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INVASION NOTE

Characterizing an invasion assemblage: first comparison of insect communities on native and introduced subspecies of *Phragmites australis* in Ontario, Canada

R. B. deJonge · M. J. McTavish · S. M. Smith · R. S. Bourchier

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Abstract Introduced *Phragmites* (*Phragmites australis* ssp. *australis*) forms part of an invasion assemblage in North America that includes non-native insect herbivores and parasitoids, some of which are now found on both the introduced and native subspecies of *Phragmites* (*P. australis* ssp. *americanus*). This insect assemblage is key to understanding the impact of *P. australis* invasion and interpreting the efficacy of biological control used against introduced *P. australis*. Our study provides the first dedicated comparison of insect assemblages associated with native and introduced *P. australis* in Canada. From a 2016 to 2017 survey of 28 geographically paired sites across Ontario, Canada, fourteen insect taxa were recorded from both subspecies. Genotype had no effect on α-diversity but stem attack rates from at least one herbivore were higher on native populations than on paired introduced populations (+ 18.6%). We report the first record of *Chaetococcus phragmitis* (Homoptera: Pseudococcidae) and *Rhizedra lutosa* (Lepidoptera: Noctuidae) in Canada and of *R. lutosa* and *Lasioptera hungarica* (Diptera: Cecidomyiidae) on native *P. australis* in North America.

Keywords *Archanara neurica* · *Lenisa geminipuncta* · *Phragmites australis* ssp. *australis* · *Phragmites australis* ssp. *americanus* · Biological control · Biocontrol

Introduction

Phragmites australis ssp. *australis* Cav. Trin ex Steud. (common reed, hereafter "introduced *P. australis*") is one of the most invasive plants in North America, forming dense monocultures that alter wetland function and biodiversity (Wails et al. 2021), including displacement of native *P. australis* ssp. *americanus* Saltonst., P.M. Peterson & Soreng (hereafter "native *P. australis*") (Saltonstall 2002). Classical biological control of introduced *Phragmites* using two stem-boring noctuid moths from Europe (*Archanara neurica* (Hübner) and *Lenisa geminipuncta* (Haworth)) has recently been approved in Canada and is continuing through the review process in the United States (Blossey and Casagrande 2016; Blossey et al. 2018).

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R. B. deJonge · M. J. McTavish (✉) · S. M. Smith
Faculty of Forestry, University of Toronto, 33 Willcocks St., Toronto, ON M5S 3B3, Canada
e-mail: michael.mctavish@alum.utoronto.ca

R. S. Bourchier
Agriculture and Agri-Food Canada-Lethbridge Research and Development Centre, 5403-1st Avenue S., Lethbridge, AB T1J 4B1, Canada

To effectively implement a biological control program and understand the impacts of introduced *P. australis* on native biodiversity, it is important to characterize the insect communities associated with both native and introduced *P. australis* genotypes (Blossey et al. 2018). Characterizing and comparing these communities will provide a baseline of insect herbivory, allow detection of changes in insect communities after the release of biological control agents, identify species that may interact positively or negatively with biological control agents, and identify new potential biological control agents (Lambert et al. 2007; Cronin et al. 2015; Allen et al. 2015, 2017; Bhattarai et al. 2017).

Research in the United States has identified at least 26 species of insect herbivores and parasitoids associated with both plant genotypes, many of which are mono- or oligophagous on *P. australis* (Tewksbury et al. 2002). At least 21 of these species are of European origin, representing a broader "invasion assemblage" consisting of introduced *P. australis* and a subset of its native insect communities that has been introduced alongside it (Tewksbury et al. 2002). However, prior to confirmation of distinct native and introduced *P. australis* lineages in North America (Saltonstall 2002), records do not provide subspecies-specific descriptions of insect assemblages (e.g., Tewksbury et al. 2002) and newer studies that have done so are focused in the United States (e.g., Cronin et al. 2015; Allen et al. 2015; Bhattarai et al. 2017) and have large geographic gaps.

Our study provides the first dedicated comparison of the insect assemblages associated with native and introduced *P. australis* in Canada. We address two primary research questions: (1) What insect taxa are present on native and introduced *P. australis* in Canada? And (2) Are certain insect taxa more or less common on native or introduced *P. australis*?

Methods

28 sites with *P. australis* arranged in 14 pairs of nearby native and introduced populations were identified across a 3.13° latitudinal gradient in southern Ontario, Canada from historical records of *P. australis* populations and roadside searches (Catling and Mitrow 2011; EDDmaps 2017). Each site pair consisted of one patch of native and introduced *P. australis* (mean area: 700 m^2) no more than 1.5 km apart (see Online Resource 1 for site details and Online Resource 2 for a site map). Between September and November 2016, sites were sampled using five quadrats (0.5 m × 0.5 m) spaced equidistant around the roadside edge and placed 5 m into the patch. All stems were cut to the substrate, counted, sealed into paper bags, and stored in a cooler until dissection. Mean live stem density across all quadrats was $40 \pm 26\ m^{-2}$ (mean ± SD) and did not differ between native and introduced populations (Wilcoxon Signed-Rank Test for symmetric, non-normal data, $V = 64$, $p = 0.501$). In July 2017, all sites were re-visited to collect root-feeding insects by digging holes ($\sim$ 0.5 m × 0.5 m × 0.5 m, volume 0.125 m^3) with a shovel at the site of each aboveground quadrat. Because of high standing water levels, roots were collected at only 18 of the 28 sites (nine of each genotype). Roots and rhizomes were sealed in plastic bags and stored in a cooler until dissection.

In the lab, each stem, root, and rhizome was carefully cut lengthwise (i.e., top to bottom) and pried apart while inflorescences were shaken over a sheet of white paper for 5 s to search for insects. Adult insects were preserved in 95% ethanol and larvae reared to emergence in the lab in 3.5 L plastic containers covered with mesh and provided weekly fresh *P. australis* stem and root tissue. Insects were identified over a period of $\sim$ 3 months using an online key prepared by Patrick Häfliger at CABI (https://www.cabi.org/phragmites/key_online.html) and by specimens sent to the Canadian National Collection. Specimens were identified to species where possible and variably to genus, family, or order if not. Therefore, all results and subsequent analyses refer to different levels of taxonomic resolution and broadly consider diversity across these taxonomic levels.

To address our research questions of taxon presence/absence and because it is more meaningful for comparing taxa that differ greatly in abundance (e.g., 1–100 + individuals per internode), insects were recorded based on overall presence/absence at each site and stem attack rate (% of stems collected from a quadrat containing at least one individual). α-diversity was calculated from stem attack rates by taxon using richness R, Shannon's Diversity Index H, and Simpson's Diversity Index D and compared between paired introduced and native *P. australis* sites using paired t-tests. β-diversity was calculated using Søresen's Dissimilarity Index (β_{SOR}) for multi-site comparisons across all sites, all introduced sites, and all native sites, and pairwise comparisons between paired sites (Baselga 2013). Presence/absence of each taxon was compared across sites using Fisher's Exact Test (a test of independence similar to Pearson's chi-squared test but more robust to smaller sample sizes). Stem attack rates of each taxon, all herbivores grouped together, and all parasitoids together were compared between paired native and introduced sites using Wilcoxon Signed Rank Tests for non-normal, symmetric data and Sign Tests for non-normal, non-symmetric data.

All analyses were carried out at $\alpha = 0.05$ in R (R Core Team 2019), including the base *stats* package for Fisher's Exact Test, Wilcoxon Signed Rank Test, and Sign Test, *vegan* package for α-diversity indices (Oksanen et al. 2020), and *betapart* package for β-diversity indices (Baselga et al. 2021). Means in the text are presented as mean ± standard deviation (SD). Figures were prepared in R and Microsoft Excel. Data can be accessed online through the Zenodo repository (deJonge et al. 2021).

Results

Fourteen insect taxa (with Acari counted separately in stems and florets) were recorded across native and introduced *P. australis* populations, including 11 herbivorous taxa and 3 parasitoids (Table 1). The grain midge *Tetramesa phragmitis* Erdös (Hymenoptera: Eurotymidae) was the most common taxon (found at 64–71% of sites), while *Chaetococcus phragmitis* Marchal (Homoptera: Pseudococcidae) was the rarest, found only in a single introduced *P. australis* population. Overall attack rates were highest from gall flies *Lipara* spp. (Diptera: Chloropidae) (10–22% of stems).

Overall, genotype had no effect on α-diversity in terms of taxonomic richness R (5.3 ± 2.1, paired t-test $t = 0.97$, $df = 13$, $p = 0.348$), Shannon's Diversity Index H (1.23 ± 0.45, paired t-test $t = 0.74$, $df = 13$, $p = 0.472$), or Simpson's Diversity Index D (0.62 ± 0.19, paired t-test $t = 1.11$, $df = 13$, $p = 0.288$). In terms of β-diversity, Sørensen's Index of Dissimilarity β_{SOR} indicated relatively high dissimilarity across all sites (0.87), native sites (0.79), and introduced sites (0.82), with lower dissimilarity between geographically paired introduced and native sites (0.39 ± 0.18).

Insect assemblages had substantial overlap but also differed between native and introduced *P. australis* populations based on taxon presence/absence (Table 1) and stem attack rates (Fig. 1). *Lipara* spp. were found at 2.4 times as many native sites as introduced sites, while *Rhizedra lutosa* Hübner (Lepidoptera: Noctuidae) was only found at native sites (five out of nine sampled). Attack rates for at least one herbivore were higher on native *P. australis* populations than paired introduced populations (+ 18.6%, Wilcoxon Signed-Rank test, $V = 98$, $p = 0.023$) but did not differ for parasitoids (Sign Test, $p = 1.00$) (Fig. 1). Attack rates of the grass flies Chloropidae (Diptera: Chloropidae) and *Lipara* spp. were 21.4% and 11.4% higher respectively on native populations than on paired introduced populations, while attack rates of the gall midge *Lasioptera hungarica* (Diptera: Cecidomyiidae) were 5.9% higher on introduced populations (Fig. 1).

Discussion

In the first dedicated survey comparing insect assemblages on native and introduced *P. australis* in Ontario, Canada, we recorded fourteen taxa (including stem- and floret-collected Acari counted separately), all of which have been previously documented on *P. australis* in North America (Tewksbury et al. 2002; Ahee et al. 2013; Allen et al. 2017). Our survey is the first record of: (a) the reed mealy bug, *C. phragmitis*, in Canada; (b) the noctuid moth, *R. lutosa*, in Canada; (c) *R. lutosa* on native *P. australis*, formerly found on unspecified but likely introduced *P. australis* (Casagrande et al. 2003); and (d) *L. hungarica* on native *P. australis*, formerly documented only on introduced (Park and Blossey 2008) and hybrid populations (Saltonstall et al. 2014).

Similar to the United States (Tewksbury et al. 2002), the insect communities observed on both native and introduced *P. australis* comprise an invasion assemblage of predominantly non-native herbivores and parasitoids (Table 1). Despite no differences in overall α-diversity between genotypes and relatively low β-diversity dissimilarity between paired introduced and native sites, there were taxon-level insect differences between paired populations. Although

Table 1 Taxonomy, feeding location and specificity (M: monophagous, O: oligophagous, P: polyphagous), and status in North America (Tewksbury et al. 2002; Ahee et al. 2013), and presence (% of sites) and attack rates (% of stems, mean ± standard deviation) of insect taxa at native and introduced *P. australis* sites (n = 14 sites per genotype and taxon, except for n = 9 sites for *R. lutosa*)

Taxon	Order: Family	Status	Feeding		Presence			Attack		
			Location	Specificity	Native (%)	Introduced (%)	*p* value	Native (%)	Introduced (%)	*p* value
Tetramesa phragmitis (grain midge)	Hymenoptera: Eurotymidae	Introduced	Stem (parasitoid)	M	71	64	1.000	5.5 (5.9)	5.0 (6.6)	1.000[*]
Lipara spp. (gall flies)	Diptera: Chloropidae	Introduced	Stem	M	**86**	**36**	**0.0183**	**21.5** (25.6)	**10.1** (19.3)	**< 0.001**
Gambrus ultimus (parasitic wasp)	Hymenoptera: Ichneumonidae	Native (?)	Stem (parasitoid)	–	57	57	1.000	6.5 (10.9)	3.9 (5.2)	1.000
Stenodiplosis phragmicola (gall midge)	Diptera: Cecidomyiidae	?	Floret	–	64	43	0.450	5.8 (8.1)	4.7 (6.8)	0.754
Thysanoptera (thrips)	Thysanoptera: Thripidae	–	Floret	–	57	50	1.000	13.2 (18.9)	7.2 (16.0)	0.754
Aphididae (aphids)	Hemiptera: Aphididae	–	Floret	–	43	57	0.706	8.4 (22.9)	3.1 (4.0)	1.000
Lasioptera hungarica (gall midge)	Diptera: Cecidomyiidae	Introduced	Stem	M	29	57	0.252	**1.1** (2.2)	**7.1** (9.3)	**0.0244**[*]
Acari [florets] (mites)	–	–	Floret	–	29	50	0.440	2.3 (5.7)	2.8 (5.0)	0.180
Eurytomidae (Chalcid wasps)	Hymenoptera: Eurytomidae	–	Stem (parasitoid)	–	36	36	1.000	2.6 (4.8)	1.8 (3.1)	0.688
Microlasioptera flexuosa (small gall midge)	Diptera: Cecidomyiidae	Introduced	Stem	M	36	21	0.678	6.1 (13.5)	0.9 (2.0)	1.000
Chloropidae (grass fly)	Diptera: Chloropidae	Introduced	Stem	M, P	43	7	0.0768	**21.5** (30.4)	**0.1** (0.5)	**0.0312**
Rhizedra lutosa (large wainscot moth)	Lepidoptera: Noctuidae	Introduced	Rhizome	M	**56**	**0**	**0.0294**	–	–	–
Acari [stems] (mites)	–	–	Stem	–	14	14	1.000	0.5 (1.3)	1.1 (3.0)	1.000

Table 1 continued

Taxon	Order: Family	Status	Feeding		Presence			Attack		
			Location	Specificity	Native (%)	Introduced (%)	*p* value	Native (%)	Introduced (%)	*p* value
Chaetococcus phragmitis (reed mealybug)	Homoptera: Pseudococcidae	Introduced	Stem	O	0	7	1.000	0.0 (0.0)	2.7 (10.1)	1.000

P values are provided for comparing presence (Fisher's Exact Test) and attack (Wilcoxon Signed Rank Tests [*] or Sign Tests) between native and introduced sites with significant p-values and data in bold

non-native, these taxa were generally more prevalent on native *P. australis* populations compared to geographically paired introduced populations in terms of both presence (*Lipara* spp., *R. lutosa*) and stem attack rates (*Lipara* spp., Chloropidae) (Fig. 1, Table 1). The greater susceptibility of native *P. australis* to this invasion assemblage compared to the introduced subspecies has been observed in other populations in the USA (Lambert and Casagrande 2007; Lambert et al. 2007; Park and Blossey 2008; Allen et al. 2017). These results are consistent wit the hypotheses of other research that apparent competition (Bhattarai et al. 2017) and enemy release (Keane and Crawley 2002) are key mechanisms underlying the invasion of introduced *P. australis* in North America.

These data provide an important baseline understanding of insect communities on native and introduced *P. australis* that is key to interpret the impacts of new biological control agents (Blossey et al. 2018). These baseline data should be used to assess potential shifts in insect communities following the release of biological control agents. For example, changes in plant architecture, such as an increase in thin-stemmed side shoots produced by biocontrol agent feeding (Tscharntke 1990), may have negative impacts on species that need larger stem diameters (e.g., *Lipara* spp.) (Lambert et al. 2007). These data may also be used to inform tests of other interactions between biological control agents and the existing insect community that may influence the overall effectiveness of management. For example, while the noctuid *R. lutosa* has been determined to have minimal impacts on introduced *P. australis* in isolation, if found in these populations it may have a synergistic impact when combined with biological control, as has been observed with multiple noctuid species in Europe (Häfliger et al. 2006).

Overall, this first Canadian comparison of introduced and native *P. australis* insect communities provides important baseline data that should be supplemented by ongoing monitoring of insect communities of *P. australis* before, during, and after biological control.

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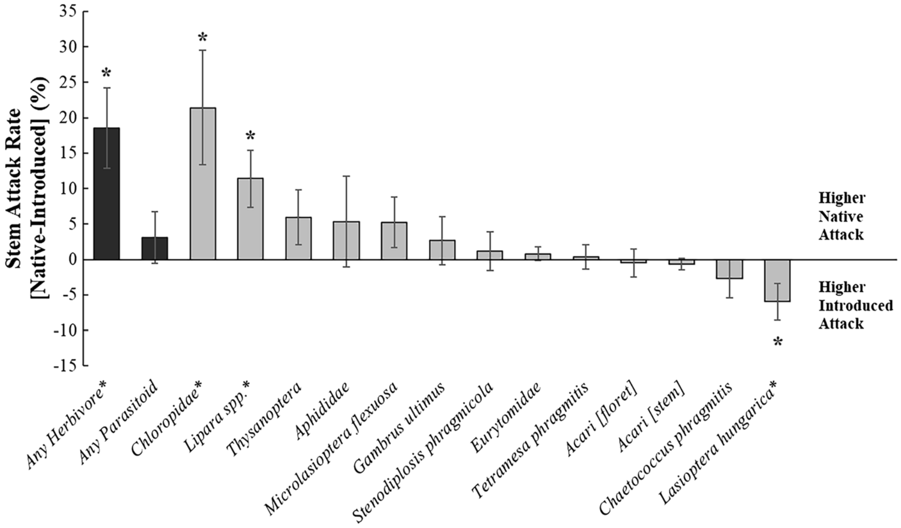

Fig. 1 Differences in attack rates between paired native and introduced *P. australis* (% of stems ± standard error) (n = 14 patch pairs). Black bars depict taxa grouped as herbivores or parasitoids; light grey depict separate taxa. Asterisks indicate differences significantly different from zero based on Wilcoxon Signed Rank or Sign Tests (Table 1)

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Availability of data and material The datasets generated and/or analysed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.5589793.

Code availability Not applicable.

Declarations

Conflict of interest None.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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