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Host specificity and risk assessment of *Archanara geminipuncta* and *Archanara neurica*, two potential biocontrol agents for invasive *Phragmites australis* in North America

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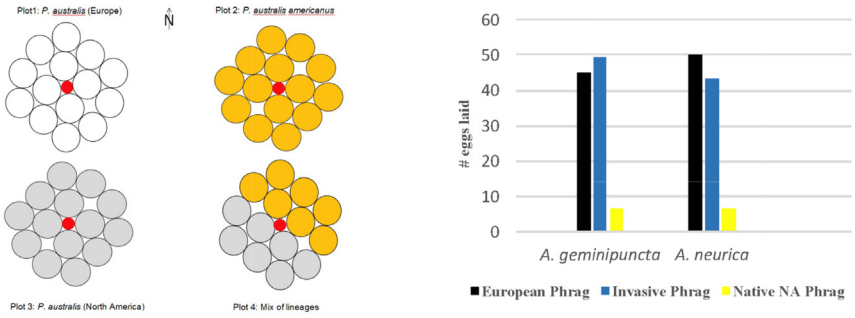
GRAPHICAL ABSTRACT



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ABSTRACT

Invasive *Phragmites australis* is widespread in North America and despite decades of management and large annual expenditures (> 5 million US$) using physical and chemical means, local populations and the species range are expanding. Allowing continued expansion does not only threaten native wetland biota but also an endemic North American subspecies *Phragmites australis americanus*. We used extensive multi-pronged investigations in Europe and North America to evaluate host specificity and impact of two European stem mining noctuid moths, *Archanara geminipuncta* and *A. neurica*. Both moth species are specific to the genus *Phragmites* and both show a very strong, but not absolute, preference for invasive *P. australis* over endemic *P. australis americanus*. No-choice tests or tests in small cages provided inconsistent results, but both moths showed consistently high preferences for introduced *P. australis*. Open field multiple-choice oviposition tests affirmed this; moths laid 6.5% of their eggs on native *P. australis americanus*. The native subspecies is further safeguarded by increased mortality of eggs and larvae when laid on, or developing in *P. australis americanus*. *Phragmites* populations in the southern US, particularly along the Gulf of Mexico, occur outside the climate range of these two temperate moth species. We consider potential threats to *P. australis americanus* demography due to *A. geminipuncta* and *A. neurica* attack to be far smaller than allowing expansion of invasive *P. australis* to continue. We therefore recommend release of these two biocontrol agents in North America.

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1. Introduction

Phragmites australis (Cav.) Trin. ex Steud. (Arundinoideae: Poaceae, common reed) is a clonal wetland grass with an almost cosmopolitan distribution (Clevering and Lissner, 1999; Lambertini et al., 2012b). Arrival of European *P. australis* added a third lineage to existing diversity of the genus in North America that includes the endemic subspecies *P. australis americanus* Saltonstall, P.M. Peterson and Soreng and a lineage of unresolved origin distributed along the Gulf Coast and ranging into South America *P. australis berlandieri* (E. Fourn.) C.F. Reed (Guo et al., 2016; Lambertini et al., 2012a; Saltonstall et al., 2004). Introduced genotypes of European origin have swept through much of temperate North America since the early 1800s but have not expanded into Mexico (Colin and Eguiarte, 2016; Saltonstall, 2002; Saltonstall and Meyerson, 2016). Hybridization between European and native North American lineages has been confirmed in wetlands in New York and Nevada (Saltonstall et al., 2014; Saltonstall et al., 2016).

Rapid range expansion of European *P. australis* in North America has worried land managers for decades due to anticipated negative ecological impacts (Marks et al., 1994). For decades, wetland managers have tried to control *P. australis* without notable success. Only restoration of regular tidal inundation at typical salt water salinity concentrations (3.5%) has been shown to successfully suppress the species in coastal areas (Chambers et al., 1998). Elsewhere, short-term suppression is possible using herbicides, but eradication is extremely difficult and only achievable for the smallest populations ($< 100\ m^2$) (Lombard et al., 2012; Quirion et al., 2018). US annual herbicide expenditures for *P. australis* control were estimated at US$ 4–5 million before 2010 (Martin and Blossey, 2013). From 2010 to 2015 The Great Lakes Restoration Initiative alone provided > US$25 million for *P. australis* management (https://www.glri.us//). Despite these large investments, assessments focus on monetary expenditure and areas treated while failing to report ecological outcomes (GLRI, 2016; Hazelton et al., 2014; Martin and Blossey, 2013). Self-reporting by managers indicated that while short-term improvements (measured as *P. australis* cover reductions or area occupied) were observed, improving ecological conditions for native species over extended time periods were elusive (Martin and Blossey, 2013). Recent analyses suggest that herbicide treatments may exacerbate threats to native species, and in some instances native species of conservation concern did better in the presence of introduced species than in areas treated with herbicide (Kettenring and Adams, 2011; Lazaran et al., 2013; Louhaichi et al., 2012; Rinella et al., 2009).

High expenditures, continued range expansion, lack of long-term ecological improvements following treatments, and the desire to replace potentially harmful effects of repeated herbicide use on wetland biota and function triggered research to assess potential for classical biocontrol in 1998 (Tewksbury et al., 2002). Through the past two decades, the biocontrol program has faced some unique challenges, including discovery of the endemic subspecies *P. australis americanus* in 2002 (Saltonstall, 2002). Consequently, any potential biocontrol agent must show specificity at the sub-species level, as currently assigned by taxonomists. Furthermore, literature reviews and surveys identified > 150 herbivores and inquilines attacking *P. australis* in Europe, while 26 species are reported to attack *Phragmites* lineages in North America, and 21 of these are accidental introductions (Tewksbury et al., 2002). Two native species and a Japanese scale (*Nipponaclerda biwakoensis* Kuwana) have been added to the known North American *Phragmites* fauna in the last decade (Ahee et al., 2013; Eichiner et al., 2011; Ramsey and Rangoonwala, 2017). Any purposeful introduction of biocontrol agents would increase already complex interactions among wetland plants, herbivores, natural enemies of herbivores and their predators (Blossey, 2003a).

We initially chose nine European herbivores for further investigations based on feeding niche, damage inflicted, and reported host-specificity but narrowed this list to the four most promising species (Häfliger et al., 2005; Häfliger et al., 2006a; Häfliger et al., 2006b; Tewksbury et al., 2002). Here we assess host specificity of *Archanara geminipuncta* (Haworth) and *Archanara neurica* (Hübner), two stem mining noctuid moths we prioritized because they are widespread, abundant, and with the largest documented and cumulative impact (Häfliger et al., 2006b). The particular focus of our investigations was assessment of potential risks to populations and demography (here defined as an assessment of how environmental factors and ecological interactions, for example competition, disease or herbivory, may affect plant populations by altering survival, growth, development and reproductive rates of plant individuals) of *P. australis americanus*.

2. Methods and materials

2.1. Study species

2.1.1. Phragmites australis

Phragmites australis is able to thrive under a wide range of conditions from oligohaline tidal to freshwater wetlands, marshes, ditches and roadsides (Kettenring et al., 2012; Kettenring et al., 2016; McCormick et al., 2010). Clonal expansion and new shoot emergence continues throughout the growing season with two thirds of biomass allocated to extensive rhizome-systems. Seed set is variable and not all populations produce viable seed each year, but seeds are important in colonization of new habitats (Albert et al., 2015; Kettenring et al., 2016; McCormick et al., 2010).

Different *Phragmites* lineages in North America can be distinguished genetically (Lambertini et al., 2012a; Lambertini et al., 2012b; Saltonstall et al., 2014; Saltonstall et al., 2004) and using morphological traits (Blossey, 2003b). Although there is regional morphological variation in *P. australis americanus* (Blossey, unpublished data), all native genotypes start to lose their leaf sheaths in mid- to late-summer beginning at the lowest internodes. Stems continue to drop their leaves and also their leaf sheaths into the fall and through the winter. In contrast, leaf sheaths on introduced genotypes remain tightly wrapped around stems, even after leaves have fallen due to senescence, and they remain attached on standing old stems for multiple years. This feature allows separation of native and introduced genotypes at all times of the year. These morphological distinctions are important characters affecting oviposition by *A. geminipuncta* and *A. neurica* (see below).

A particular lineage (Type I), now formally recognized as *P. australis berlandieri* (Saltonstall and Hauber, 2007; Saltonstall et al., 2004) is of unresolved origin (Guo et al., 2016; Lambertini et al., 2012a) and this subspecies occurs in the southern US and south through Mexico into South America (Colin and Eguiarte, 2016; Saltonstall and Meyerson, 2016). There are no reports of any specialized insects attacking this lineage in North America and stem dissection of specimens from Arizona and the Mississippi Delta revealed no internal feeders (Blossey, unpublished data), a strong indication that the species may be a relatively recent arrival to North America. The northern range margin for populations of this lineage appear to be restricted to areas where average temperatures range from 17 to 21 °C (Casagrande et al., 2018).

2.1.2. Archanara geminipuncta and A. neurica

Detailed life-histories of *A. geminipuncta* and *A. neurica* are well described (Häfliger et al., 2006b). Eggs overwinter under leaf sheaths and hatch as *P. australis* shoots begin to emerge in early spring. Larvae initially feed gregariously (*A. geminipuncta*) or individually (*A. neurica*) in soft, nutrient- rich tissues in stems above the growing points. Larvae need to change stems 3–4 times to complete development and all later instars are solitary feeders in uppermost stem portions where they consume growing points and prevent stem elongation. Larval development takes several weeks and mature larvae pupate in lower stem sections. Short-lived nocturnal adults emerge between June and August in Western Europe, mate and lay eggs (100–150/female) in individual rows under leaf sheaths. *Archanara neurica* phenology precedes *A. geminipuncta* by about 2–3 weeks (Häfliger et al., 2006b).

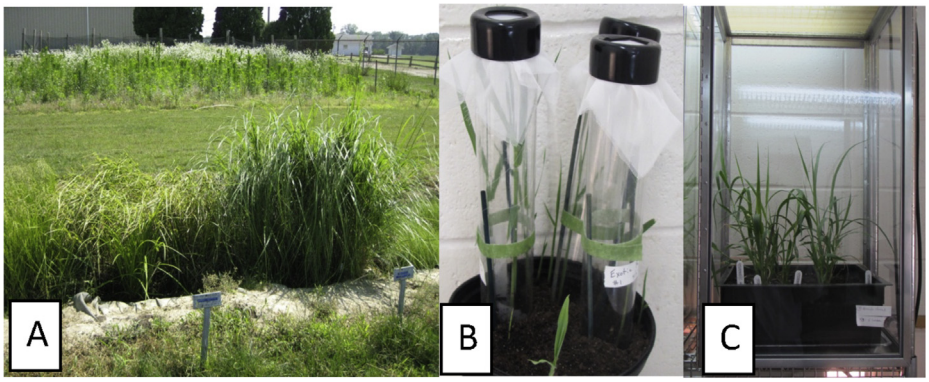

Fig. 1. Common garden for growing test plants (A) and design of Stage 1 (B) and Stage 2 (C) larval host specificity testing of *A. geminipuncta* and *A. neurica* at URI.

Stems attacked early wilt and often die completely; stems attacked later wilt at the top, lose stem tips, and often develop side shoots (Häfliger et al., 2006a). Reduction in shoot heights and aboveground biomass can be substantial, typically 20–60% (Häfliger et al., 2006a). Outbreaking moth populations causing extensive reed dieback have been reported in Europe, particularly for *A. geminipuncta* (Mook and van der Toorn, 1985) and large population fluctuations show outbreak cycles of 3–4 years with stem attack reaching 100% (Michel and Tscharntke, 1993; Tscharntke, 1990). Due to extensive shoot damage in Europe, *A. geminipuncta* is considered a keystone species influencing trophic structure and abundance of reed biota from insects to birds (Tscharntke, 1989; Tscharntke, 1992a; Tscharntke, 1999).

2.1.3. Non-target plant species selection

In determining appropriate plant species for host-range testing, we followed nomenclature according to the USDA PLANTS database (USDA NRCS, 2017). Our host-plant testing followed established guidelines using plant phylogeny, species of conservation or agricultural concerns, plants that are attacked by congeners of potential biocontrol agents, and plants with similar chemistry. We further prioritized plants that co-occur in habitats invaded by *P. australis* if multiple candidate species were available (USDA, 2000; Wapshere, 1974). We considered two additional key biological filters. First, both *A. geminipuncta* and *A. neurica*, are stem feeders and neither can develop outside of host plant stems. Head capsule width of mature larvae (*A. geminipuncta*: 2.07–2.19 mm, 95% CI, N = 13; *A. neurica*: 1.56–1.79 mm, 95% CI, N = 5) or pupal width (*A. geminipuncta*: 4.30–4.77 mm, 95% CI, N = 22; *A. neurica*: 2.96–3.33 mm, 95% CI, N = 7) are limiting physical factors that do not allow larvae to develop or pupate in plants with narrower stems. Second, both species overwinter as eggs under leaf sheaths, which excludes most plants from serving as hosts, including many row crop cereals that are harvested annually. Nevertheless, to comprehensively address USDA-APHIS TAG test-plant categories (USDA, 2000), we included cereals in host range tests despite larval size limitation and lack of any reports that either *A. neurica* or *A. geminipuncta* attack crops in Europe (Tewksbury et al., 2002). We also included species that initiate growth later in the season and we tried to synchronize larval emergence to match plant and insect phenology. This resulted in a TAG approved list of 43 species, and we added several different *P. australis americanus* haplotypes from across North America to our testing sequence. The full list is available in the associated Data in Brief paper (Blossey et al., 2018).

2.2. Host specificity testing

We conducted host specificity testing in quarantine at the University of Rhode Island (URI), where we focused on ability of neonate larvae to penetrate and survive in stems of potted test plant species. We conducted additional tests in Europe at CABI in Delémont, Switzerland (CABI) focusing on important agricultural crops and field tests to achieve more reliable results impossible to obtain in quarantine (Blossey, 1995; Blossey et al., 1994b; Briese, 2005; Clement and Cristofaro, 1995; Cullen, 1990; Marohasy, 1998).

2.2.1. Plant propagation

For our experiments at URI, we field-collected common plants locally in New England and obtained others through the nursery industry and from colleagues in other regions. We obtained introduced haplotype M *P. australis* locally in Galilee, Rhode Island and a collection of native genotypes from populations of known haplotypes (confirmed by Kristin Saltonstall) from the Montezuma National Wildlife Refuge in New York (Type E); the Holt Research Forest in Arrowsic, Maine (Type E); Memramcook, New Brunswick, Canada (Type S); Block Island, Rhode Island (Type AB); and Dover, Delaware (Type F).

We initially transplanted field-collected or greenhouse propagated plants into potting soil (SUNGRO Metro-Mix 510) in 20 L nursery containers (C2100 Nursery Supply Inc., Chambersburg, Pennsylvania, USA) held outdoors. Wetland species were held in shallow wading pools. Growing plants outdoors avoids common greenhouse pests and plants are better able to develop their normal growth and chemical characteristics (Blossey et al., 1994a; Blossey et al., 1994b). Furthermore, growing container plants provided flexibility to move plants into quarantine, but these plants often failed to achieve large stem diameters. We therefore transitioned our plant production to a wetland garden using plastic-lined trenches (1 × 1 m × 0.8 m deep) (Fig. 1A). We initially stocked our garden using at least 1–2 individuals for each non-target species to allow for genetic variation, but established many more individuals for introduced *P. australis*. This change in growing venue resulted in plants with larger stem diameters that *A. geminipuncta* and *A. neurica* larvae prefer and thus provided valid positive controls.

Collaborators in Europe and North America provided field collected rhizomes to supplement our field collected stock at CABI. We propagated all plants in 15L nursery containers (Soparco, Max Schwartz AG, Villingen, Switzerland) with a soil mixture containing commercial potting soil (Selmaterra, Eric Schweizer AG, Thun, Switzerland), recycled potting soil, sand, and vermiculite (VTT, Muttenz, Switzerland; approximately 6:8:3:1) with 1 g/L slow-release NPK fertilizer (Hauert Tardit 6 M, Grossaffoltern, Switzerland) in a common garden (Table 1). For most of the year we kept plants in a shallow pool (6.5 × 3.5 m) lined with plastic and 5–10 cm of water. We grouped individual populations but randomly intermixed native, introduced and European populations to avoid position effects. We annually pruned rhizomes that grew out of their pots and frequently divided and repotted plants.

2.2.2. No-choice larval feeding and survival experiments at URI

We obtained *A. geminipuncta* and *A. neurica* eggs from captive colonies (originally collected near CABI) maintained at CABI and held outdoors in Switzerland before shipment to URI. We kept eggs in an

Table 1
Origin of potted *Phragmites* plants and their haplotype (when known) propagated in Switzerland and use of the number of pots/population in open-field oviposition tests with *A. geminipuncta* and *A. neurica* from 2011 to 2015. Letter code in parentheses indicates haplotype (where known). Two label numbers faded but plants could be assigned to origin based on label color code and plant morphology.

Population	Haplotype	Number of pots			
		2011	2013	2014	2015
Native *P. australis americanus*					
Beldens Landing, California	–	7	–	2	–
Astoria, Oregon	E	–	4	2	2
Sun Lakes Park, Washington	D	–	–	3	1
Medicine Lake, Montana	E	–	3	–	1
Saratoga Springs, Utah	–	–	–	5	–
Savage Fen, Minnesota	E	–	2	4	2
Seminary Fen, Minnesota	S	7	5	1	3
Spring Bluff, Illinois	–	7	–	1	1
Montezuma, New York	E	–	4	3	2
Hillsborough, New Brunswick Canada	S	–	3	–	2
North American *P. australis*					
Novato, California	M	–	4	4	1
Rock Ford, Washington	M	–	5	1	4
Moses Lake, Washington	M	7	–	–	–
Saratoga Springs, Utah	–	–	4	2	2
Forest Lake, Minnesota	M	–	6	–	6
Galeville, New York	M	7	–	–	–
Island Farm, Virginia	–	7	–	–	–
Assunpink Lake, New Jersey	–	–	–	3	
Cape May, New Jersey	–	–	–	4	1
New Haven, Connecticut	M	–	2	5	–
Unknown (faded label)	–	–	–	2	–
European *P. australis*					
Surrey, UK	–	–	–	–	1
Krautsand, Germany	–	–	–	–	1
Delémont, Switzerland	–	4	6	4	3
Magadino, Switzerland	–	–	–	2	1
Yverdon, Switzerland	–	–	–	2	2
Dobanovci, Serbia	–	–	4	–	4
Hodmezovasarhely, Hungary	–	5	–	3	–
Iasi, Romania	–	5	4	3	2

incubator (4 °C) until we had sufficient and appropriate plant material available for tests and then brought eggs to room temperature where they hatched within days. This allowed us to stagger tests, easing logistical and space constraints and enabled synchronization with field-grown plants. However, egg survival greatly declined the longer we kept eggs in incubators past initial spring conditions.

We excavated plants or cut plant sections immediately once stems started to grow in our wetland garden and potted them into 20 L nursery containers to allow for appropriate testing conditions. Timing for this process was extremely important, as larvae only accept shoots of appropriate diameter and development stage. We used a two-stage protocol where Stage 1 measured acceptance of neonate larvae of a test plant over a 5-day period in a no-choice test. When test plants showed signs of internal feeding, we conducted a second no-choice test (Stage 2) and measured acceptance and larval feeding over a 10-day period.

In Stage 1 we measured height and basal stem diameter before placing a transparent tube (5 cm diameter; 30.5 cm or 46 cm tall, depending on plant height; Fig. 1B) covered with mesh to allow ventilation over 1–3 stems. We supported each tube with a bamboo stick and buried tubes in soil to prevent larval escape. We placed an individual neonate *A. geminipuncta* or *A. neurica* larva at the base of a plant using a wet fine paint brush. After 5 days we dissected all stems, recording feeding marks, entrance or exit holes, presence of frass and larval survival. We classified external scraping or cutting a hole into stems without larvae entering stems and feeding as “no internal feeding”. We only considered a particular sequence of tests valid, if larvae attacked and survived on introduced *P. australis* set up simultaneously as controls. We used 15 to 30 replicates for each plant species (including for controls) but limited availability reduced replication to 14 for *Secale cereale* L. (Poaceae) testing *A. geminipuncta* and 11 replicates of *Aristida purpurea* Nutt. (Poaceae) testing *A. neurica*. Plants showing internal feeding and larval survival advanced to Stage 2.

We conducted Stage 2 testing using 5 (2012) or 6 (2013) replicates/plant species in a vinyl cage (46 × 46 × 76 cm high, Fig. 1C) using multi-stemmed plants grown in 46 × 36 × 8.5 cm high flats (Christie Enterprises, Kenilworth New Jersey, USA). We placed 5 neonate larvae individually on stems and after 10 days we recorded results as in Stage 1.

Phragmites australis berlandieri is a subtropical plant and test individuals did not develop into typical specimens in Rhode Island or Switzerland that *A. neurica* or *A. geminipuncta* adults or larvae would encounter in the field (compare Fig. 3D and Fig. 8A–C). We obtained fresh stems and rhizomes collected in Fort Pierce, Florida on 22 February 2016 and confined neonate *A. geminipuncta* and *A. neurica* on stem sections of newly emerged shoots as in Stage 1 at URI on 23 February 2016 to complement tests using greenhouse grown individuals. *Phragmites australis berlandieri* develops an abundance of thinner side shoots, therefore we also confined larvae on side shoots and growing tips of Florida plants but needed to substitute with greenhouse-grown plants from the same site in Florida to achieve appropriate replication. We used introduced *P. australis* from our outdoor common garden as controls and measured larval feeding and survival five days after inoculation.

2.2.3. No-choice host specificity larval feeding and survival experiments at CABI

Work at CABI allowed us to test a subset of important crop and wetland plants available in Europe in a common garden or a greenhouse. We conducted these tests from 2006 to 2011 as plants and larvae became available. We carefully transferred 3–6 neonate larvae onto shoots of each test plant and controls (European *P. australis*) using six replicates. When we tested annual crop species, we planted four or five seedlings together in one pot to achieve stem densities resembling *P. australis* controls. We arranged pots randomly, each covered with gauze bags supported by two wire frames to avoid larval dispersal. We checked stems under a stereo microscope for larval presence and feeding activity after two weeks.

2.2.4. No-choice larval establishment and larval development tests on different Phragmites lineages at CABI

To assess whether larvae discriminate between *P. australis* and native *P. australis americanus*, we obtained neonate *A. geminipuncta* (N = 24) and *A. neurica* (N = 29) in spring 2003, and transferred a single larva onto potted European *P. australis* or native *P. australis americanus* in our common garden. We covered each pot with a gauze bag for 5 days to prevent larval dispersal (Fig. 2D). Two weeks after larval transfer, attacked shoots could be recognized by wilting tips. We cut these shoots at the soil surface and kept them upright in transparent plastic cylinders (10 cm diameter; 37 cm high) covered with gauze lids under ambient outdoor conditions. We recorded days until emergence of larvae and larval weight (mg) (Mettler Toledo AE160, Greifenbach, Switzerland).

In spring 2004 we used a similar design, but this time we followed larval development through to pupation and adult emergence. We

Fig. 2. Design of wooden frame gauze cages (A), larval dispersal multiple-choice cage (B), field-oviposition cage (C), and no-choice larval development on potted plants (D), to test host specificity of *A. geminipuncta* and *A. neurica* at CABI.

released 10 neonate larvae onto potted European *P. australis* and native *P. australis americanus* (N = 10 replicates/lineage). We cut attacked stems after about a week and held them in plastic containers (see above) until larvae emerged. We recorded days to larval emergence and larval weight (mg) and then returned larvae to the plants they had originated from. In a few instances when stems of appropriate diameters were unavailable on the original plant, we chose an alternate pot from the same population. We allowed larvae to enter stems, and then once more removed attacked stems to record time to second and third shoot change, and larval weight. We followed the same procedure until final shoot change and pupation. We allowed mature larvae to pupate and dissected pupae out of stems, weighed them and allowed them to emerge in small cups (5–6 cm diameter) provided with a layer of vermiculite and a moist cotton pad. We recorded survival and length of development of each stage until pupation and adult emergence.

2.2.5. Multiple-choice larval dispersal and feeding tests with A. geminipuncta at CABI

In 2005 we conducted a multiple-choice experiment (N = 6 replicates) in two greenhouses using potted plants to assess preferences of dispersing neonate larvae among different *Phragmites* lineages and *Phalaris arundinacea* L. (Poaceae). We included *P. arundinacea* in this experiment because it was accepted for oviposition in a no-choice test (see below). We used sprouting plants propagated in our common garden and we arranged them in six groups of four (Fig. 2B) using native *P. australis americanus*, European *P. australis*, North American *P. australis* and *P. arundinacea* each represented by one pot in each group. We individually labelled each stem and measured its diameter and height before sequentially releasing 40 neonate larvae between 2 and 5 May 2005 onto a vertical wooden stick placed onto a cardboard platform connecting the pots (Fig. 2B). We covered the four plants with a gauze cage for 24 h to prevent larval dispersal. After 5 days we cut all attacked shoots (recognizable by wilting tips) and placed them into transparent plastic cylinders for larval emergence to record days to emergence and larval weights (see above). After 4 weeks we dissected all shoots and recorded presence and numbers of head capsules or dead larvae.

2.2.6. Oviposition tests at CABI

Archanara larvae of all ages have very limited dispersal abilities, thus oviposition choices are critical in determining fate of offspring. Furthermore, oviposition site selection may constitute an additional safety feature in protecting non-target species, even if larvae would be able to successfully complete development on certain plant species not selected for oviposition. We focused on testing a few wetland pant species in no- and multiple-choice tests, but our main focus was on oviposition choice among *Phragmites* lineages. We tested oviposition preferences over multiple years using various no-choice, single-choice and multiple-choice approaches using cut shoots or potted plants of European or North American *P. australis* or native *P. australis americanus*. In many years we were limited by availability of test plants or the number of females compromising our ability for appropriate replication and statistical analyses.

We conducted tests in confinement using cut shoots or potted plants from 2003 to 2005 by releasing a single (cut shoots) or five *A. geminipuncta* or *A. neurica* pairs (potted plants) (Fig. 2C). In tests with cut shoots we exchanged shoots and recorded all eggs laid every two days; in tests with potted plants we recorded the number of eggs laid on each plant a few weeks after adult releases in early September.

Testing oviposition preferences at CABI allowed us to use free-flying *A. geminipuncta* and *A. neurica* (Fig. 3) from 2011 to 2015 while testing different *Phragmites* lineages from across North America (Table 1). In mid-July of each year, we mowed a 10 × 10 m section in a meadow at Delémont (Fig. 3A) and placed groups of 14 potted *Phragmites* into the four corners (N = 56 pots total; 2 m × 2 m area, pot rims touching; Fig. 3A and B). Three of the four groups of 14 pots contained either only European *P. australis*, only North American *P. australis*, or only native *P. australis americanus*. The fourth group consisted of seven pots each of North American *P. australis* and *P. australis americanus* with pots clustered by lineage (Fig. 3B). Within each lineage we used at least three different populations and their position within each group was randomized, but we used different populations within each lineage over the years (Table 1) due to changes in plant availability.

We released mated pairs or females evenly divided among pots in each corner of our array as they became available in July or early August. We tested *A. neurica* in 2013 and 2015 (N = 52 females in each year) and *A. geminipuncta* in 2011 (N = 8 females), 2014 (N = 44 females) and 2015 (N = 28 females). Attempts to test *A. geminipuncta* in 2013 largely failed due to poor rearing success (N = 8 females). Poor weather conditions resulted in loss of adults and we recovered only three egg batches. In 2011 and 2013 we marked females with fluorescent powder (Fig. 3C). We established in pre-trial experiments that this procedure did not affect female longevity or oviposition. Color-coding allowed us to track egg batches according to original female release location since fluorescent powder remained on eggs batches. Two weeks after releasing the last moth, we harvested all stems, measured their height and basal diameter, and recorded the number of eggs.

In 2015, we replaced the group containing seven North American *P. australis* and seven native *P. australis americanus* with 14 pots of Type I *P. australis berlandieri* obtained from Zellwood, Florida (Fig. 3D). We kept this subspecies over winter in a greenhouse that was unheated except to prevent freezing. Although these plants grew and developed side shoots as is typical for this lineage, they did not resemble the vigorous and tall stems found in the field in North America (Fig. 3D).

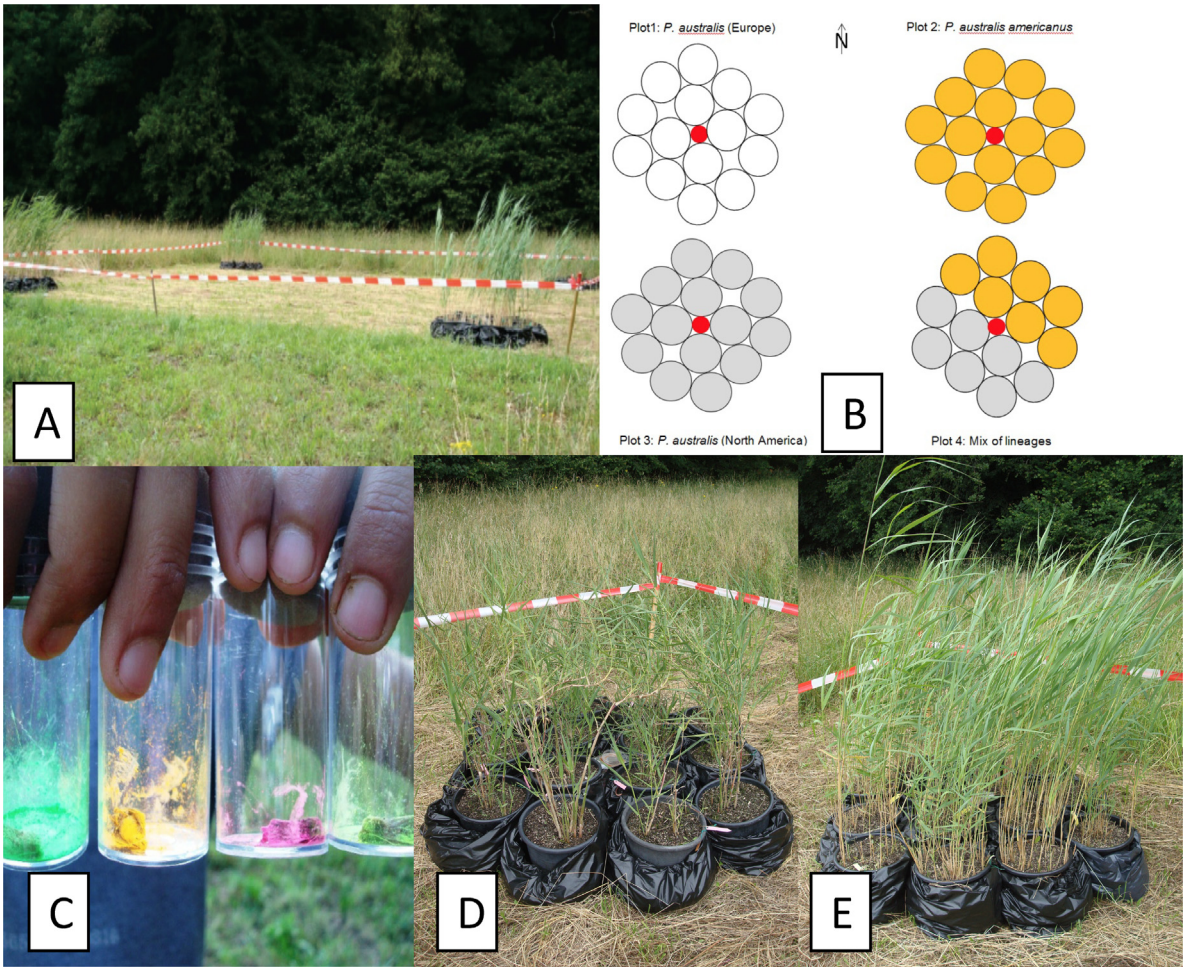

Fig. 3. Experimental design of multiple-choice open-field oviposition tests with free-flying *A. geminipuncta* and *A. neurica* (A, B). Choices included European *P. australis* (Plot 1); endemic North American *P. australis americanus* (Plot 2); North American *P. australis* (Plot 3), and a mix of *P. australis americanus* and North American *P. australis* (Plot 4). Each plot consists of a mix of plants from different collection locations (see Table 1). Depicted is the design used in 2013, in other years the position of different *P. australis* lineages differed to avoid position effects. Small red circles in the center of each plot indicate adult release locations. In some years moths were marked with fluorescent powder (C) to indicate their release patch. Type I specimens tested in 2015 grew poorly (D, compare to Fig. 8), while introduced *P. australis* (E) achieved near normal growth.

2.2.7. Winter survival of A. geminipuncta eggs at CABI

Lower leaf sheaths of *P. australis americanus* are starting to separate from stems and drop to the ground at the time *A. geminipuncta* and *A. neurica* oviposit. Eggs laid on *P. australis americanus* stems that subsequently drop to the ground may experience increased mortality compared to eggs sheltered above ground on standing stems due to litter predators, freeze–thaw cycles and different moisture conditions, including flooding. We tested differences in egg survival by either taping leaf sheaths with 10 attached *A. geminipuncta* eggs (we removed surplus eggs to standardize replicates) to *P. australis* stems in pots in October 2006 or by placing loose leaf sheaths containing 10 eggs directly onto potting soil (N = 12 replicates/treatment). Retrieving eggs, or assessing egg hatch would have been impossible for those exposed in the litter over the winter. Therefore, we covered each pot with gauze bags (37 × 37 × 160 cm) in April 2007 and then dissected all stems in early May 2007 recording the number of larvae for each pot to establish overwintering survival.

The distribution of *P. australis berlandieri* in the U.S. is restricted to areas south of 35° latitude, while the two moths occur only north of 35° latitude in Europe (Casagrande et al., 2018). To investigate the potential of the two moths to establish in climates where *P. australis berlandieri* currently occurs, we set up an egg overwintering experiment with *A. geminipuncta* and *A. neurica* at URI in October 2017. We placed eggs into Petri-dishes (N = 10; 10 replicates/species) set to photoperiod and fluctuating average day and night temperatures of Fort Pierce, Florida and Basel, Switzerland (Blossey et al., 2018) in two incubators (Percival I-36LL, Percival, Perry, Iowa, USA). We reprogrammed incubator conditions twice a week to follow seasonal changes at Fort Pierce and Basel. We started to check for larval emergence weekly starting in February 2018, until we terminated the experiment in May 2018.

2.3. Statistical analyses

We conducted our tests over many years, at various times of the year and under different conditions, but all were run with a *P. australis* control. Formal statistical analyses are often, but not always, inappropriate under such circumstances (frequent low sample size when survival was poor in treatments), therefore we provide summarized findings but also report raw data (Blossey et al., 2018).

We applied Generalized Linear Models (GLM's) with binomial errors for each moth species to evaluate if larval establishment success in no-choice tests in 2003 differed between native *P. australis americanus* and European *P. australis*. We applied log-likelihood tests to evaluate the hypothesis of no effect of *P. australis* lineage on establishment success (Section 2.2.4). We applied Generalized Linear Mixed Models (GLMM) with normal errors to evaluate effects of larval development stage and *Phragmites* lineage on development time, larval weight and diameter of chosen stem and a GLMM with binomial errors to evaluate effects on

Table 2
Test plants attacked by *A. geminipuncta* and *A. neurica* in Stage 1 (1 larva/stem) or Stage 2 tests (5 larvae on potted plants with multiple stems) at URI. We only show species where feeding occurred; a list of all test plant species is available (Blossey et al., 2018). Feeding (%) indicates proportion of replicates (reps) showing internal feeding, and survival (%) represents proportion of replicates with larval survival. Data shown are means of 15–30 replicates/test plant species (Stage 1) or 25–55 larvae in 5 – 6 replicates/test plant (Stage 2). Typically, only species showing feeding and survival in Stage 1 advanced to Stage 2. Only tests with corresponding positive controls (larvae initiating feeding and surviving in introduced *P. australis* stems are considered valid and are included here). NT = not tested.

Test plant species	Stage 1			Stage 2			
	Reps N	Feeding %	Survival %	Larvae N	Reps N	Feeding %	Survival %
A. geminipuncta							
P. australis M	120	86	57	130	21	95	29
P. a. americanus (ME)[1]	15	67	33	NT			
P. a. americanus (NB)[1]	15	73	33	NT			
P. a. americanus (NY)[1]	15	53	20	NT			
Arundo donax	15	40	7	55	11	36	0
Cortaderia selloana	15	33	0	55	11	36	0
Spartina alterniflora	15	33	13	50	10	60	10
Spartina cynosuroides	15	27	7	55	14	18	0
Zizania aquatica	24	4	4	50	10	0	0
Triticum aestivum	15	27	7	55	11	0	0
Arundinaria tecta	15	7	0	25	5	0	0
Schoenoplectus americanus	15	13	0	50	10	10	0
A. neurica							
P. australis M	197	41	22	30	6	100	67
P. a. americanus (ME)[1]	10	50	10	NT			
P. a. americanus (NB)[1]	25	48	12	NT			
P. a. americanus (NY)[1]	15	47	7	NT			
P. a. americanus (RI)[1]	10	0	0	NT			
P. a. americanus (DE)[1]	10	30	10	NT			
Spartina alterniflora	25	4	4	30	6	0	0
Eragrostis trichodes	25	4	0	NT			
Zizania aquatica	30	27	10	30	6	0	0
Saccharum officinarum	15	13	0	30	6	0	0
Phalaris arundinaceae	25	16	0	30	6	0	0
Glyceria striata	25	24	0	NT			
Schoenoplectus acutus	25	12	0	30	6	0	0

[1] State and Province two-letter abbreviations for collection location of *P. australis americanus* and their haplotype in parentheses: NB = New Brunswick (S), ME = Maine (E), NY = New York (E), RI = Rhode Island (AB); DE = Delaware (F).

larval survival (Section 2.2.4) in 2004. We ran independent models for *A. geminipuncta* and *A. neurica*. Models included pot and larval ID as random terms. We applied log-likelihood tests to evaluate significance of model terms.

We evaluated effects of *Phragmites* lineage on larval survival in multiple-choice tests (Section 2.2.5) with Negative Binomial GLMs. We used negative binomial models because data were overdispersed (mean = 5.2; variance = 20.2). We evaluated differences in larval weight, and stem diameter in multiple-choice tests with one-way ANOVAs. We tested for difference in larval attack rates in our overwintering experiment (Section 2.2.8) where eggs under leaf sheaths remained attached to *P. australis* stems (protected) or were exposed to conditions on the soil (unprotected) using an Independent-Samples *t*-test using SPSS 12.0 (SPSS Inc., Chicago, Illinois). Our data satisfied assumptions of normality and homogeneity of variance on all t-tests and ANOVAs.

We evaluated effects of *P. australis* origin (European, introduced North American, native *P. australis americanus* and Gulf Coast *P. australis berlandieri*), stem diameter and stem height on oviposition of free flying *A. neurica* and *A. geminipuncta* in Switzerland (Section 2.2.6) with zero-altered negative binomial models. These are coupled models in which one component predicts oviposition occurrence (following a binomial distribution) and the second component predicts abundance or number of eggs laid (following a negative binomial distribution) (Zuur, 2009). We chose the negative binomial distribution because it is a better choice for overdispersed data, as in the case of egg count data.

For all *Phragmites* lineages, stem diameters and stem height were positively correlated (0.33; $P < 0.001$) and hence we evaluated each measurement with independent models. Here we present results for diameter only as this measure explained a larger proportion of the variation. We ran independent models for *A. neurica* and *A. geminipuncta* and only fitted models for years where we had sufficient data (2014 for *A. geminipuncta* and 2015 for *A. neurica*). We fitted all models in R (Core Team, 2016) using the hurdle function in the MASS package (Venables and Ripley, 2002).

3. Results

3.1. No-choice larval survival tests on non-Phragmites host plants at URI and CABI

Neonate larvae are extremely selective in their choice of stems and we had to abandon many early efforts when larvae failed to establish in apparently healthy and appropriately-sized stems of *P. australis* controls invalidating our tests. Only including the tip and growing point of *P. australis* shoots, achieved good establishment success and larval survival. Of the 43 different plant species included in our host specificity testing 32 showed no signs of any external or internal larval feeding in no-choice Stage 1 tests (Blossey et al., 2018). Very few test plant species allowed initial larval survival under no-choice conditions at URI (Table 2) or in Switzerland (Table 3).

Our tests demonstrated that under no-choice conditions native *P. australis americanus* will be accepted by both *Archanara* species, although Stage 1 larval survival was substantially lower on native haplotypes than on introduced *P. australis* (Table 2). These results precipitated a number of additional tests, particularly in Switzerland, to further evaluate ability of larvae to discriminate or develop successfully in native *P. australis americanus*.

In no-choice tests a small proportion of *A. geminipuncta* larvae initiated feeding or survive for short periods of time on some test plants in

Table 3
Two-week survival (%) of *A. geminipuncta* and *A. neurica* neonate larvae in no-choice tests on potted plants in Switzerland. Data are means ± SE with N = 6 replicates/plant species each initially receiving 5 larvae. A "–" indicates that the species was not tested. European *P. australis* served as control.

Test plant species	*A. geminipuncta*	*A. neurica*
European *Phragmites australis*	60	44
Phalaris arundinacea	0	0
Arundo donax	0	0[1]
Cortaderia selloana	4	0[1]
Avena sativa	0	0[1]
Triticum aestivum	0	0[1]
Zea mais	0	0[1]
Hordeum vulgare	0	0[1]
Oryza sativa	4	–
Saccharum officinarum	0	–
Typha latifolia	0	0
Eragrostis trichodes	0	–
Schoenoplectus acutus	0	12
Schoenoplectus americanus	0	–
Lolium perenne	0	–
Spartina cynosuroides	20	0
**Spartina pectinatus*	–	0
Agropyron cristatum	–	0
Iris versicolor	–	0
Glyceria striata	0	–
Setaria italica	0	–
Zizania aquatica	0	–

[1] Only 3 larvae transferred/replicate.
* Only tested in Europe.

Table 4
Five-day survival of neonate *A. neurica* and *A. geminipuncta* larvae in no-choice tests using Gulf Coast Type I *P. australis berlandieri* main shoots, side shoots of field collected and greenhouse grown plants with field grown North American *P. australis* as control at URI. Data are means of 3–15 replicates with 1 larva/replicate.

Test plant species	*A. neurica*		*A. geminipuncta*	
	N	Survival	N	Survival
		No.		No.
Field-collected North American *P. australis*	15	3	25	6
Field-collected *P. australis berlandieri*				
New main shoots	12	1	25	1
New side shoots	15	0	15	1
Tips of older plants	5	0	5	3
Greenhouse-grown *P. australis berlandieri*				
New main shoots	3	1	4	4
New side shoots	7	1	8	5
Tips of older plants	6	1	6	4

Stage 1 or 2, but they did not survive beyond a few days on *Zizania aquatica L.*, *Triticum aestivum* L., or *Arundo donax* L.; only *Spartina alterniflora* Loisel allowed 10% of larvae to survive for 10 days in Stage 2 (Table 2) but larvae did not grow. Tests in Switzerland showed some, albeit greatly reduced larval survival on *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn., *Spartina cynosuroides* L. (Roth) and *Oryza sativa* L. (Table 3), but these species were either not attacked (*O. sativa*) or larvae never survived in Stage 1 and 2 testing at URI (Table 2).

Archanara neurica showed an even more restricted acceptance of species outside the genus *Phragmites* in no-choice tests. At URI, larvae probed seven species, and for two (*Z. aquatica* and *S. alterniflora)* a small proportion of larvae survived for five but none for 10 days (Table 2). Tests in Switzerland confirmed these results, however, a few larvae survived (but did not grow) for two weeks, but not beyond, on *Schoenoplectus acutus* (Muhl.), a species that was not attacked at URI (Tables 2 and 3).

3.2. No-choice larval survival tests on P. australis berlandieri at URI

Larvae of both *Archanara* species accepted greenhouse propagated *P. australis berlandieri*, but survival was greatly reduced in field grown stems, particularly for *A. neurica* (Table 4). *Archanara neurica* showed limited survival in side shoots and tips of older greenhouse grown plants, while *A. geminipuncta* fared slightly better (Table 4), but these tissues are not typical larval feeding locations.

3.3. No-choice larval establishment and larval development tests with A. geminipuncta and A. neurica on different Phragmites lineages at CABI

We found no significant differences in establishment success or weights of surviving *A. geminipuncta* larvae at first shoot change between *P. australis americanus* and European *P. australis* (log-likelihood ratio test: deviance = 0.14; P = 0.7; Table 5) in 2003. In contrast, *A. neurica* showed significantly reduced establishment success in native *P. australis americanus* (log-likelihood ratio test: deviance = −6.8, P = 0.01; Table 5) and greatly reduced larval weights with increased development times to first shoot change, but the low number of surviving larvae did not allow us to evaluate these differences statistically (Table 5).

In our no-choice experiment following larval development to adult emergence in 2004, we found no significant differences in development times ($p > 0.05$) for larvae developing in European *P. australis* or native *P. australis americanus* (Fig. 4). While survival significantly decreased over time for both species (*A. geminipuncta:* $X^2 = 109.04$, df = 3, $P < 0.001$; *A. neurica*: $X^2 = 6.22$, df = 2, P = 0.05), stage-specific survival rates did not differ between *Phragmites* lineages ($p > 0.05$; Fig. 4). Larval weight significantly increased over time for both species ($X^2 = 289.7$, df = 3, $P < 0.001$; *A. neurica*: $X^2 = 80.4$,

Table 5
Establishment success (%) of neonate *A. geminipuncta* and *A. neurica*, larval weight at first shoot change (mg), and time to first shoot change on potted European *P. australis* and native *P. australis americanus* plants at CABI. Data are means ± SE.

Lineage	Establishment success %	Larval weight mg (No of larvae)	Development time days
A. geminipuncta			
European *P. australis*	66.7	10.3 ± 0.9 (6)	14.1 ± 0.9
Native *P. australis americanus*	50.0	10.3 ± 2.5 (6)	14.8 ± 0.8
A. neurica			
European *P. australis*	87.5	16.7 ± 1.3 (11)	15.3 ± 0.3
Native *P. australis americanus*	40.0	11.6 ± 2.32 (4)	18.0 ± 0.6

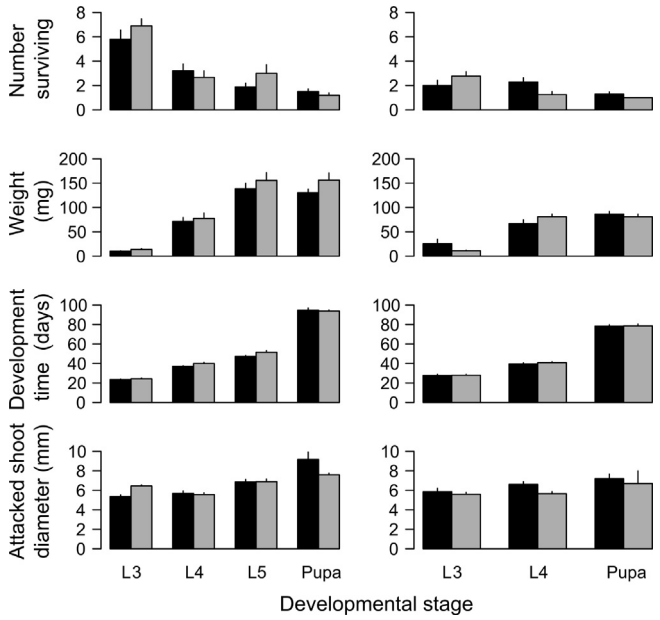

Fig. 4. Survival, larval or pupal weights (mg), and development time (days) and selection of shoot diameters (mm) for *P. australis* (black columns) and *P. australis americanus* (grey columns) by *A. geminipuncta* and *A. neurica* larvae in no-choice tests. Data are means (± SE) of 10 replicates (each receiving a single neonate larva) for either *Phragmites* lineage and number of larvae surviving through various shoot changes to pupation and adult emergence.

df = 2, P < 0.001), but it did not differ between *Phragmites* lineages (P > 0.05; Fig. 4). *Archanara neurica* larvae chose stems of similar diameter between stages (P > 0.05), but *A. geminipuncta* selected significantly wider stems as larvae matured and transitioned from stem to stem in European *P. australis* but not in native *P. australis americanus* (Fig. 4; significant stage x *Phragmites* lineage interaction: X^2 = 18.8, df = 3, P < 0.001).

3.4. Multiple-choice larval dispersal and feeding tests with A. geminipuncta

Foraging *A. geminipuncta* larvae accepted all *Phragmites* lineages but avoided *P. arundinaceae* (Table 6). Successful establishment was not affected by available stem diameters (r^2 = 0.004; P = 0.799) and there was no significant difference in establishment rates between North American and European *P. australis* (Table 6). However, establishment rates were nearly 50% lower in *P. australis americanus* (Table 6). Neither larval survival nor larval weight at first shoot change was affected by *Phragmites* lineage or stem diameter (Table 6).

3.5. Oviposition tests in Europe

In our no-choice oviposition test using cut shoots, *A. geminipuncta* laid a few eggs on *A. donax* and *P. arundinacea* but none were laid on these plants in multiple-choice tests in field cages using potted plants (Table 3 in Blossey et al. 2018) and no larvae survived in our Stage 1 or Stage 2 tests (Table 3). We found 10 *A. geminipuncta* eggs on *Typha latifolia* L. in our multiple-choice oviposition test using potted plants (Table 3, Blossey et al. 2018) but there is no record of this plant being attacked in the field and larvae did not attack this species in our Stage 1 tests (Table 3). Overall our different approaches testing oviposition choice of *A. geminipuncta* and *A. neurica* resulted in inconsistent results among years and venues that all point to problems with validity of tests using confined insects that are unable to leave or express their full behavioral repertoire. In the interest of full disclosure of all tests we conducted, we report detailed results for each test in the associated open access Data in Brief (Blossey et al., 2018) as they are informative when placed into the overall context of our evaluations.

The failure of tests in confinement to produce consistent results led to open-field oviposition experiments that are known to be superior to cages or quarantine studies in producing reliable results regarding field host ranges (Blossey, 1995; Blossey and Schroeder, 1995; Blossey et al., 1994b; Clement and Cristofaro, 1995). From 2011 to 2015 we released a total of 59 *A. geminipuncta* and 104 *A. neurica* females and recorded a total of 508 eggs (8.6 eggs/female) for *A. geminipuncta* and 680 (6.5 eggs/female) for *A. neurica*. Typical numbers of eggs laid per females are approximately 150 for *A. geminipuncta* and 120–130 for *A. neurica*, of which most are laid 2–3 days after adult emergence and mating (Häfliger et al., 2006b). Our results suggest that females sampled and oviposited on plants in our experimental array, but chose to migrate in search of other *Phragmites* patches. Our fluorescent marking revealed that several females laid eggs in adjacent patches, but overall our small experimental stands were not attractive enough to discourage female dispersal.

Both *A. geminipuncta* and *A. neurica* oviposited on all *P. australis* lineages (Table 7) and summarizing results over all years (excluding *P. australis berlandieri*, which was only tested in 2015) European *P. australis* received 44.9% of *A. geminipuncta* eggs, North American *P. australis* 49.2% and *P. australis americanus* only 6.5% (Table 7, Fig. 7). European *P. australis* received 50.3% of *A. neurica* eggs, North American *P. australis* 43.2% and *P. australis americanus* only 6.5% (Table 7, Fig. 7). We found that *A. geminipuncta* laid 20 eggs (33% of total eggs oviposited by the species in 2015) and *A. neurica* laid 57 eggs (12.6% of all eggs laid by the species in 2015) on *P. australis berlandieri*. However, the plant specimens we were able to offer in Switzerland, with greatly reduced heights and stem diameters, do not resemble the tall and vigorously growing plants we typically encounter in the field, questioning the validity of our test results. Stem diameters of *P. australis berlandieri*

Table 6
Number of surviving larvae, larval weight (mg) at first shoot change and shoot diameter of attacked shoots in multiple-choice tests using potted plants and dispersing neonate *A. geminipuncta* larvae (N = 40/replicate). Data are means ± SE of 6 replicates/treatment.

Plant/Lineage	No. surviving larvae	Larval weight (mg)	Shoot diameter (mm)
European *P. australis*	5.3 ± 1.3	6.3 ± 0.8	3.3 ± 0.4
North American *P. australis*	4.0 ± 1.3	6.1 ± 1.1	3.4 ± 0.4
Native *P. australis americanus*	2.0 ± 1.6	6.6 ± 0.8	4.5 ± 0.4
Phalaris arundinacea	0	0	3.0 ± 0.2
Statistics	P = 0.461[1] P = 0.067[2]	P = 0.966[2]	P = 0.0804[3]

[1] GLM Negative Binomial GLM comparing ancestral and introduced *Phragmites* lineages.
[2] Results from same model comparing ancestral and native NA *Phragmites* lineages; *P. arundinacea* was not included in the model.
[3] One way ANOVA.

Table 7
Number of stems attacked, number of eggs laid/stem and total number of eggs laid by free flying *A. geminipuncta* and *A. neurica* on European *P. australis,* North American *P. australis*, native *P. australis americanus*, and Gulf Coast *P. australis berlandieri* in open field multiple-choice oviposition experiments between 2011 and 2015. Please note that different species were released in different years using different number of females and with varying success rates (see text for details).

	A. geminipuncta			*A. neurica*		
	No attacked stems	Mean no. eggs/stem ± SE	Total no eggs 2011–2015 (%)	No. attacked stems	Mean no eggs/stem ± SE	Total no eggs 2011–2015 (%)
European *P. australis*						
2011	1	10				
2013	0			16	6.2 ± 1.1	
2014	15	12.9 ± 2.8				
2015	2	7.5 ± 3.5		28	7.4 ± 1.3	
Total			219 (44.9)			306 (50.3)
North American *P. australis*						
2011	3	10 ± 2				
2013	1	17		12	10.4 ± 2.5	
2014	7	16.9 ± 3.4				
2015	2	9.5 ± 6.5		26	5.9 ± 0.9	
Total			184 (49.2)			278 (43.2)
Native *P. australis americanus*						
2011	0					
2013	2	12 ± 1		1	6.0	
2014	1	14				
2015	1	8		7	4.7 ± 1.7	
Total			34 (6.5)			39 (6.5)
Gulf Coast *P. australis berlandieri*[*]						
2015	2	10 ± 1	20	10	5.7 ± 1.6	57

* This species not used to calculate distribution (%) of eggs among lineages as it was only used in 2015.

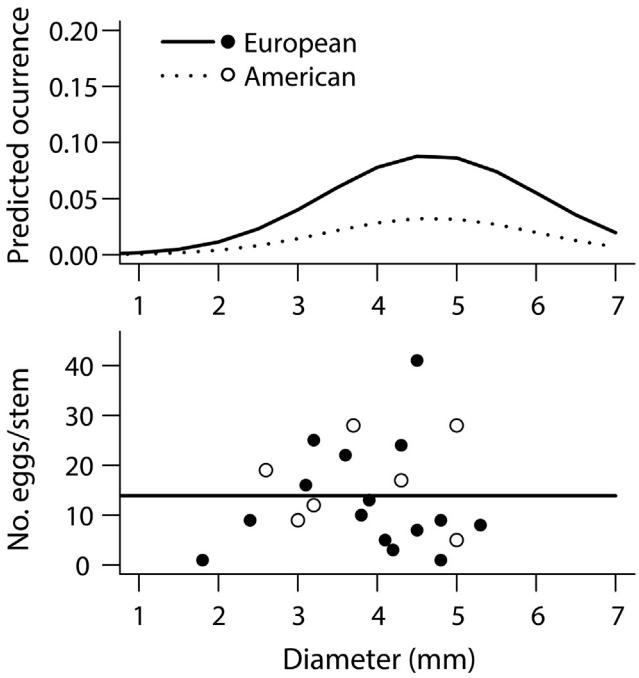

Fig. 5. Predicted occurrence (top) and abundance (number of eggs/stem; bottom) of *A. geminipuncta* eggs in 2014 as function of stem diameter of North American and European *P. australis*. *Archanara geminipuncta* did oviposit on a single native *P. australis* stem in 2014. Line on bottom panel indicates model predictions for European and North American *P. australis* (no significant effect of *P. australis* origin or stem diameter). For model results see Table 11.

in the field are typically at least double those achieved under Swiss growing conditions (compare Figs. 3 and 8).

In 2014, *A. geminipuncta* oviposited on a single *P. australis americanus* stem, vs. 17 European or North American *P. australis* stems. In 2014 the number of eggs laid on ancestral European shoots was significantly higher than on North American *P. australis* (Fig. 5, Table 8), however, the proportion allocated to these two lineages was nearly identical when summarized from 2011 to 2015 (Table 7, Fig. 7).

Table 8
Zero-altered negative binomial model results for effects of different *Phragmites* lineages and stem diameter (cm) on oviposition occurrence and egg abundance of *A. geminipuncta* in 2014 and *A. neurica* in 2015. Non-significant factors were dropped from selected models and are not included.

	Estimate	Standard error	Z value	Pr(> |z|)
A. geminipuncta				
Abundance (# of eggs)				
Intercept	2.6	0.17	15.1	< 0.001
Occurrence				
Intercept	−4.3	0.77	−5.6	< 0.001
Origin (introduced NA)	−1.03	0.47	−2.3	0.03
Stem diameter	0.4	0.20	−1.9	0.04
A. neurica				
Abundance (# of eggs)				
Intercept	1.70	0.15	11.8	< 0.001
Occurrence				
Intercept	−5.73	1.30	−4.38	< 0.001
Origin (introduced NA)	0.51	0.30	1.69	0.09
Origin (*P. australis americanus*)	−0.93	0.47	−1.97	0.04
Origin (*P. australis berlandieri*)	−0.85	0.44	−1.92	0.05
Stem diameter	1.53	0.61	2.52	0.1
Stem diameter (squared)	−0.15	0.07	−2.21	0.02

Oviposition was positively correlated with stem diameter (Fig. 5 top, Table 8) and stem height (data not shown), but females avoided thin stems and stems > 7 mm (Fig. 5). Once a stem was selected, the number of eggs laid per stem averaged 14.6 ± 2.0 (1SE) and was not a function of *Phragmites* lineage, stem diameter, or stem height (Fig. 5 bottom, Table 8).

In 2015, *A. neurica* successfully oviposited on all *Phragmites* lineages but the species preferred stems of European or North American *P. australis* over *P. australis americanus* (Table 7). Female *A. neurica* preferred midsize stems (4–6 mm in diameter) and oviposition was significantly higher on taller stems of intermediate diameter (Table 8, Fig. 6 top). Once a stem was selected, the number of eggs laid per stem averaged 6.3 ± 0.6 (1SE) and was not a function of the *Phragmites* lineage, stem diameter, or stem height, (Table 8; Fig. 6 bottom). As a result, we found significantly fewer eggs on native *P. australis americanus* and the Gulf Coast lineage *P. australis berlandieri* (Fig. 6).

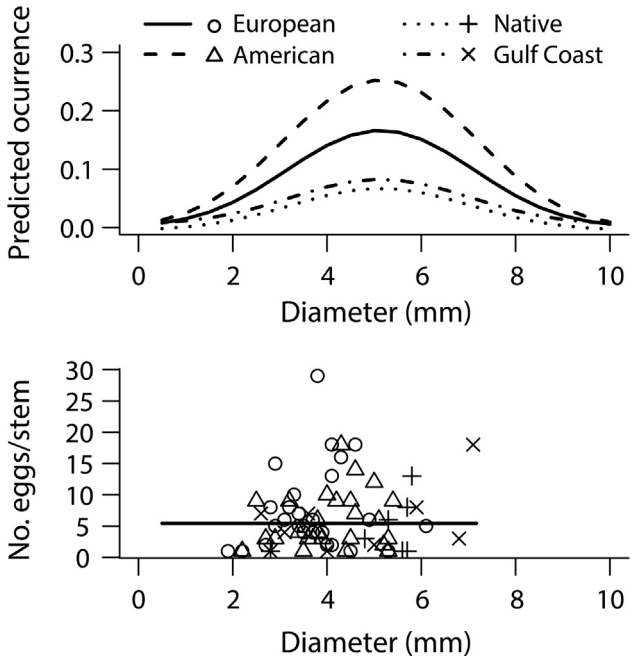

Fig. 6. Predicted occurrence (top) and abundance (number of eggs per stem; bottom) of *A. neurica* eggs in 2015 as function of stem diameter of North American or European *P. australis*, native *P. australis americanus* (Native) and Gulf Coast (Gulf Coast) *P. australis berlandieri*. Prediction lines for Native and Gulf Coast lineages are slightly jittered to allow visualization. Line on bottom panel indicates model predictions for all *Phragmites* lineages (no significant effect of *P. australis* origin, stem diameter or height). For model results see Table 11.

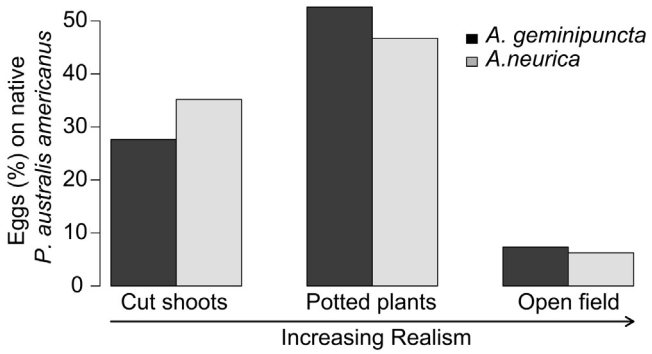

Fig. 7. Eggs (%) laid on native *P. australis americanus* in single- or multiple-choice oviposition tests offering either European or North American *P. australis* or both as alternate choices to *A. geminipuncta* or *A. neurica*. We offered cut shoots and potted plants in cages, open field tests allowed adult dispersal. Data are summaries of tests conducted from 2003 to 2015 (see text for details).

When we summarily compared the distribution of eggs laid from 2003 to 2015 on cut shoots, potted plants or in field-oviposition tests, the proportion of eggs laid on native *P. australis americanus* dramatically decreased when we switched from containments to allowing dispersal (Fig. 7). This was true for both moth species, further supporting previous analyses of oviposition tests in other biocontrol systems (Blossey, 1995; Blossey and Schroeder, 1995; Blossey et al., 1994b; Clement and Cristofaro, 1995) and illustrating the importance of conducting more realistic tests.

3.6. Winter survival of *A. geminipuncta* eggs

Of the eggs kept under leaf sheaths attached to stems 97% hatched and 68% of the neonate larvae successfully attacked a *P. australis* shoot. We have no data on hatch rates of eggs for leaf sheaths placed directly on soil due to difficulties in relocating these eggs. However, we found highly significant differences in attack rates (t (22) = −2.926, P = 0.008) with 3.5 ± 0.7 larvae/pot where eggs overwintered on the ground, compared to 6.6 ± 0.6 larvae/pot where eggs overwintered under leaf sheaths attached to upright stems.

Not a single egg hatched when we kept them in growth chambers resembling Fort Pierce, Florida climate conditions. In contrast, eggs kept at temperatures resembling Central European conditions (15 °C August-mid-October, 2 °C mid-October to February) remained viable and larvae emerged from 54 ± 6.36% (*A. neurica*) and 84 ± 6.18% (*A. geminipuncta*) (data are means ± SE from 10 replicates/treatment for each species). These data are identical in outcome to work we previously conducted at CABI in 2016/2017 but we lost data record sheets for this experiment and repeated the experiment at URI.

4. Discussion

4.1. Risks of non-target attack for species outside the genus *Phragmites*

Two decades of host specificity testing for *A. geminipuncta* and *A. neurica* at CABI and URI demonstrate that no species outside the genus *Phragmites* is a host. Despite some rare oviposition and early larval development on non-*Phragmites* test plants, no species outside this genus allowed extended larval development. We consider limited early larval development in wild and domestic rice, *Z. aquatica* and *O. sativa* to be of no concern because stems of these annuals are either harvested or submerged preventing overwintering of *Archanara* eggs. Furthermore, *Archanara* larvae hatch early in spring before these species start growing, and stem diameters of both species are too small to allow larval development or pupation. Hence, neither species is at risk of sustained or marginal attack and neither moth is reported as a pest of cultivated rice. *Cortaderia selloana* itself is an introduced species (USDA NRCS, 2017) that can become a problem, and *Archanara* larvae were unable to develop in this species.

Three species, *S. alterniflora*, *S. cynosuroides* and *S. acutus* allow initial larval establishment, with S. *acutus* attacked by *A. neurica* only, and at a greatly reduced rate on potted plants in Switzerland but not at URI; we consider this species not to be a host. Both *S. alterniflora* and *S. cynosuroides* allowed early larval development in no-choice tests. All three species have a delayed phenology relative to early-season *P. australis* growth rendering them unsuitable for attack by neonate *A. geminipuncta* or *A. neurica* larvae. We kept eggs refrigerated to allow for synchronization of larval and stem emergence to test these species. This is not an absolute guarantee as stems will become available for later instars should they grow in the vicinity of *P. australis*, but neither of these well-known noctuid species has ever been reported from any *Spartina* species in Europe. In conclusion, we consider only species within the genus *Phragmites* to be within the fundamental or physiological host range of *A. geminipuncta* or *A. neurica*.

4.2. Risks of attack of *P. australis berlandieri* (Type I) and other Gulf Coast lineages

Multiple lines of evidence suggest that *P. australis* lineages growing in southern climates occur outside of the climate envelope of *A. geminipuncta* and *A. neurica*, two species that only occur in temperate climates (Casagrande et al., 2018). Furthermore, our egg overwintering experiment demonstrated that climate conditions around the US Gulf

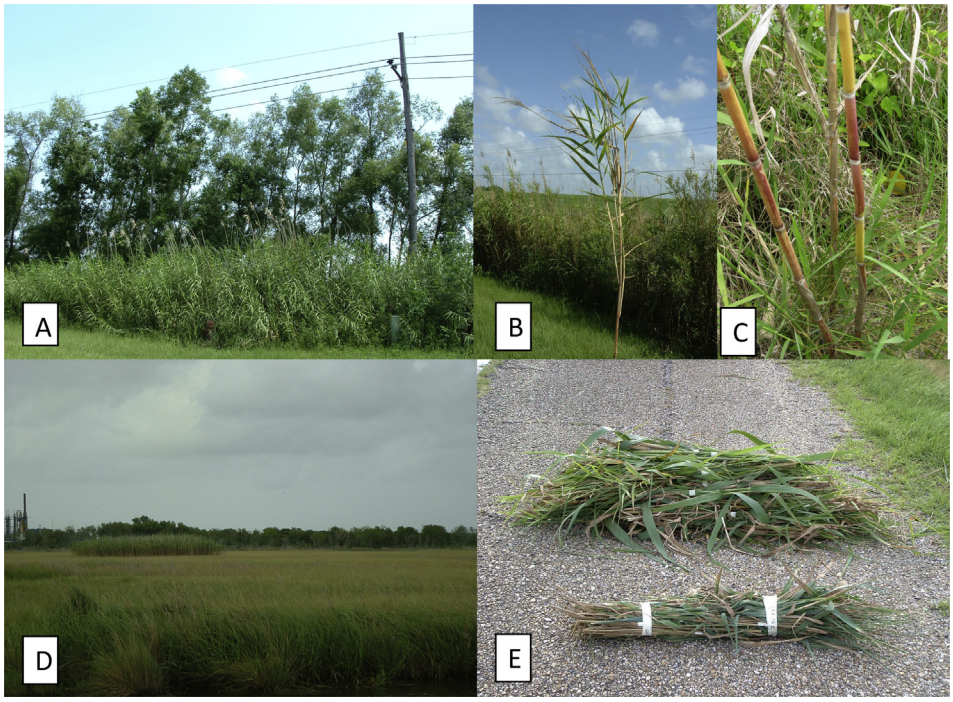

Fig. 8. Different *Phragmites* lineages in the Mississippi Delta. (A) Type I *P. australis berlandieri* growing in upland under power line; (B) appearance of upper portions of *P. australis berlandieri* stem with abundant side shoots, and (C) smooth polished lower sections of large diameter stems lacking leaf sheaths; (D) introduced *P. australis* clone invading *Spartina patens* marshes and typical biomass production (1 m^2) of introduced *P. australis* (front) and *P. australis berlandieri* (back).

Coast do not allow survival of *A. geminipuncta* or *A. neurica* eggs, even if females migrated south and laid eggs on *P. australis berlandieri* or other introduced genotypes that are rapidly expanding in the region.

We found limited oviposition and larval development to occur on Type I stems or plants, however, we were unable to grow specimens at CABI or URI that resemble field-grown plants (Fig. 8). Plants we had available for oviposition tests in Switzerland (Fig. 3D) were small and bushy, about 1 m tall compared to the often 4–5 m tall stems moths would encounter in the field (Fig. 8). Furthermore, when we offered greenhouse grown plants, larval survival increased (Table 4) suggesting that stems did not express the typical traits (stem diameters, toughness, etc.) that otherwise prevent oviposition and larval survival. Field-grown *P. australis berlandieri* stems are very tall, have very hard and tough stems with large diameters (mean 11.5 mm for 55 stems measured from Mississippi, Arizona and Florida; range 9.5–20 mm; Blossey, unpublished data) that show little physical resemblance to plants we had available.

Both *A. geminipuncta* and *A. neurica* accept a narrow range of stem diameters for oviposition (Figs. 5 and 6) and in our oviposition tests we found no eggs on stems with diameters exceeding 10 mm. Few Type I stems have diameters < 10 mm (see above), further reducing their suitability as hosts. This oviposition choice may be an evolved response reflecting the force (as a function of stem diameters) required by neonate or older larvae to penetrate and consume stem tissues. In our European surveys we occasionally recorded larval attack on stems exceeding 10 mm in diameter, but not > 12 mm (Häfliger et al., 2006a). We cannot categorically exclude lack of attack on Type I should moth and *P. australis berlandieri* distributions overlap at some point in the future since the species can be utilized under no-choice conditions if plants are grown in the greenhouse, but we consider probability of such attacks to be non-existent at the present time and extremely low in the future.

Introduced *P. australis* has expanded greatly in the Gulf Coast region overlapping in range with *P. australis berlandieri*, and it is now making up a substantial portion of the vegetation in outer marshes of the Mississippi Delta, where it is believed to prevent wetland loss (Hauber et al., 2011). At the same time, *P. australis* expansion into interior wetlands, and lack of integration into local food webs raises concerns for many wetland managers that mirror concerns in other parts of North America (Hauber et al., 2011; Kettenring et al., 2012). Threats to Mississippi Delta ecosystems not only include plant invasions but also geological forces such as sinking of the Delta, climate change, sea level rise, saltwater intrusion, channelization of the river, reduced sediment inputs (Day et al., 2007), and herbivory by native muskrat, *Ondatra zibethicus* L, deer *Odocoileus virginianus* (Zimmermann), and nutria *Myocastor coypus* (Molina) (for an extensive review see Keddy et al., 2007). While both nutria (Milholland et al., 2010; Prigioni et al., 2005) and muskrat (Vermaat et al., 2016) consume *Phragmites*, how their local feeding preferences may affect long term plant community composition in the Mississippi Delta and adjacent wetlands is unknown. Although exclosure studies show dramatic effects of mammalian herbivores, they were done without *P. australis* (Keddy et al., 2007). The extensive damage by nutria and occasionally muskrats with dramatic “eat-outs” is interacting with other stressors and “this combination of increased flood duration and increased salinity are likely to convert fresh water swamps and marshes to salt marshes and open water, a process well documented in historical photographs” (Keddy et al., 2007).

A widespread dieback of *P. australis*, particularly in the furthest outlying marshes of the Delta is widely attributed to outbreaks of the introduced scale *N. biwakoensis* (Baurick, 2018a; Baurick, 2018b; USGS, 2017), but aerial photography suggests an ongoing multi-year process of declines in *P. australis* vigor due to multiple, yet currently unidentified stressors (Ramsey and Rangoonwala, 2017). That declines are particularly pronounced in the furthermost outlying marshes, while the scale is widely distributed without causing similar declines (Baurick, 2018a; Baurick, 2018b), suggests that increasing salinity levels may be an important contributor to reduced *P. australis* vigor in the Mississippi Delta. Along Atlantic Coast marshes, *P. australis* colonization and distribution are limited by frequency of tidal flushing and increasing salinity levels (Chambers et al., 2003). There appears to be no further associated research to gauge the impact of *N. biwakoensis* on *P. australis*, yet introduction of scale-resistant *P. australis* genotypes or introduction of biocontrol agents are being considered (Baurick, 2018a; Baurick, 2018b), in an apparent rush to prevent further wetland loss. At a minimum, a clear experimental link of *P. australis* declines and *N. biwakoensis* abundance in the field should be established along a salinity gradient with data then informing an appropriate risk assessment of any such activity.

While further research is required to find mechanisms for the ultimate causes of *P. australis* declines in the Delta, pinning hopes for Delta survival and revival on an introduced species (Ramsey and Rangoonwala, 2017; Stevenson et al., 2000) does not appear to be a successful strategy in light of the multitude of threats (Day et al., 2007). Furthermore, *P. australis* is poorly suited (Lissner and Schierup, 1997) to deal with the Delta's increased salinity levels that are better tolerated by typical saltwater marsh plants, such as various *Spartina* species. *Phragmites australis* declines may provide a window of opportunity for restoration with native species.

Based upon life history and climate preferences of *A. geminipuncta* and *A. neurica*, these temperate climate insects are unlikely to survive in southern Louisiana and would have no bearing on the status of introduced *P. australis* or *P. australis berlandieri* growing along the Gulf Coast. A potential introduction of *A. geminipuncta* and *A. neurica* is unlikely to affect the perceived benefit of *Phragmites* in reducing effects of sea level rise and sinking of the Mississippi Delta (Kettenring et al., 2012). However, *A. geminipuncta* and *A. neurica* are also unlikely to prevent further encroachment of *P. australis* into important bird habitats in the region requiring managers to seek additional potential biocontrol organisms with a more southern distribution.

4.3. Risks to native P. australis americanus

Our experiments indicate that *P. australis americanus* genotypes are within the physiological or fundamental host range of *A. geminipuncta* and *A. neurica*. In no-choice tests at URI (Table 2) and in Switzerland (Table 5), neonate larvae established in *P. australis americanus*, although survival rates were reduced, typically by 40–50%. Furthermore, dispersing neonate larvae (we tested only *A. geminipuncta*) favor European or North American *P. australis* over *P. australis americanus* (Table 6). We cannot categorically exclude the possibility of attack on *P. australis americanus* after field release. However, female oviposition preferences further reduce the possibility of larval attack on *P. australis americanus* (Fig. 7). When we allowed females to forage and disperse, both *A. neurica* and *A. geminipuncta* laid only 6.5% of all eggs on native *P. australis americanus* (Tables 7 and 8). Eggs laid on *P. australis americanus* suffer increased mortality when leaf sheaths drop to the ground, further reducing the probability of attack. We demonstrated this effect in our experiments, and we expect that predators and flooding, a typical event in many North American wetlands, will increase this mortality substantially.

In summary, the probability of attack on native *P. australis americanus*, while not zero, appears substantially reduced at all life history stages of *A. geminipuncta* and *A neurica*. Nearly 95% of eggs are laid on *P. australis*; eggs laid on *P. australis americanus* suffer high mortality (> 40%); of emerging larvae, few will choose to attack *P. australis americanus,* and for those that do, increased mortality will result. Both *Archanara* species need to change shoots multiple times to complete larval development, thus foraging larvae need to successfully locate new *Phragmites* stems. Endemic *P. australis americanus* generally, but not always, occurs in mixed wetland plant communities further reducing the probability for foraging larvae to successfully locate new stems (Crawley and Gillman, 1989). These factors combine to greatly reduce potential risk and impact to individual *P. australis americanus* plants and their populations.

We cannot categorically exclude that rapid evolutionary changes (Carroll et al., 2007; Williams et al., 2016; Williams and Jackson, 2007) may affect defense syndromes in *P. australis* (native or introduced) and in insect host preference. However, the major factor determining insect host choice in our system involves female choice, a behavioral trait in response to loose leaf sheaths on native *P. australis americanus* and thus a less likely candidate to be overcome by strong selective forces.

Even if larvae were able to improve their preference for and performance on *P. australis americanus*, substantial attack rates would be needed to affect plant performance (Häfliger et al., 2006a) and subsequently demography. Ultimately, our weed biocontrol targets *P. australis* demography; reductions in invasive *P. australis* population growth rates and either no change or ideally an increase in population growth rates for *P. australis americanus*, a subspecies that has greatly declined, due to competition with invasive genotypes (Saltonstall, 2002). Substantial stem mortality and reduction in rhizome growth is required to affect *Phragmites* performance (Häfliger et al., 2006a) and the low number of eggs laid on *P. australis americanus*, combined with low egg survival and larval avoidance of native stems seems very unlikley to result in negative demographic consequences. This is the case for many weed biocontrol programs where agents establish but their impact does not reduce populations (Myers and Sarfraz, 2017). This high level of sub-species host specificity is not unique to *P. australis* (Casagrande et al., 2018), and lack of a demographic threat, even if the proposed biocontrol agents would attack native *P. australis americanus*, would be acceptable to the vast majority of land managers (Martin and Blossey, 2013).

Should biocontrol agents ever attack *P. australis americanus*, overwintering eggs can be eliminated using a mow/mulch regiment or controlled burns. This management method is currently employed after herbicidal treatment of *P. australis* in North America and successfully and frequently used to reduce insect pest populations in commercially managed reed beds in Europe and Asia (Branson et al., 2015; Brix et al., 2014).

4.4. Risks to other biota, particularly predators

Larvae and pupae of both *Archanara* species are important food sources for a number of European birds as well as insect parasitoids (Tscharntke, 1992a; Tscharntke, 1992b). There is no record of toxicity of the species and their cryptic coloration suggest that their defense is camouflage, not chemistry. There are four native North American species initially classified as *Archanara* spp. but now placed in the genus *Capsula* (*C. oblonga*, *C. subflava*, *C. alameda* and *C. laeta*) (Lafontaine and Schmidt, 2010). The species variously attack wetland plants in the genera *Typha*, *Scirpus*, *Juncus*, *Schoenoplectus*, and *Sparghanium* and none of these species, while widespread, have been recorded as toxic or causing adverse impacts on predators. This should allay concerns about potential harm to consumers of *Archanara* larvae, pupae or adults, may they be birds, or bats, or other vertebrates or invertebrates.

Considering potential threats to other wetland biota by these moths, we are left with concerns regarding potential of rapid and widespread death of extensive *P. australis* stands. Such widespread death of plants that constitute valuable habitat was a concern that halted herbicide management of invasive *Spartina* in California to safeguard endangered California rails (Lampert et al., 2014). Concerns over potential negative effects on the endangered southwestern willow flycatcher *Empidonax traillii extimus* following widespread defoliation of *Tamarix* spp. by the biocontrol agent *Diorhabda carinulata* Desbrochers led to a withdrawal of release permits (Dudley and Bean, 2012). European evidence documents the potential for outbreaking *Archanara* populations, a desirable outcome to reduce invasive *P. australis* populations and allow return of native wetland biota. However, unlike herbicide or mechanical treatments (both often followed by spring burns), death due to attack by biocontrol agents will leave dead standing biomass. We strongly discourage winter or spring burning of such stands as this will eliminate overwintering biocontrol agents. Dead stems will continue to provide shelter and cover for wetland biota such as marsh birds or invertebrates foraging in flooded or moist soils and those dependent upon decomposer foodwebs. The marsh surface or soil foodweb is the most important contributor to wetland function in invaded areas since few native species utilize green *P. australis* tissue (Tewksbury et al., 2002). We anticipate increased light penetration following years of noctuid outbreaks, a desired outcome to allow native wetland plant recruitment currently suppressed by invasive *P. australis*.

4.5. Contemporary P. australis management as threat to native biota

Despite large expenditures, there are few data to assess success of invasive *P. australis* management beyond superficial metrics of area treated and resources expended (Hazelton et al., 2014; Martin and Blossey, 2013), which is not unique to *Phragmites* (Blossey, 2016; Buckley and Han, 2014; Foxcroft et al., 2014; Reid et al., 2009). Long-term assessments suggest that herbicide treatments may eradicate only very small populations, creating the need for repeated treatments to suppress large populations (Quirion et al., 2018). Management efforts to suppress invasive *P. australis* in perpetuity divert conservation resources from other projects. We know almost nothing about ecological outcomes of herbicide treatments on native biota, including on native *P. australis americanus*. Invasive *P. australis* continues to spread across the continent threatening remaining native *P. australis americanus* populations (Kettenring et al., 2012; Saltonstall, 2002) and unless biocontrol can be successfully implemented, long-term herbicide treatments will likely become unacceptable (Hazelton et al., 2014; Martin and Blossey, 2013).

5. Conclusions

Invasive *P. australis* is widespread in North America (Chambers et al., 1999; Kettenring et al., 2012; Saltonstall and Meyerson, 2016) with many decades of failed management at great expense and with a dearth of information about impacts of management (Hazelton et al., 2014; Marks et al., 1994; Martin and Blossey, 2013; Quirion et al., 2018). We question the wisdom of continued widespread herbicide campaigns without long-term evaluation of project outcomes and impact on native biota. Results from evaluations of invasive plant management programs using herbicide suggest existence of unintended but serious negative consequences. Native biota may be worse off in areas treated by herbicide compared to areas where the invasive species was left untreated (Baker et al., 2009; Keeley, 2006; Kettenring and Adams, 2011; Skurski et al., 2013). Furthermore, current management practices are allowing continued expansion of the invasive lineage jeopardizing native wetland biota and threatening endemic *P. australis americanus* (Saltonstall, 2002).

In summary, we have extensively evaluated the host specificity and potential impact of *A. geminipuncta* and *A. neurica* if a release were to occur in North America. We conclude that releases pose no risk to plants outside the genus *Phragmites*, predators or foodwebs. We further provide evidence for very strong, but not absolute, preference of the two moth species for invasive *P. australis* over *P. australis americanus*. These preferences became more pronounced as realism of our test design approximated field conditions with 6.5% of eggs laid on *P. australis americanus*. We consider the potential threat to *P. australis americanus* demography by *A. geminipuncta* and *A. neurica* to be far smaller than allowing continued expansion of invasive *P. australis*. Thus, we will proceed with a petition for field release of both species in North America. However, *Phragmites* population in the southern US are outside of the climate envelope of *A. geminipuncta* and *A. neurica* requiring additional research to develop biological control of *P. australis* in southern climates.

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