig. 6). Phylogenetic relationships among plants could not explain the use of *T. geniculata* (Marantaceae), suggesting that other factors such as plant chemistry (Bernays and Chapman 1994) or phylogenetic relationships among *Ischnodemus* spp. might be involved in *I. variegatus* host selection. Field sampling in St. Lucie and Sarasota counties indicated that *I. sallei* is commonly found associated with *T. geniculata* (R. Diaz unpublished data; Table 1). Future studies could evaluate potential competition, sharing or displacement of *I. sallei* by the exotic *I. variegatus* on *T. geniculata* in Florida.

Other *Hymenachne* spp., *T. geniculata*, *P. repens* and *S. bicolor* occur in the native range of *H. amplexicaulis*, allowing possible interaction with *I. variegatus* (New York Botanical Garden 2007; Soreng et al. 2007). However, collections in the native range have only found *I. variegatus* in association with *H. amplexicaulis* (Slater and Wilcox 1969; Baranowski 1979). This suggests that despite the presence of potential alternative hosts,

I. variegatus sustains persistent populations only on *H. amplexicaulis*. The recognition of primary hosts and potential spill-over to secondary hosts in members of the family Blissidae was recognized by Slater (1976, 1987), who has studied the phylogeny and host associations of this group for several decades. According to Slater and Wilcox (1973), most blissids tend to use one host species, but the temporary use of a secondary host has been observed. Furthermore, these authors suggested that most secondary hosts do not meet all physiological and ecological requirements of the insects, and that after a short time “colonies on such hosts tend to die out”.

The population dynamics of *I. variegatus*, coupled with host quality of *H. amplexicaulis*, may provide insight into the timing of spill-over. Field monitoring of *I. variegatus* and *H. amplexicaulis* in Sarasota Co. indicated that (1) insect populations increase during the summer until reaching a peak in September and October and (2) *H. amplexicaulis* grows more aggressively in open, deep water marshes with high sediment influx compared to shaded, low water wetlands (R. Diaz unpublished data, Csurhes et al. 1999). Therefore, we hypothesize that the risk of spill-over on suboptimal hosts may be higher in shaded areas experiencing high insect densities later in the growing season (fall in Florida). Further periodic sampling of suboptimal hosts growing close to as well as separated (Schooler et al. 2003) from *H. amplexicaulis* would be required to test this hypothesis. According to other studies, spill-over of weed biological control agents may occur when populations reach certain threshold levels (Dhileepan et al. 2006; Schooler et al. 2003; Blossey et al. 2001). The presence of *I. variegatus* on *H. amplexicaulis* stands in Florida provides an ideal field setting for studies on host switching and permanent utilization of suboptimal species predicted by the fundamental host range.

Changes in host utilization by specific insect herbivores depend on the level of novel selection pressures and the genetic variation upon which the selection will act (van Klinken and Edwards 2002). In the present system *Ischnodemus variegatus* had a much higher fitness when feeding on *H. amplexicaulis* compared to other hosts; and the fortuitous arrival of *I. variegatus* into Florida suggests that the local population could have a low genetic variability. Under this scenario, a selection pressure for improved performance, and consequently permanent utilization,

of suboptimal hosts (native plants) in Florida may be unlikely. However, selection pressures favoring utilization of suboptimal hosts could be related to the availability of enemy free-space (Bernays and Graham 1988), a reduction in primary host density and seasonal availability during primary host absence. The permanent utilization of suboptimal hosts by *I. variegatus*, and the impact of *I. variegatus* on these hosts in Florida wetlands should be determined by long term monitoring studies.

Conclusion

Field surveys in the native range indicated that *I. variegatus* had been collected only from *H. amplexicaulis* (Baranowski 1979; Baranowski and Slater 2005). Nevertheless, the presence of *I. variegatus* in Florida allowed us to study the interactions of a putatively monophagous herbivore with other plant species. Overall, *I. variegatus* had a narrow fundamental host range. Higher adult survivorship and longevity as well as higher fecundity were found on *H. amplexicaulis* compared to other species. Field and laboratory tests suggested that *H. amplexicaulis* plant quality and *I. variegatus* density affect the degree of spill-over to suboptimal host plants. We conclude that feeding on species other than *H. amplexicaulis* results in high fitness costs to *I. variegatus* which may be a strong selection pressure for maintenance of a narrow host range.

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