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Folivory and disease occurrence on *Ludwigia hexapetala* in Guntersville Reservoir, Alabama

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ABSTRACT

We report leaf feeding, disease occurrence, and associated indigenous herbivore/fungal pathogen communities on the introduced wetland species *Ludwigia hexapetala* at Guntersville Reservoir, AL. Plant populations were sampled on three dates from May to September 2014. A complex of indigenous herbivore and fungal taxa, mostly known from other *Ludwigia* spp., resulted in peak feeding and disease occurrence on 88% and 92% of sampled leaves, respectively. Herbivore damage declined over the growing season from 78 to 21% of sampled leaves, and disease symptom occurrence increased from 0 to 80%. Total leaf damage (percent leaf area) from both herbivory and disease was determined by software image analyses of floating and aerial leaves and reached 14% total reduction in photosynthetic tissues by September 2014. Aerial leaves were more commonly affected by disease symptoms, whereas floating leaves had a greater incidence of herbivore damage. Fourteen insect herbivore and seven fungal taxa were associated with *L. hexapetala* at Guntersville Reservoir. Despite the diverse assemblage of herbivores and fungi associated with *L. hexapetala*, damage was relatively low and the weed continues to persist as a nuisance species at this and other sites in southeastern United States. However, these results along with past surveys and literature review demonstrate the ability of common *Ludwigia* arthropod herbivores in the United States to host shift between *Ludwigia* spp. This begs the question as to how difficult it will be to locate potential biocontrol agents of *L. hexapetala* outside the United States that will be sufficiently host specific to present little to no risk to native *Ludwigia* spp.

Key words: aquatic weed, biological control, invasive species, natural enemies, new associations, plant pathogens.

INTRODUCTION

Ludwigia hexapetala (Hook & Arn.) Zardini, H. Y. Gu, & P. H. Raven (Uruguayan primrose-willow; Onagraceae) is one of three invasive *Ludwigia* spp. threatening wetland ecosystems in the United States (Grewell et al. 2016). Introduced from South America, *L. hexapetala* is now widespread in the southeastern United States, with disjunct populations in California and Oregon (Grewell et al. 2016). Invasive populations also exist outside the United States in France, Belgium, Italy, Spain, Greece, the United Kingdom, and The Netherlands (Dandelot et al. 2005, Thouvenot et al. 2013). Closely related *Ludwigia* are difficult to distinguish morphologically, and conflicting diagnostic characters have been presented by various authors (Nesom and Kartesz 2000). *Ludwigia hexapetala* is decaploid ($2n = 80$; Zardini et al. 1991), a characteristic that may contribute to relative invasiveness over other *Ludwigia* spp. (Pandit et al. 2011, Grewell et al. 2016).

Management of *L. hexapetala* in the United States is a concern as the number and distribution of infestations increase. *Ludwigia hexapetala* causes economic damage through disruption of flood control, irrigation water delivery, and mosquito control (Okada et al. 2009) and large seasonal biomass accumulation leads to anoxic conditions under the dense mats, which are harmful to aquatic animals (Dandelot et al. 2005). Manual and mechanical removal and herbicide application are currently used to control *L. hexapetala* spread and distribution with some success (Grewell et al. 2016). Although not operational, biological control of invasive *Ludwigia* spp. is under examination in South America as a self-sustaining alternative to other management technologies (Hernandez and Cabrera Walsh 2014).

Primary management of *L. hexapetala* in the United States is difficult to determine, but likely comes in the form of herbicide applications, of which there are efficacious formulas available (Richardson et al. 2008). Various *Ludwigia* spp. have been treated successfully with 2,4-D, diquat, triclopyr, glyphosate, imazamox, and imazapyr (Aquaplant 2014). Large infestations on the Kissimmee Chain of Lakes in Florida are currently being managed with a nonselective combination of glyphosate and flumioxazin, yet spraying large mature stands can result in extended periods of dead woody tissue remaining intact for months after application (M. Netherland, pers. comm.).

Ludwigia spp. in the United States have been previously examined for insect herbivores, leading to documentation of several species associated with the genus (e.g., Harms and Grodowitz 2009, 2012). Specialist herbivores have a narrow diet breadth and are generally restricted to hosts that are chemically related (often species within a single family or genus) (Jaenike 1990, Becerra 1997). Therefore, the question of whether herbivores of native *Ludwigia* taxa may be useful in managing populations of introduced *Ludwigia* has been posed (McGregor et al. 1996, Harms et al. 2012). Indeed, at least two common insect herbivores of *Ludwigia peploides*

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(Kunth) P.H. Raven, *Lysathia ludoviciana* Fall and *Altica litigata* Fall (Coleoptera: Chrysomelidae), but no fungal pathogens, have been documented to attack the invasive *Ludwigia grandiflora* Michx. Greuter & Burdet in the United States (McGregor et al. 1996, Carruthers et al. 2011). Despite the diversity of known *Ludwigia* herbivores in the United States and limited observations of impacts to *L. hexapetala*, little is known about specific impacts from native insects/fungi or whether their presence during invasion may limit successful establishment of invasive *Ludwigia* spp. (i.e., biotic resistance). For this reason, a study was designed to document herbivorous insects and potential fungal pathogens on *L. hexapetala* and quantify leaf damage over a single growing season. Information gathered during this study may be useful to provide a taxa list of indigenous invertebrates and fungi that could be studied further for use as management tools on *L. hexapetala* populations in the United States as well as providing additional insights into the ecology of *L. hexapetala* and native herbivores.

MATERIALS AND METHODS

Populations of *L. hexapetala* within three areas (Site 1, 34.70215 N, 85.9054 W; Site 2, 34.68713 N, 85.9312 W; Site 3, 34.63402 N, 85.9784 W) of Guntersville Reservoir, AL were examined on three dates (29 May, 24 July, and 4 September) during 2014. Sites were chosen based on historical occurrence of *L. hexapetala*, accessibility by airboat, and water depth, such that sampling could take place by wading into infestations. Infestations varied in size, from less than 0.40 hectare to approximately 4 hectares. Total linear distance from Site 1 to Site 3 was approximately 10 km, with Site 2 located between Sites 1 and 3.

On sampling dates, a general assessment of above-water infestation was made at each site within an approximate 6-m arc of the airboat. The airboat remained near the edge of the infestation so as not to disturb plants and insects prior to examination and collection. Measurements taken at each site included a visual estimate of total vegetative coverage, percent *L. hexapetala* coverage, percent *L. hexapetala* in creeping or emergent form, other plant species present, and a qualitative health assessment of *L. hexapetala* plants. Visual estimates of coverage were made by two observers and averaged.

Insects were collected by three methods, standardized across sites. At each site, a 5-min search was conducted by wading through plant material and hand-collecting any potential herbivores present. Although this method potentially disturbs the mat, and could cause flying insects to leave before we can to collect them, we did not observe such an exodus and believe that wading, in combination with our other collection techniques, was adequate to capture a range of associated herbivores. On the first sample date, 10 sweeps with a muslin cloth sweep net through emerged plant material was used to collect insects. On subsequent sample dates the net was no longer used because it did a poor job in the near-water habitat (i.e., plant material at the water's surface or near the surface) and was difficult to keep from wetting, thereby limiting insect collection. A battery-powered backpack Prokopack Aspirator[1] fitted with 0.5-mm mesh was also used to collect insects. On the first sample date, the vacuum method was only used at Site 2 because of equipment malfunction, but was used on the second and third dates at all sites. The 30-s vacuum collection was replicated three times per site. All insects were preserved in 70% ethanol and identified to the lowest practical taxon, usually species. Determination on which species represent potential herbivores of *L. hexapetala* was made by referring to literature and through discussions with taxonomists. Specimens are vouchered at the Engineer Research and Development Center (Vicksburg, MS) and Louisiana State University (Baton Rouge, LA)

Within sites, 25 to 35 aerial and floating leaves were haphazardly collected to quantify visible herbivore and pathogen damage. Leaves were blotted dry, kept cool during transport, and subsequently scanned with a digital flatbed scanner[2] at 600 dpi. Adaxial and abaxial leaf surfaces were imaged to determine whether damage occurred differentially based on leaf face. Once scanned, images were processed with the use of ImageJ image analysis software (Rasband 2014). To estimate undamaged leaf dimensions, leaf margins were hand drawn, with the use of an Intuos tablet/stylus PC interface,[3] with best judgment on all leaves that exhibited cutting or chewing damage to their perimeter. Images were subsequently processed by thresholding, a software technique that allows a user-defined color range (in this case, shades of green) to be isolated, producing a binary image in which all damaged portions of the leaf (i.e., nongreen) are coded white and undamaged portions are black. After thresholding, particle analysis was used to generate whole-leaf and whole-leaf without-damage measurements. These measurements were in turn used to calculate 1) leaf size (mm^2), and 2) percent leaf damage ([area removed from the leaf/ leaf size] $*$ 100). In addition, the proportion of sampled leaves with signs of herbivory or pathogens (referred to as damage occurrence) was determined for each site at each date by categorizing each scanned leaf as 'herbivore', 'pathogen', or 'both'. It was not possible to determine the proportion of damage within each category on each leaf because of the similarity of leaf color in herbivore or pathogen damaged leaves.

On the second and third sampling dates, leaves were collected from diseased plants and returned to the U.S. Army Engineer Research and Development Center (USA-ERDC, Vicksburg, MS) for pathogen isolation and identification. Leaves were not collected in May for pathogen detection because no disease symptoms were observed in the field. Sections of tissue that appeared to be diseased were excised and surface sterilized in 10% Clorox® for 1 min, then rinsed in sterile water. The pieces were subsequently inserted into slits cut into Martin's agar (Martin 1950) plates and incubated in the dark at room temperature (20 to 22 C) for approximately 1 wk. Fungal isolates that emerged from the diseased tissue were transferred to potato dextrose agar (PDA) and corn meal agar[4] slants for preservation. They were also plated onto PDA and potato carrot agar (Dhingra and Sinclair 1995) for identification. Viral and bacterial pathogens were not assessed.

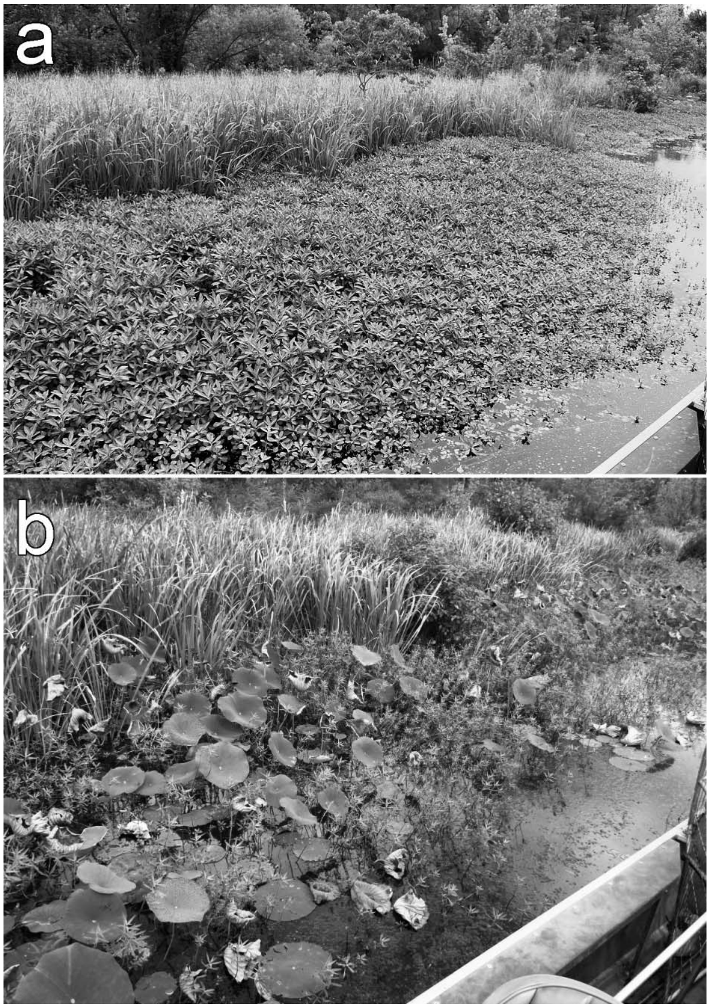

Figure 1. *Ludwigia hexapetala* at Guntersville Reservoir, AL in (a) May 2014 and (b) September 2014. Note substantial decline in *L. hexapetala* coverage with corresponding increase in other plant species.

STATISTICAL ANALYSES

Differences in leaf damage (percent damage and damage occurrence) between adaxial and abaxial leaf face and leaf types (floating or aerial) were determined by *t*-test[5] on data that were averaged by leaf type, per site and date. Statistical differences were determined at $P \leq 0.05$ and means are presented as $\bar{X} \pm$ SE. To determine whether pathogen and herbivory occurrences, as determined by leaf examination, were correlated, product-moment correlation analysis was used. Because occurrence data consisted of presence/absence records, we averaged all leaves per site, per date, and used the resulting means for the correlation analysis.

RESULTS AND DISCUSSION

Qualitative plant-health assessments throughout the study indicated healthy, mostly undamaged plants during the May and July sampling, but health declined by September, which was observed as near-total leaf abscission and decrease in plant abundance. It is unclear whether this represented a normal presenescent stage in *L. hexapetala* phenology or damage from herbivores and/or pathogens, which contributed to leaf loss. Both are plausible and many plant species are known to abscise damaged or diseased leaves (Faeth et al. 1981). However damage levels were low during this study, so the ultimate impact of herbivory is unclear.

In May, *L. hexapetala* stands consisted of approximately 78% aerial material that increased by September to > 95 %. Water surface coverage (*L. hexapetala* as a percent of all plants) decreased from 70% in May to 42% in September. Over time, surface coverage of *L. hexapetala* declined at sites from 61% in May to 25% in September (Figure 1). Surface coverage of alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.], another common species at sampling sites, increased from 10 to 28%. Additionally, percentage of open water also increased from 15 to 32% over time.

We hypothesized that upper and lower leaf faces would be differentially impacted by herbivores or pathogens because some insects are commonly found on the underside of leaves during the day and some fungal spore stages, particularly the rusts and powdery mildews, may more commonly be found on lower leaf surfaces (Agrios 2005). Data did not support this hypothesis, as we found no statistical differences in the damage assessment of upper and lower leaf faces for damaged leaves, so only upper leaf faces were used for analyses.

Overall, mean leaf damage was minimal (5% ± 0.4%) in May and reached a maximum of 14% ± 1.5% in September. Attack frequency on aerial and floating leaves was 85 and 75%, respectively (Figure 2a), whereas mean leaf damage of aerial leaves was twice that of floating leaves (10 vs. 5%). Aerial and floating leaves were differentially attacked by herbivory and disease; herbivory was more common on floating leaves (72% floating leaves, 38% aerial; $t = -2.28$, df $= 13$, $P = 0.04$; Figure 2b) but disease symptoms were not significantly different between the two (57% aerial leaves, 18% floating; $t = 2.018$, df $= 13$, $P = 0.06$; Figure 2c). Despite different attack rates between aerial and floating leaves, percent damage of leaves with herbivore damage was not different between leaf types. However, damage to leaves with disease symptoms was significantly greater on aerial leaves than floating (14% damage to aerial leaves versus 3% to floating; $t = 4.09$, df $= 7$, $P = 0.004$).

Although % leaf damage was seemingly low, occurrence was high; 77% of all leaves were damaged in May, 81% in July, and 87% in September. High leaf damage occurrence but low damage levels to leaves may be explained two ways: 1) herbivore/disease communities are generalists and only sometimes attack *L. hexapetala* but have a more-preferred host, or 2) leaf turnover rates in *L. hexapetala* are high enough to influence lower overall damage estimates. Because population impacts were visible by September, it is possible that rapid leaf turnover is responsible for our lower-than-expected damage estimates. Additionally, sap-feeding hemipterans were collected in relatively high numbers during July and September and their damage was difficult to identify or quantify, so it is possible we underestimated their impacts to *L. hexapetala* leaves.

It was uncommon for both categories of damage to be present on a single leaf, a trend that continued throughout

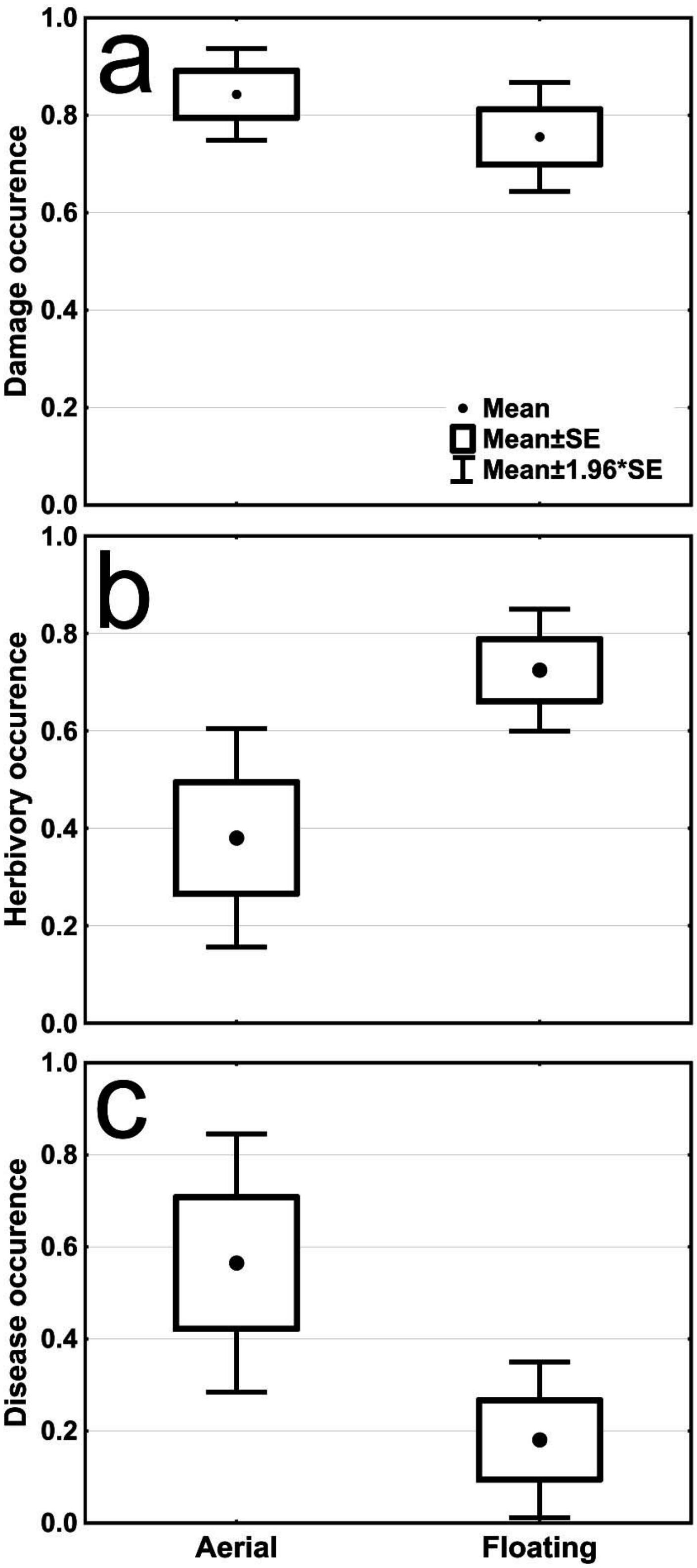

Figure 2. (a) Proportion of aerial or floating leaves exhibiting either type of damage, (b) herbivore damage alone, and (c) disease symptoms alone on leaves of *Ludwigia hexapetala* at Guntersville Reservoir. Plots represent means, boxes are standard error (SE) and bars are 1.96 ⁎ SE.

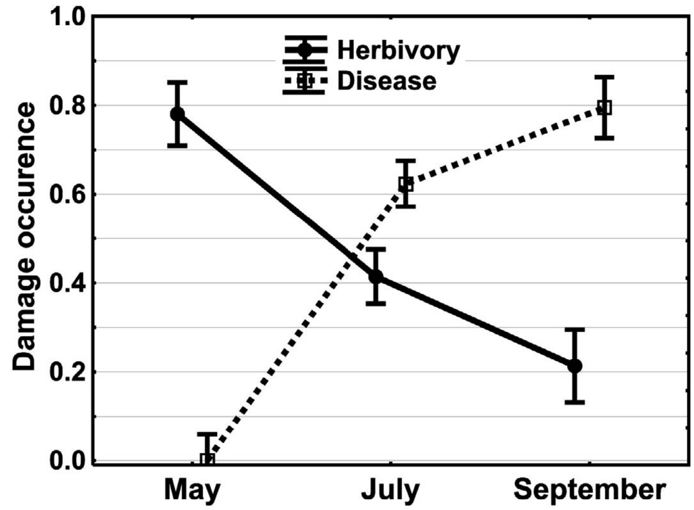

Figure 3. Proportion of leaves (mean ± SE) with herbivory and disease (both leaf types combined) over the course of the study period.

the study. In fact, herbivore and disease damage were negatively correlated. When examined together, 85% of damaged aerial and floating leaves displayed only one type of damage. Additionally, less than 20% of aerial and 40% of floating leaves exhibited signs of both herbivory and disease on the same leaf. As mentioned previously, it was difficult to assign damage from plant hoppers, so it is possible we underestimated the number of leaves that were attacked by both pathogens and herbivores. Overall, significantly more leaves with a single damage type were observed compared to both damage types ($t = 7.86$, df $= 16$, $P < 0.001$). Whether this is true resource partitioning between insects and fungi is unknown, although a clear declining trend in occurrence of herbivory during the study with a corresponding increase in disease occurrence was evident (Figure 3). Possibly, infected *L. hexapetala* leaves are less suitable for insect herbivores (Hatcher et al. 1995a). In contrast, damaged or stressed plants of some species may provide higher nutritive value (through increased soluble nitrogen) and contribute to increased performance of herbivores (Lewis 1979, White 1984, Hatcher et al. 1995a). Alternately, leaves/plants damaged by herbivory may not represent an ideal host for infectious fungi because the physical response of some plants to herbivory negatively impacts certain developmental stages of fungi (Hatcher et al. 1995b).

Leaf size

A benefit of using software analysis is the ability to measure leaf size quickly and accurately. Overall, mean leaf size of aerial and floating leaves declined over time. Aerial leaves declined from a mean of 1,388 ± 82 mm^2 in May to 426 ± 20 mm^2 by September. Decrease in average leaf size is probably related to high leaf turnover during times when combined effects of herbivory and disease produce leaf abscission (Hatcher 1996). Floating leaves, although not present in September, also decreased in size, from 930 ± 28 mm^2 in May to 402 ± 19 mm^2 in July.

Table 1. Insect herbivores and fungi associated with *L. hexapetala* during May, July, September at Guntersville Reservoir. Shaded rows indicate taxa that have been previously reported from *Ludwigia* spp. Collection techniques include hand collection (HC), aspiration (A), and sweep net (SW).

Taxon	May	July	September	Primary Collection Technique
Insects				
Coleoptera: Curculionidae				
Perigaster cretura	×	×	×	HC
Auleutes sp.	×		×	A
Tanyspherus lemnae[1]	×			A
Coleoptera: Chrysomelidae				
Altica sp.	×			A
Lepidoptera: Crambidae				
Elophila obliteralis	×	×		A
Hemiptera: Cicadellidae				
Draeculacephala inscripta	×	×	×	A
Graminella nigrifrons		×		A
Homalodisca vitripennis	×			SW
Hemiptera: Delphacidae				
Isodelphax basvitta		×	×	A
Pissonotus sp.		×	×	A
Hemiptera: Miridae				
Lygus lineolaris		×		A
Diptera: Ephydridae				
Hydrellia sp. a			×	A
Orthoptera: Tettigoniidae				
Conocephalus sp.		×	×	A
Fungi				
Euascomycetes				
Cylindrocarpon heteronema		×		
Alternaria alternata		×	××	
Phoma sp.		×		
Curvularia lunata			×	
Pestalotiopsis guepinii			×	
Dematiaceous ascomycete			×	
Pithomyces chartarum			×	

[1]*Tanyspherus lemnae* (Fabricius) (duckweed weevil) was likely an incidental collection.

Disease

Seven fungal species were isolated from *L. hexapetala* leaves during the study (Table 1). Of these, only the genus *Alternaria* had been previously reported as occurring on a *Ludwigia* sp., specifically *Ludwigia decurrens* (Walter) (Farr et al. 1989). *Alternaria alternata* (Fr.) Keissler, *Curvularia lunata* (Wakker) Boedijn, and *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis are all cosmopolitan species occurring on a variety of plants and other substrates (Ellis 1971) and were probably secondary invaders. *Cylindrocarpon heteronema* (Berkeley & Broome) Wollenweber, *Pestalotiopsis guepinii* (Desm.) Stey., and *Phoma* sp. are all known pathogens often on woody hosts (Sinclair et al. 1987, Farr et al. 1989, Agrios 2005) and could have caused lesions on *L hexapetala*. Because the dematiaceous Ascomycete (Table 1) did not sporulate, it was impossible to know if it was in a genus that contained pathogenic species.

Although no disease symptoms were observed in May, by July 89% of aerial and 36% of floating leaves exhibited symptoms, contributing to a change in overall (combined herbivory and disease) leaf damage of 5% in May to 14% in September. Of leaves that had only disease symptoms (and no signs of herbivory), percent damage ranged from a mean of 11% in July to 17% in September. Mean leaf damage of aerial leaves was 15%, whereas floating leaves was 5%; aerial leaves were nearly six times as likely to show disease symptoms as floating leaves (186 aerial leaves showing symptoms versus 38 floating). These analyses include floating leaves from only May and July because few floating leaves were present at sites during the September sampling, so collections were not made.

Insect herbivores

In contrast to an increase in disease occurrence during the study, herbivore damage ranged from 75% (May) to 21% (September) on aerial leaves and 81% (May) to 64% (July) on floating leaves.

The type and number of collected insect taxa were directly related to the sampling technique used. For example, *Draeculacephala inscripta* Van Duzee (Hemiptera: Cicadellidae) adults were overwhelmingly collected by aspiration (312 specimens by aspiration vs. 20 by hand collection), whereas adult and larval *Perigaster cretura* (Herbst) (Coleoptera: Curculionidae) were primarily collected by manual searching (97 specimens by hand collection, 8 by aspiration). Only four taxa [*P. cretura*, *Elophila obliteralis* (Walker) (Lepidoptera: Crambidae), *D. inscripta*, and *Conocephalus* sp. (Orthoptera: Tettigoniidae)] were collected by hand collection; although *P. cretura* was collected more efficiently by hand, others were collected in higher numbers by aspiration. Additionally, 14 taxa (Table 1; 3 curculionid, 1 chrysomelid, 1 crambid, 6 hemipteran, 1 orthopteran, and 1 ephydrid species) were collected by aspiration. Although not surprising, the differences in taxa collected by the different sampling techniques emphasizes the need for balanced sampling techniques when conducting herbivore surveys to capture the range of taxa associated with the study plant.

Despite the diversity of herbivores collected on *L. hexapetala* during this study, most were generalists and so their value as a biological control tool is dubious. Additionally, several are known previously from other *Ludwigia* spp. (Harms and Grodowitz 2009, 2012). For example, *Pissonotus piceus* Van Duzee (Hemiptera: Delphacidae) has been previously reported from *Ludwigia* sp. and *Polygonum* sp. (Haag et al. 1986). *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae; tarnished plant bug) is a generalist, with over 300 reported host species (Young 1986). *Perigaster cretura* is known from at least four *Ludwigia* spp., including *Ludwigia alterniflora* L., *Ludwigia octovalvis* (Jacq.) P.H. Raven, *L. peploides*, and *Ludwigia repens* Forst (Mitchell and Pierce 1911, Knab 1915, Clark 1976, Center et al. 1999, Harms and Grodowitz 2012). *Draeculacephala inscripta* (the waterlettuce plant hopper) is known to feed on a number of aquatic plants, including *L. peploides* (Harms and Grodowitz 2009, Center et al. 1999). The large numbers collected during our study (over 100 individuals in July), coupled with the collection of multiple life stages, suggest that *L. hexapetala* is more than adequate as a host plant for *D. inscripta*. Only collected during the May sampling, *Altica* sp. (Coleoptera: Chrysomelidae) is probably oligophagous; it has been reported from at least three *Ludwigia* hosts and other species in multiple families (Center et al. 1999, Pettis and Braman 2007, Harms and Grodowitz 2012). *Graminella nigrifrons* Forbes (Hemiptera: Cicadellidae) has not been reported previously from *Ludwigia* spp. but has a large host range (Redinbaugh et al. 2002). However, *G. nigrifrons* is known to transmit viruses in agricultural crops (Redinbaugh et al. 2002) so could potentially contribute to disease spread in *L. hexapetala*. Despite previous reports of *Lysathia ludoviciana* (Fall) (Coleoptera: Chrysomelidae) on *L. hexapetala* in Alabama (McGregor et al. 1996), we did not collect any specimens during this study. *Lysathia ludoviciana* has been reported from several host plant species (Haag et al. 1986) so is not an ideal biological control candidate.

Of the herbivore taxa represented by our sampling, *Auleutes* sp. (Coleoptera: Curculionidae) may have value towards invasive *Ludwigia* management. *Auleutes bosqi* Hustache feeds on *L. peploides, L. grandiflora, L. hexapetala, Ludwigia elegans* (Cambess) H.Hara, and *L. leptocarpa* (Nutt.) H. Hara in South America (Hernandez and Cabrera Walsh 2014) and, although previously suggested as a biocontrol agent (Cordo and Deloach 1982), appears to have a broad host range within *Ludwigia*. We have previously collected an unknown *Auleutes* sp. from *L. peploides* in southeastern United States (Harms and Grodowitz 2012). Host specificity of this species is unknown; taxonomy of this group has recently undergone revision and proper identification is currently being sought.

There is clear crossover potential of native folivorous insects between *Ludwigia* spp., including several herbivore species we have previously identified from the closely related, and questionably native (Grewell et al. 2016), *L. peploides*. None of the species associated with *L. hexapetala* in this study appear to be host specific, but records are lacking for several. These findings do beg the question as to whether insect natural enemies may be identified outside the United States that could be used domestically and not pose a risk to the nearly 30 native *Ludwigia* spp. (USDA, 2014).

It may be prudent to examine indigenous herbivore and pathogen species for their usefulness in managing introduced *Ludwigia* spp. because of their availability, abundance at some sites in southeastern United States, and apparent absence in western states, where *Ludwigia* spp. are spreading and causing economic and environmental damages. This approach has been used previously with other species (e.g. the introduction of *Prokelisia marginata* Van Duzee (Hemiptera: Delphacidae) from California into Washington for control of *Spartina alterniflora* Loisel.; Grevstad et al. 2003). The use of native and naturalized insects for control of invasive *Ludwigia* spp. has been considered previously (Freedman et al. 2007, Harms and Grodowitz 2012) and likely faces similar hurdles to classical biological control introductions, including risk assessment and host-specificity studies.

An undeniable gap in our study is that below-water tissues were not examined for herbivore/disease damage. It has been reported that up to 80% of biomass in *L. hexapetala* exists as submersed tissue (Grewell et al. 2016), which represents a substantial energy resource for primary consumers. However, successful biological control of other species (e.g., alligatorweed) has been obtained by agents that attack only above-water portions (Buckingham 1996) and it has been suggested that when management (i.e., herbicide application or attack by biological control agents) coincides with depletion of energy stores for rapid growth (as occurs in spring or following dieback from herbicide application), control is most likely (Pesacreta and Luu 1988). Although levels of folivory observed in the current study may contribute to population-level impacts to *L. hexapetala*, agents that attack underwater structures may provide additional control if they are able to reduce vegetative spread or nutrient uptake. Therefore, a reasonable future research priority is examination and quantification of impacts to these plant parts.

SOURCES OF MATERIALS

[1]Prokopack Aspirator Model 1419, John W. Hock Company, Gainesville, FL.

[2]Digital flatbed scanner, Canon CanoScan LiDE 30, Canon USA, New York.

[3]Intuos tablet/stylus PC interface, Wacom, Vancouver, WA.

[4]Corn meal agar, Difco, Detroit, MI.

[5]Statistica 64 version 12, StatSoft Inc., Tulsa, OK.

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