C-15 -07

Phase 2 Biological Control of Invasive *Phragmites australis*

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DISCLAIMER

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16. Abstract

Non-native *Phragmites australis australis* negatively affects public safety and interferes with New York State Department of Transportation (NYSDOT) maintenance operations and causes negative ecological impacts. Successful management using mowing or herbicides is only effective for small infestations and requires repeat applications. Development of biological controls using specific European herbivores offers a promising alternative. From 2017-2022 we focused on host-specificity, obtaining a federal release permit and development of follow-up assessments for two stem boring moths, *Archanara geminipuncta* and *Archanara neurica*. The application for field release was approved by TAG in 2019, but only Canadian authorities granted field release. In the US, USDA/APHIS is requiring additional work on potential benefits of introduced *Phragmites* and further assurance that native lineages of *Phragmites* are safe. We have advanced (but not fully completed) mass production techniques using a combination of artificial diet and cut stems, made important advances in developing a demographic model, novel monitoring techniques using acoustic recorders and vegetation analyses but could not assess different release procedures and local impacts or fully finalize these products that require field assessments. Releases in Canada are promising and both moth species have established facilitating opportunities for assessment of host specificity under field conditions.

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EXECUTIVE SUMMARY

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INTRODUCTION

Non-native *Phragmites australis australis* establishment and rapid clonal expansion causes negative ecological impacts, and also negatively affects public safety and interferes with New York State Department of Transportation (NYSDOT) maintenance operations. Successful eradication using herbicides has only been achieved for small infestations while larger populations require continuous herbicide treatments to maintain suppression (Quirion *et al.* 2018). These practices pose problems to the maintenance budget and can cause negative ecological side effects for non-target plants. Consequently, several sponsors have helped to fund the development of biological controls using specific herbivores from the European native lineage of invasive *P. australis*. This included Phase 1 research sponsored by NYSDOT that helped identify four stem-boring moths, *Archanara geminipuncta*, *Archanara neurica*, *Archanara dissoluta* and *Arenostola phragmites* as potential biocontrol agents. The purpose of Phase 2 was to continue and potentially conclude these investigations focusing on completing host specificity screening tests for the two most promising species (*A. geminipuncta* and *A. neurica*); preparing a TAG petition; and begin preparations for field release including mass production, development of long-term monitoring protocols and approaches for technology transfer. This project was carried out in close collaboration with partners at the University of Rhode Island and CABI Switzerland and involved collaborators from Canada (independently funded by Canadian grants).

SCOPE OF WORK

The scope of work over the 5-year duration of the contract is divided into numerous tasks and subtasks. I will use this division to report on project progress.

TASK 1: PROJECT MANAGEMENT

The project work team at Cornell was anticipated to include a research associate (Dr. Andrea Dávalos) and a graduate student. Dr. Dávalos accepted a tenure track position at SUNY Cortland requiring some changes in project personnel to still achieve the needed supervision and task accomplishments. Dr. Dávalos continued to be part of the research team, and part of her summer salary is paid off the contract to retain her expertise in modeling and demography. She has also engaged undergraduate students from SUNY Cortland in developing small research projects to help address some of the questions regarding plant demography and the influence of insect herbivores. To replace Dr. Dávalos, I recruited Dr. Stacy Endriss as a post-doc and she joined our team in February 2018. Stacy has extensive experience in weed biocontrol and plant invasions and she took over some of my responsibilities in leading the various field projects, particularly the development of monitoring and assessment protocols. Stacy collaborated closely with Dr. Dávalos but she also moved on to a faculty position in NC as of July 2022. We used additional small-scale exploratory funding from the US Fish and Wildlife Service to engage in a collaboration with the Bioacoustics program at the Cornell laboratory of Ornithology (led by Dr. Holger Klinck) in exploring use of stationary recorders to assess presence of vocalizing birds and amphibians in *Phragmites* stands. And we obtain some funding from the US Army Corps of Engineers to assess dispersal of *Archanara* adults.

Professor Casagrande retired effective July 2017 but he is emeritus at URI. Much of his responsibility in this contract was supervision of Lisa Tewksbury, who is continuing to take care of the immediate needs in the program. Dr. Casagrande, while not involved in the day-to-day operations, continues to work closely with me in project completion, supervision, and writing of petitions and publications. The team and our Canadian collaborators also met at the Annual Meeting of the North American Invasive Species Management Association (NAISMA) held in Saratoga Springs in 2019. We discussed progress, joint monitoring and research proposals and briefed a number of other interested individuals from around North America on the status of the program. Both Stacy Endriss and I delivered talks highlighting aspects of the *Phragmites* biocontrol program, the development of demographic tools, and long-term assessment methods during a special session that my team organized, and which was extremely well attended.

The work program, particularly the field work was, and to some extent still is, significantly affected by Covid-19 restrictions. While work at URI with newly hired undergraduate students was possible in 2020, such arrangements were not allowed at Cornell until later in the fall, and only on a limited basis. We took advantage of the possibilities at URI and completed the *Phragmites* herbivore inventory in RI (see Subtask 5d), while work at Cornell was largely maintaining common gardens, data analyses and report and publication advancements that could be accomplished with existing personnel or remotely. We held regular project team meetings, including overseas collaborators at CABI and our Candian counterparts via zoom. Canadian authorities allowed field releases of both *Archanara* species in 2019, but despite a recommendation by TAG to allow field releases in the US, APHIS has required additional work before granting release of the species in the USA. I will detail these below (Task 2), but it required a slight re-orientation of the work program.

All subcontractual arrangements with CABI, URI and Victoria Nuzzo (for vegetation monitoring) were executed by August 2016. All required Annual and SPR Quarterly Status Reports were delivered.

While not directly related to (or funded by) the work program on *Phragmites*, my team is developing on a number of publications to help land managers, interested citizens and those funding invasive species management efforts better understand and appreciate the use of biological control. One of my graduate students (Wade Simmons) completed an online survey on attitudes towards invasive species management that has been submitted to Conservation Letter recently.

TASK 2: OBTAIN A USDA/APHIS PLANT PROTECTION & QUARANTINE (PPQ) 526 PERMIT FOR BIOLOGICAL CONTROL.

To be able to release biological control agents, extensive documentation of their host specificty and potential environmental impacts are required. Finding host-specific control agents for invasive *P. australis* was further complicated by the existence of endemic native genotypes currently recognized as subspecies *P. australis americanus*. Much of the past 20 years were dedicated to this research. Submission of the full petition for field release to TAG (within USDA/APHIS) had been delayed repeatedly due to various circumstances, including for strategic reasons to address new developments, such as widespread declines on invasive *P. australis australis* in the Mississippi River Delta that raised concerns about the future of the Delta for commercial and conservation purposes. We detailed some of those concerns and our response to it in the 2017 Annual Report and in multiple publications but will not repeat the arguments here as they are contained in many publications. Over the past years we responded to fundamental opposition to biological control of *P. australis australis* (Bhattarai *et al.* 2016; Cronin *et al.* 2016) by trying to debunk unfounded fear mongering (Blossey & Casagrande 2016a; Blossey & Casagrande 2016b). We have also used a Special Issue of the journal BioControl to address the issue of subspecies level specificity and biocontrol of grasses specifically using the *P. australis australis* program as a showcase (Casagrande *et al.* 2018). But the opposition by the same set of individuals has not ceased and in summer 2019 another paper deemed a review was published in Biological Invasions (Kiviat *et al.* 2019). Together with a number of collaborators, I led the writing of a response to correct the blatant mistakes and this paper was quickly accepted by Biological Invasions (Blossey *et al.* 2019b) and is Open Access to allow widespread distribution. While this is unlikely to convince the fundamentalist opposition, it provides a rebuttal and a much-needed counterpoint.

In addition to these papers, we have developed the idea of using demography (which is part of this contract, see Task 5) as part of weed biocontrol programs using a review of the literature and our work on water chestnut (*Trapa natans*) (Blossey *et al.* 2018b). While not directly using *Phragmites* as a case study, this paper introduces the concept of demography as the important "measuring stick" when it comes to assessing success and failure of biocontrol programs, as well as potential non-target effects on other plant species. We have further promoted this idea during the 2019 NAISMA conference. Having these papers and further

advancement of these ideas in the literature provides important background and a foundation to argue our case for release of specific *Phragmites* herbivores.

Furthermore, often reports and TAG petitions include data that are not fully analyzed or peer reviewed leading to differences, however slight they may be, in results, interpretations or even statistical treatments with later published peer reviewed papers. Due to the potential problems this may create for a potentially contentious case such as *P. australis australis*, I spent much of 2018 carefully analyzing and summarizing host specificity data to make sure that the published record and the TAG petition offer the same sophisticated analyses and reviews. I greatly underestimated the amount of time and dedication that it took to bring all these data into succinct manuscripts, had them re-analyzed by a biostatistician (A. Dávalos) and written up. It has taken months more than I previously estimated but the papers were published in 2018 (Blossey *et al.* 2018c; Blossey *et al.* 2018d) and we made them Open Access, so that they are not hidden behind a paywall and allow access by land managers or others.

Part of these efforts to close potential "loopholes" and submit a well thought out TAG petition were some additional host specificity tests that we conducted at URI using *Spartina* spp. and *Phragmites australis berlandieri* (Fig. 1), as well as assessing the ability of overwintering eggs to survive under southern US climate conditions (see also Task 6). The results of these tests came in on time to be part of the revisions of the publications and the details are reported there (Blossey *et al.* 2018c; Blossey *et al.* 2018d). In short, all species appear safe and do not support larval development and the climate in the Gulf Coast does not support completion of the life cycle of the two *Archanara* species, which are temperate region insects.

Fig. 1. From left to right: six first instar *A. geminipuncta* larvae before being inserted into the base of test plant stems using size 00 paint brushes, size comparison of Type M (left) and Type I *Phragmites* side shoots, *Spartina alterniflora* stem with a hole hollowed using an awl tool, and dead first instar *A. geminipuncta* larva dissected from a *S. alterniflora* stem. There was no evidence of internal feeding or molting.

Lastly, several months were required to review all >1,500 threatened and endangered species management and recovery plans, including a new listing that appeared in October 2018 in the federal register for black rails. While species that occur outside the current range of *P. australis australis* or the predicted climate envelope of *A. geminipuncta* and *A. neurica* could be

excluded, only species occurring in Hawaii, Puerto Rico and entirely marine species were excluded from review leaving approximately 1,000. This avoided any potential bias or changes in distribution due to climate change, or range expansion of invasive *P. australis australis*. This review is now necessary due to a guidance document issued by USFWS regarding their Section 7 review of TAG petitions for proposed field releases (USFWS 2015). Every single species will either be categorized as likely or potentially being affected or not, and the reasons can be direct use of the habitat, food web interaction, predation etc.

After all of these plans were reviewed the final petition was submitted to TAG in early October 2018 (Blossey *et al.* 2018a) and after undergoing review we received a positive decision in spring 2019. Rob Bourchier who submitted the TAG petition to Canadian authorities, also received approval from Canadian federal authorities, this included a permission for field releases. The first releases have occurred in Ontario in late summer 2019 and have continued and the species appear to have established at multiple release sites (see Task 8 for some details and updates). In the US, USDA/APHIS did not allow us to proceed to field releases as TAG recommended (this is the usual process and not unique to the *Phragmites* system), but we were not alerted to this "non-decision" and further requirements until 2021. This means the status of the permit is basically as "on-hold, requiring further work". Thus, the permit has not been denied, but we will need additional work and data to proceed.

In discussions with regulators, specifically Dr. Robert Pfannenstiehl at USDA/APHIS, it has become clear that there is significant political pressure (from the LA delegation) to address the *Phragmites* declines reported in the Mississippi Delta, and the safety of native *P. australis americanus*. An ongoing USDA/ARS research project is doing research to potentially import natural enemies (parasitoids) of the Japanese scale (*Nipponaclerda biwakoensis*) that is now considered a threat to introduced *Phragmites* in the Delta. We have addressed all these issues in our publications, the lack of certainty that the scale is responsible for the declines (it is more likley that the sinking of the Delta and the high salinity reduce *Phragmites* vigor), that our biocontrol agents cannot survive in LA etc. and we will not repeat these arguments here. But the political pressure is real. Dr. Pfannenstiehl suggested that for the petition to move forward, that additional work should be conducted to address the potential beneficial utility of introduced *P. australis australis* in coastal and other communities. While we have done similar work a decade ago (Martin & Blossey 2013) we will need to repeat and enhance these initial surveys to fulfill these requirement. There is little doubt about the outcome, given the focus on massive control campaigns that are ongoing across the continent.

An additional concern expressed by Dr. Pfanenstiehl were that oviposition experiments conducted at CABI in Switzerland showed low overall oviposition. At APHIS this raised questions regarding utility and power to forecast discriminatory behavior of *Archanara* adults and hence safety of native *P. australis americanus*. Part of the constraints in Switzerland is the limited availability of native *Phragmites* plants. This resulted in the inability to create larger experimental arrays, a problem difficult to overcome outside of North America. Fortunately, both *Archanara* species are now established in Canada (see Task 8), allowing work to be conducted at field sites to further assess how larvae or adults select feeding and oviposition sites. Thus, a follow-up work program with additional funding is needed to focus on two questions:

1. Are there potential beneficial effects of introduced *P. australis australis* in coastal or inland areas of the United States?

2. Can additional information be provided to safeguard native *P. australis americanus* due to the selectivity of *Archanara neurica* and *A. geminipuncta* favoring introduced *P. australis australis*?

Lastly, because both *Archanara* species were released close to the NY border (along Lake Ontario) in Canada, we anticipate rapid dispersal south after establishment. This was a deliberate decision by our Canadian collaborators because such delays at USDA/APHIS have occurred in other western weed biocontrol programs. But once dispersal of the organisms to NY has occurred and be documented, we would be allowed to further work with and distribute the organisms within state without federal permits. Although this would be initially restricted to sites within NY unless other surrounding states document arrival of the species within their jurisdiction as well. We have selected additional monitoring locations along Lake Ontario (with some small funding obtained from the US Army Corps of Engineers) to assess whether such dispersal has occurred. We will be using adult trapping techniques (black light traps) and physical observations of recognizable morphological changes (increased branching) due to larval attack (See Task 8) to assess dispersal.

TASK 3: OBTAIN PERMIT TO LIBERATE WILDLIFE BIOLOGICAL CONTROL AGENTS FROM THE NYS DEC SPECIAL LICENSES UNIT.

There is no longer a need to obtain special approval from the NYSDEC for field release of biological control agents. The state now goes along with the federal decision-making processes. Once approved by the feds, we will be able to release into *Phragmites* populations in New York.

TASK 4: OBTAIN NYSDOT HIGHWAY WORK PERMITS

To streamline obtaining HWP's in different regions, NYSDOT consolidated this into a single application process covering all field sites that fall within the jurisdiction of NYSDOT. Insurance certificates and all other required materials were provided to NYSDOT and we obtained a HWP in April 2017 which was updated in 2021.

TASK 5: DEFINE AND MONITOR CONTROL SITES, AND DEVELOP A DEMOGRAPHIC MODEL OF *PHRAGMITES AUSTRALIS*

I will report in the following sections of Task 5 both on monitoring and assessing sites before biocontrol agents have been released, as well as our attempts to develop assessment protocols and work summarizing long-term data sets we have curated on *Phragmites* and its associated herbivores. Some of this was started many years ago (for example the common garden in Phase 1) but is only now being finalized for publication. Some of the data and figures are not in their final format and I will omit (some) details of the statistical analyses.

Subtasks 5a-c: Vegetation Monitoring

In 2009, in collaboration with land managers in the NY DOT and other management agencies we selected 11 *Phragmites* sites (7 introduced and 4 native) in New York to monitor *Phragmites* growth and impact on native vegetation. In late August/early September 2009 at each site we randomly established three parallel transects, each with 5-6 permanent $1m^2$ quadrats spaced 5-10m apart, that bisected the apparent invasion front of the *Phragmites* population. Each quadrat was marked with a 1.5m PVC conduit at each of the 4 corners. Sites ranged from seasonally to permanently flooded, and water levels varied through time reflecting natural variation in precipitation patterns and beaver activity. A number of these sites are on DOT ROW's, others are owned by the DEC or The Nature Conservancy (TNC).

In early May 2017 we revisited each site and relocated the permanent quadrats. At one site (in the vicinity of Syracuse) we could not relocated permanent quadrats due to rapid and extensive growth of 3-5m tall *P. australis*. At a second site (Colwell Pond in the Lakeview WMA) we relocated permanent quadrats but the area was flooded 1m deep due to a rise in recent Lake Ontario water levels. We therefore omitted both sites from the 2017-2022 work. Our data focus on the remaining 9 sites (Table 1, Fig. 2).

Table 1. Name, location, occupancy by native and non-native *Phragmites,* and number of quadrats of long-term *Phragmites* monitoring sites in New York State. Sites are grouped by *Phragmites* origin and ordered East to West.

Site name	Location (town)	*Phragmites australis* origin	Latitude	Longitude	Number of quadrats	Location ID
Ilion	Ilion	Non-native	43.018778	-75.028162	18	1
Utica	Utica	Non-native	43.115357	-75.233779	18	2
Martens Marsh	Montezuma	Non-native	43.084364	-76.708233	14	3
Eagle Point - S	Butler	Non-native	43.020779	-76.792823	15	4
Rochester	Rochester	Non-native	43.175400	-77.768495	18	5
Bear Swamp	Sempronius	Native	42.740430	-76.292652	18	6
Lakeview WMA	Pulaski	Native	43.751584	-76.198781	18	7
Carncross	Montezuma	Native	43.082323	-76.710609	18	8
Eagle Point - N	Butler	Native	43.021354	-76.793263	15	9

In September each year we recorded *P. australis* stem density and estimated percent cover, and recorded presence and estimated percent cover of all plant species rooted within each quadrat, in 16 cover categories (midpoints: 0.01, 0.5, 1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, and 100%) in each quadrat. We compiled data by site, year, and quadrat to assess change in *P. australis* stem density and cover, species richness, and vegetation cover by origin (native and

non-native) and life form (graminoid, (bi)annual, perennial forb, woody, fern, and moss).

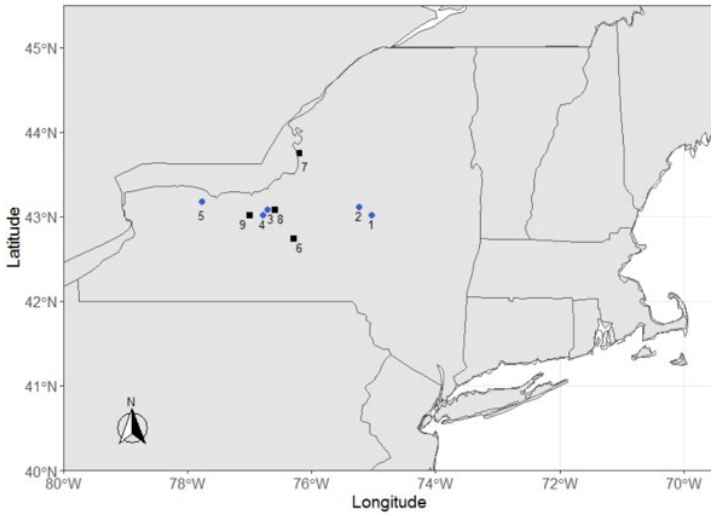


Fig. 2. Location of native (black squares) and non-native (blue circles) long term *Phragmites* monitoring sites in New York State. Numbers refer to locations referenced in Table 1.

Statistical analyses - field

We fitted all models in package glmmTMB (Brooks *et al.* 2017) in R (R Core Team 2019) and considered $P < 0.05$ statistically significant. We conducted type III analysis of variance (ANOVA) and Wald X^2 tests using the car package. We estimated marginal means and pairwise comparisons using Tukey's test with the eemeans package (Lenth 2022). We backward-eliminated non-significant terms via loglikelihood tests. We estimated conditional and marginal R^2 with MuMIn package (Bartoń 2020) estimated predictions with the ggeffects package (Lüdecke 2018) and checked model assumptions using the DHARMa package (Hartig 2020).

We modeled the proportion of quadrats occupied by *Phragmites* over time with a Generalized Mixed Linear Model (GLMM) with binomial errors. We included year, *Phragmites* origin (native or non-native), presence/absence in 2009 (at the beginning of the study) and all 2-way and 3-way interactions as fixed effects and site as a random effect (intercept) to account for repeated measures. Preliminary analyses evaluating effects of time, *Phragmites* origin and *Phragmites* presence/absence in 2009 on stem density, indicated a violation of the constant variance assumption. Variance changed over time and differed by sampling location. Thus, we fitted GLMMs with a negative binomial distribution and dispersion components for *Phragmites* presence/absence in 2009. The full model included all 2-way and 3-way interactions and a random effect of site (intercept) to account for repeated measures.

We evaluated the effects of *Phragmites* density, origin, and their interaction on native cover (%), plant species richness and Shannon diversity index with a GLMMs with negative binomial errors (cover and species richness) or Gaussian errors (Shannon index). Models included site and sampling year as crossed random effects (intercept).

We fit multivariate generalized linear models evaluating how year, *Phragmites* density, *Phragmites* origin and the interaction between *Phragmites* density and origin influenced plant communities with the mvabund package (Wang *et al.* 2022) using a negative binomial

distribution. The mvabund function does not account for nested random effects, thus we used restricted permutations (N=1000) to account for the lack of independence of quadrats within each sampling site (Szöcs *et al.* 2015). To standardize sample size across sites (N=14-18 quadrats per site) we averaged model results from 1000 iterations of the model, each time using a random set of 14 quadrats per site. We used nonmetric multidimensional scaling (NMDS) using Bray-Curtis dissimilarities in the vegan package (Oksanen *et al.* 2019) to visualize plant community composition. We excluded native and non-native *Phragmites* and plant species with less than three occurrences from plant community analyses.

Phragmites field expansion results

Both native and non-native *Phragmites* populations expanded at all field sites over the 12-year study period (Figs 3-5). Quadrats where *Phragmites* was initially absent were increasingly occupied over time; by 2021, *Phragmites* was present in 78% of initially unoccupied quadrats in native stands, and 82% in non-native stands (Fig.3A; Table 2; Conditional R^2=0.73; Marginal R^2=0.68). Every quadrat occupied in 2009 remained occupied thereafter (except for an occasional quadrat with initially only one or few stems) regardless of *Phragmites* origin (Fig.3B).

Stem density increased over time in both native and non-native *Phragmites* stands. The patterns of increases in *Phragmites* stem densities in quadrats where *Phragmites* was absent in 2009 did not differ between native or introduced populations (Fig. 3C). In all years, density of non-native *Phragmites* was significantly higher than density of native *Phragmites* in quadrats initially occupied in 2009 (Fig. 3D; Table 3; Conditional R^2=0.68; Marginal R^2=0.65).

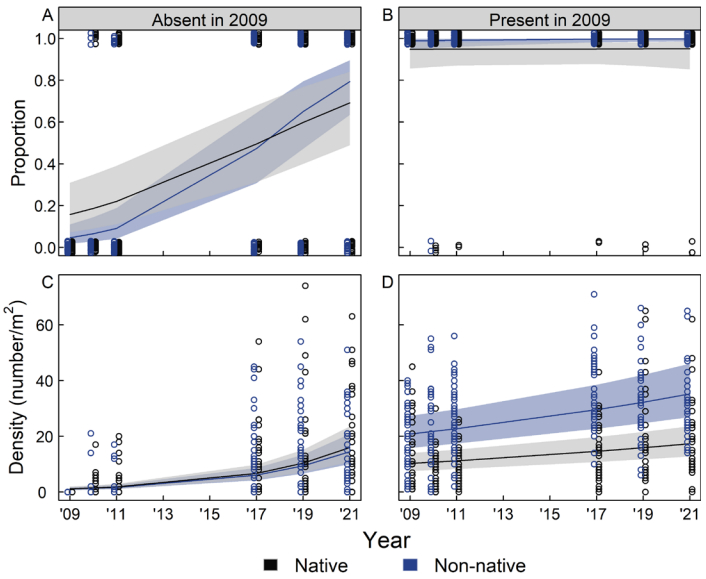


Fig. 3. Proportion of quadrats occupied by *Phragmites* (A, B) and density of *Phragmites* (C, D; number/m^2) from 2009 - 2021 at locations where *Phragmites* was (A, C) absent or (B, D) present when permanent quadrats were established in 2009. Sites were occupied by either native *P. australis americanus* (N=4) or non-native *P. australis australis* (N=5). Lines represent marginal effects from a Generalized Linear Mixed Model with binomial (presence/absence) or negative binomial (density) errors and bands depict 95% CI. Models included random effects for site (intercept) and an overdispersion component (stem density). Points are quadrat observations and jittered to allow visualization.

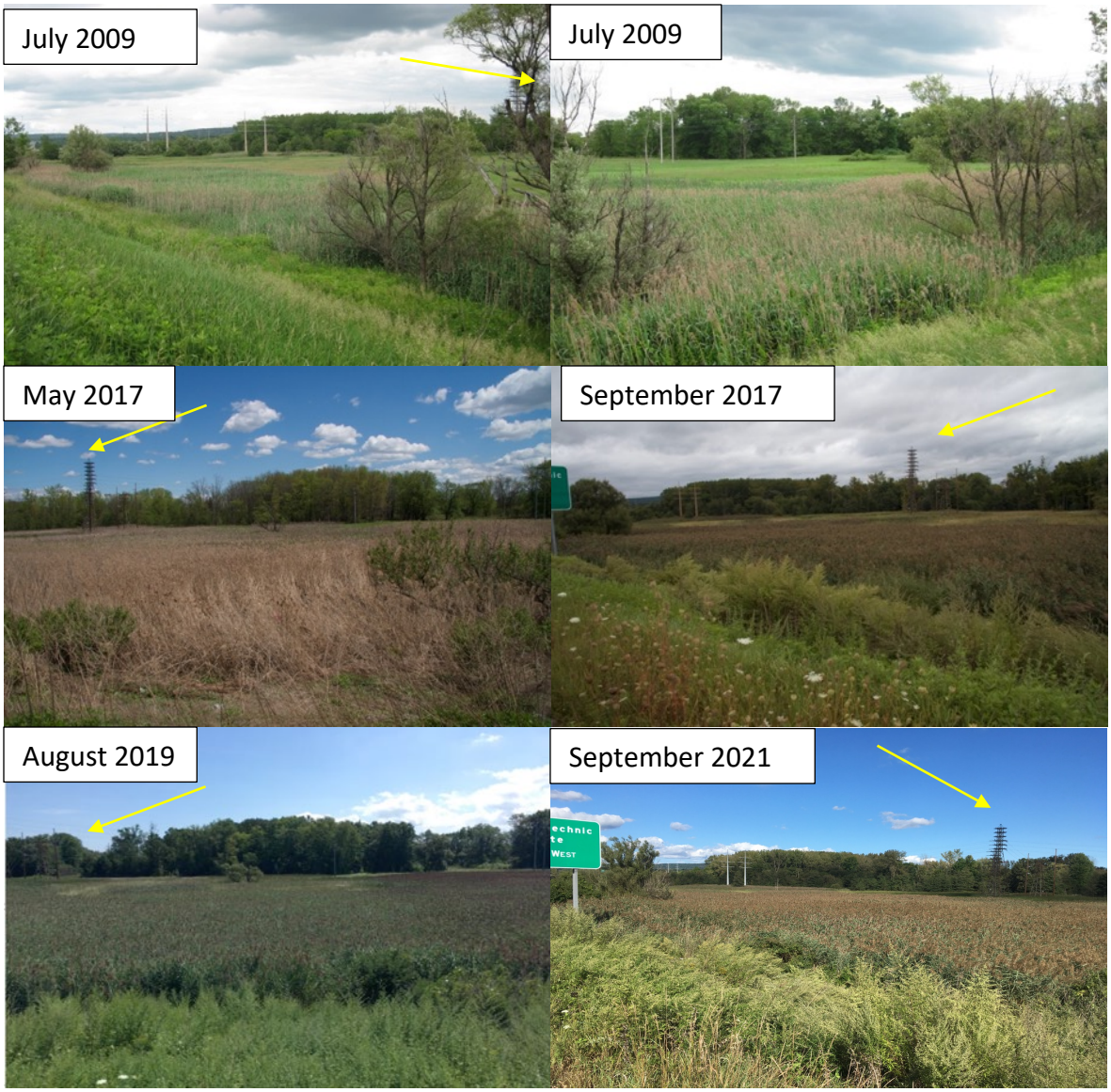


Fig. 4. Introduced *P. australis australis* at Utica in mid-summer in 2009, in May and September 2017, in August 2019 and mid-September 2021. Please note *Phragmites* expansion into the marsh with lower mixed vegetation (light green colors towards transmission line poles). By 2019, this mixed vegetation was almost entirely overrun by introduced *P. australis australis*. Yellow arrows point to a transmission line pole for reference purposes. The direction these photos were taken is slightly different and the transmission pole in the upper left-hand photo is obscured by a tree.

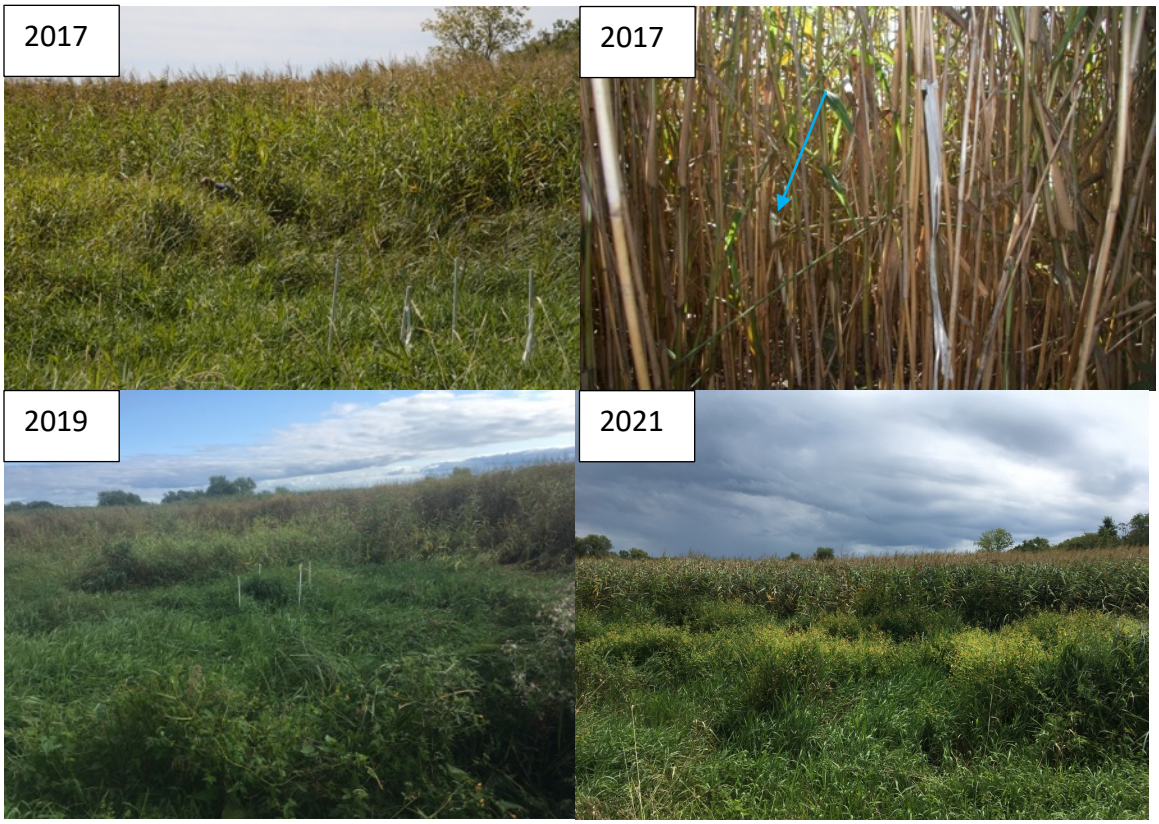


Fig. 5. Native *P. australis americanus* invasion front at Lakeview WMA in September 2017, 2019 and late August 2021 with high reed canary grass (*P. arundinaceae*) cover and permanent monitoring quadrats in the foreground. High stem densities occur in the interior of stand (upper right panel) with white flagging tape marking location of permanent monitoring quadrat (blue arrow points to a PVC corner post).

Table 2. Proportion of quadrats occupied by *Phragmites australis* over time (sampling years) as function of *P. australis* origin (native or non-native) and sampling location (*P. australis* absent/present in 2009) at sites occupied by native *P. australis americanus* (N=4) and non-native *P. australis* (N=5). Values reflect estimates and standard errors from Generalized Linear Mixed Model with binomial errors and Type III Wald X^2 tests. Model included random effects for site (intercept).

	Estimate	Std. Error	z value	Pr(>|z|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	-3.39	0.51	-6.65	<0.001	44.23	<0.001
Year	0.36	0.04	8.50	<0.001	72.24	<0.001
Origin (native)	1.51	0.68	2.21	0.03	4.87	0.03
Phragmites p/a in 2009	7.67	0.84	9.13	<0.001	83.44	<0.001
Year x Origin	-0.16	0.05	-2.92	0.004	8.52	0.004
Year x Location	-0.20	0.07	-2.95	0.003	8.69	0.003
Origin x location	-2.88	0.89	-3.24	0.001	10.51	0.001

Table 3. *Phragmites* stem density (m^2) over time (year) as function of origin (native or non-native) and *Phragmites* presence/absence (p/a) when permanent sampling quadrats were established in 2009 at sites occupied by native *P. australis americanus* (N=4) and non-native *P. australis australis* (N=5). Values reflect results of Generalized Linear Mixed Model with negative binomial errors and Type III Wald X^2 tests for fixed effects. Model included random effects for site (intercept) and overdispersion components.

Fixed effects

	Estimate	Std. Error	z value	Pr(>|z|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	-0.14	0.26	-0.53	0.60	0.28	0.60
Origin (native)	0.10	0.24	0.40	0.69	0.16	0.69
Year	0.22	0.02	11.97	<0.001	143.22	<0.001
Phragmites p/a in 2009	3.13	0.23	13.56	<0.001	183.77	<0.001
Origin x *Phragmites* p/a in 2009	-0.80	0.15	-5.25	<0.001	27.55	<0.001
Year x *Phragmites* p/a in 2009	-0.17	0.02	-9.13	<0.001	83.43	<0.001

Dispersion effects

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	3.43	0.14	24.04	<0.001
Location	-1.65	0.17	-9.83	<0.001

Table 4. Effects of *Phragmites* stem density (m^2) and origin (native, non-native) on native plant cover (%), plant species richness and Shannon diversity index at sites occupied by native *P. australis americanus* (N=4) and non-native *P. australis australis* (N=5). Values reflect results of Generalized Linear Mixed Models with negative binomial errors (cover and species richness) or Gaussian errors (Shannon diversity) and Type III Wald X^2 tests for fixed effects. Models included crossed random effects (intercept) for site and sampling year.

(A) Native Plant Cover

	Estimate	Std. Error	z value	Pr(>|z|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	3.57	0.42	8.48	<0.001	71.84	<0.001
Density	0.00	0.00	0.05	0.96	0.00	0.96
Origin (non-native)	-0.27	0.55	-0.48	0.63	0.23	0.63
Density x origin	-0.04	0.01	-8.12	<0.001	66.00	<0.001

(B) Plant Species Richness

	Estimate	Std. Error	z value	Pr(>|z|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	1.67	0.17	9.56	<0.001	91.44	<0.001
Density	0.00	0.00	-1.96	0.05	3.84	0.05
Origin (non-native)	-0.16	0.23	-0.67	0.50	0.45	0.50
Density x origin	-0.01	0.00	-6.14	<0.001	37.72	<0.001

(C) Shannon Diversity Index

	Estimate	Std. Error	z value	Pr(>|z|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	1.13	0.14	7.87	<0.001	61.96	<0.001
Density	0.00	0.00	-0.43	0.67	0.18	0.67
Origin (non-native)	-0.21	0.19	-1.09	0.28	1.18	0.28
Density x origin	-0.01	0.00	-6.43	<0.001	41.33	<0.001

Native plant cover (%; Conditional R^2=0.52; Marginal R^2=0.24), native plant species richness (Conditional R^2=0.51; Marginal R^2=0.22) and native Shannon diversity index (Conditional R^2=0.50; Marginal R^2=0.23) significantly decreased with increasing *Phragmites* density (Fig. 6; Table 4), and rate of decline was steeper at non-native *P. australis australis* sites than at native *P. australis americanus* sites (Table 4).

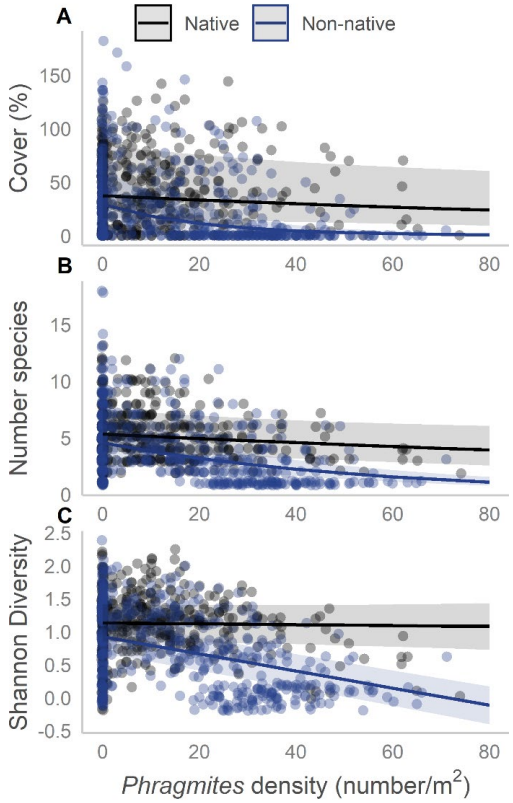


Fig. 6. (A) Native plant cover (%), (B) number of native plant species and (C) native plant Shannon Diversity index as a function of *Phragmites* stem density (number/m^2) from 2009-2021 at sites occupied by either native *P. australis americanus* (grey; N=4) or non-native *P. australis australis* (blue; N=5). Data represent marginal effects and 95% CI from a Generalized Linear Mixed Model with negative binomial error. Points are quadrat observations. Models included random effects for site and year. Points are quadrat observations.

We recorded a total of 139 plant species (107 native and 32 non-native) across all sites and years. The majority of species (61) occurred at only a single growing location, and no species was found at all sites. The most common species were the non-native reed canary grass (*Phalaris arundinacea*) which occurred at 7 of the 9 sites, and the native sedge (*Carex lacustris*) which occurred at 6 sites. While sites varied from permanently to seasonally flooded, and were located in different regions of the state, only one site (Bear Swamp) supported a unique plant community (shrub fen) distinct from the other ten sites. Plant communities were similar across years (deviance=729.53, P=0.68; mean of 1000 model iterations each with 14 quadrats per site) and at locations with native or non-native *Phragmites* (deviance=1479.58, P=0.33; Fig. 7). Increasing *Phragmites* density was associated with different plant communities (deviance=893.89, P=0.001), regardless of *Phragmites* origin (origin x density; deviance=181.23, P=0.5). Cover of 23 plant species significantly decreased with increasing

Phragmites density, while cover of remaining species (N=114) did not vary with *Phragmites* density (P>0.05 for all cases).

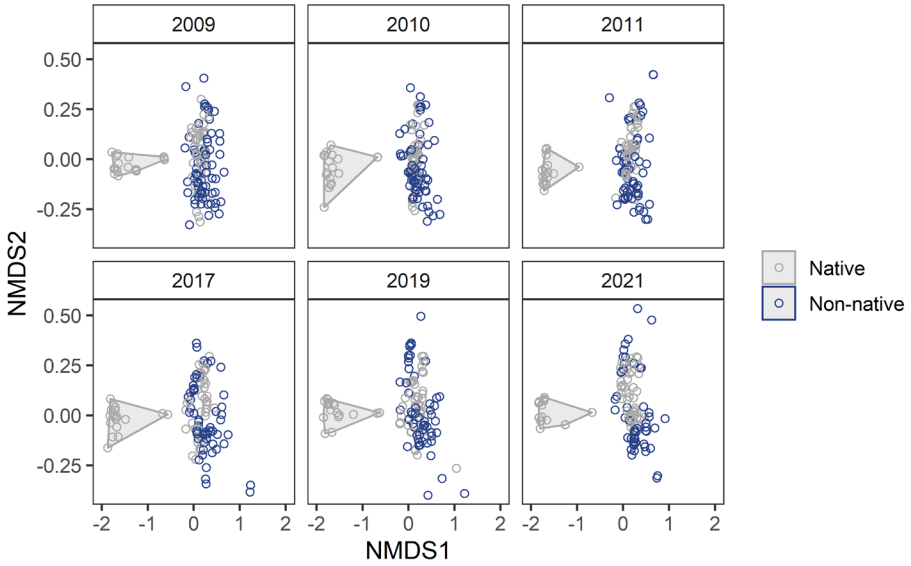


Fig. 7. Non-metric multidimensional scaling (NMDS) of plant communities at sites occupied by either native *P. australis americanus* (N=4; grey) or non-native *P. australis australis* (N=5; blue). Points are quadrat observations and polygon highlights Bear Swamp, the only site with distinct vegetation communities. Polygons for remaining sites are not shown for clarity.

Our results *Phragmites* populations expansion at all sites, regardless of origin, but this appears a slow continued process without additional disturbances. Sites that were nearly or entirely invaded appeared to remain stable over our observation period and we found no declines in quadrat occupation once a quadrat was occupied. This includes native *P. australis americanus* which shows robust and dense populations particularly at Carncross and Lakeview WMA (Fig. 5). At some sites, such as in Utica, introduced *Phragmites* has invaded nearly all remaining plant communities diminishing the value of these locations for native species (Fig. 4). Native *Phragmites* is considered increasingly rare in the US, particularly in the East, where introduced *P. australis australis* is expanding its range locally and regionally. However, native *P. australis americanus* seems to hold its own at the sites we monitored and it may only be vulnerable to introduced *P. australis australis* (see next section).

It is important to note that our data suggest that maintaining diverse wetland plant communities does not require elimination of non-native *Phragmites* but solely a reduction in stem densities and cover to allow for co-existence with native species. Based on our data *Phragmites* stem densities of < 20/m^2 may be sufficient to facilitate diverse wetland assemblages. Whether this pattern we describe here for our long-term research sites can be confirmed elsewhere by others across North America remains to be seen. Also, we will need to further assess the response of other non-plant biota to changes in *Phragmites* cover and stem densities. But it is an important reminder that what we want to achieve in invasive plant management, whether we use biocontrol or other management tools, is a reduction of negative impacts, not eradication or elimination of a species. Eradication may neither be feasible nor necessary, but this fact is often ignored when goals are articulated. Our further work on insect and other biota at these long-term monitoring sites will inform our recommendations.

Task 5e: Demographic model (including common garden expansion rates)

Here I initially report on a long-term evaluation of an experimental set-up to assess competition between native and introduced Phragmites under standardized condition. We and collaborators across North America initially collected rhizome fragments of *P. australis australis* and *P. australis americanus* at field sites across North America. We propagated rhizome cuttings for at least two years in a common garden in multiple 100L tree pots/population (BFG Supply, Lancaster, New York, USA) filled with commercial potting soil (Farfard Canadian growing mix No. 1-P, Agawam, Massachusetts, USA) randomly placed in shallow pools (5-10 cm deep to retain wetland conditions). This procedure allowed us to reduce potential environmental effects of field collection location via maternal effects. Where possible, we obtained haplotype information by submitting samples for analyses to Kristin Saltonstall.

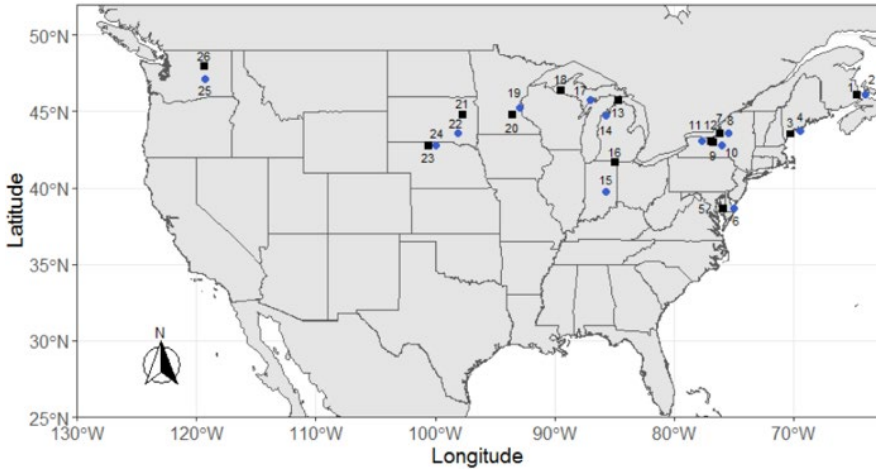


Fig. 8. Location of 13 paired populations of native (black squares) and non-native (blue circles) *Phragmites* grown in a common garden in Ithaca, New York, USA 2008-2015. Numbers refer to locations referenced in Table 5.

In summer 2008 we established a long-term common garden consisting of 75 trenches spaced 1m apart from each other at the Cornell Resource Ecology and Management Facility (REM) in Ithaca (Figs. 9, 10). Each trench (10 m long, 50 cm wide and 50 cm deep) was lined with pond-liner (45 mil EPDM [Ethylene Propylene Diene Monomer], Pondliner.com, Shawnee, Oklahoma) and filled with Cornell compost mix (Cornell University, Ithaca NY). We propagated new *Phragmites* plants from fresh rhizome cuttings taken from the established collection of plants from across North America maintained in large tree pots. We planted one individual (approx. 20cm tall with several new leaves) at each end of each trench pairing a native with an introduced population from the same region (Fig. 9).

We established 28 *Phragmites* populations (14 native, 14 introduced) at the beginning of the experiment in 2008 (Table 2), including from the Rochester long-term monitoring location. We completely randomized planting locations within our common garden with each population represented by five clonal individuals but each trench always contained a native clone at one end and an introduced clone at the other in an alternating fashion (Fig. 9). We allowed plants to grow and expand through clonal growth in each trench. We initially removed competing other plants through regular weeding but stopped this interference in 2012 when clones were well established and bare soil in trenches had filled with *Phragmites* shoots.

Table 5. Collection location of native and introduced *Phragmites* populations (N = native, I = introduced Eurasian haplotype, PQ = introduced hybrid of Asian/Australian descent) grown in the common garden at the Resource Ecology and Management Facility, Cornell University, Ithaca, New York, USA.

Location	Latitude	Longitude	Type
Antioch, CA	37.97	-121.81	PQ
Bergen Swamp, NY	43.09	-77.98	N
Clark County, SD	44.81	-97.72	N
Darr Bridge, NE	42.8	-100.63	M
Darr Bridge, NE	42.8	-100.63	N
Davidson County, SD	43.6	-98.11	M
Deer Creek, NY	43.57	-76.2	M
Deer Creek, NY	43.57	-76.2	N
Dieppe, NB	46.09	-64.75	N
Escanaba, MI	45.75	-87.06	M
Forest Lake, MN	45.28	-92.99	M
Libby River, ME	43.55	-70.32	M
Libby River, ME	43.55	-70.32	N
Long Lake, MI	44.72	-85.77	M
Mackinaw City, MI	45.77	-84.73	N
Marenisco, MI	46.4	-89.57	N
Mile Marker 59, IN	39.79	-85.77	M
Moncton, NB	46.09	-64.76	M
Montezuma, NY	43	-76.78	M
Montezuma, NY	43	-76.78	N
Moses Lake, WA	47.12	-119.29	M
Novato, CA	38.09	-122.56	M
Pipewort, IN	37	-101.89	N
Rochester, NY	43.11	-77.73	M
Seminary Fen, MN	44.82	-93.56	N
Sun Lake, WA	30.76	-85.69	N
TNC Choptank MD	38.68	-75.95	M
TNC Choptank MD	38.68	-75.95	N

We followed clonal expansion by recording the distance of the furthest above ground shoot from the original planting location until the two clones met. We were able to distinguish native and introduced shoots easily through subspecies-specific morphological characters. In most years, we harvested all above ground shoots in late fall or early winter after shoot senescence (typically in November, but occasionally also in December), counted the number of stems in each trench (separated by native or introduced status) and then dried all above ground materials and recorded dry biomass. We terminated the experiment in 2015 when introduced *Phragmites* had started to spread through the common garden, including outside of the lined trenches.

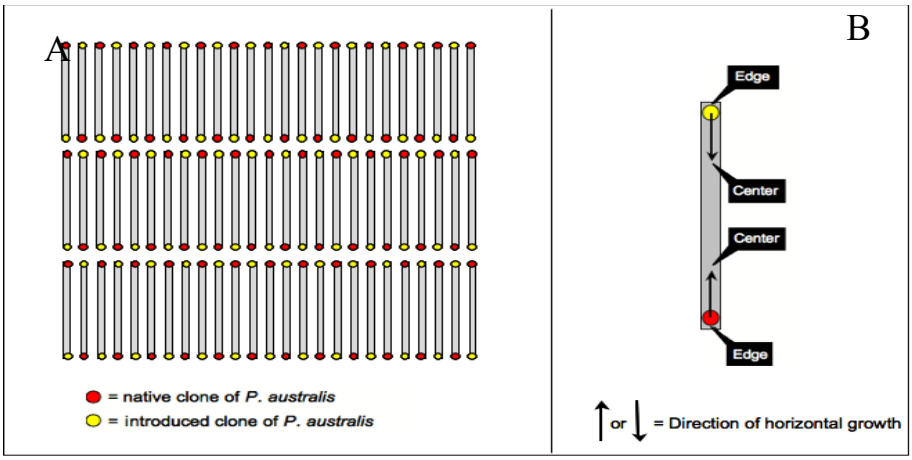


Fig. 9. (A) The *Phragmites* common garden design consisting of 3 sets of 25 trenches (0.5m wide, 0.5m deep and 10m long). We planted each trench with a native *P. australis* at one end and an introduced at the other (B).

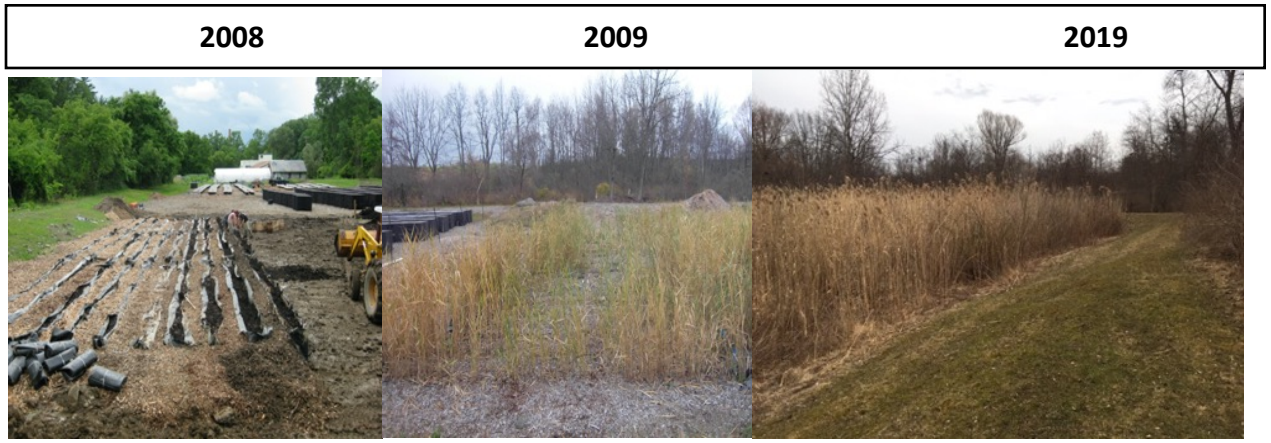


Fig. 10. Long-term experimental set up to study clonal expansion rates of native and introduced *P. australis*. Picture on left shows common garden design using linear "trenches" in July 2008 during construction.

The data we present here are both important to evaluate long-term ecological consequences of continued introduced *P. australis australis* invasion as well as background information to populate our demographic model (see below). The data from this experiment has been combined with the field monitoring data presented above and will be submitted to Journal of Ecology this fall.

Statistical analyses - common garden

We fitted all models in package glmmTMB (Brooks *et al.* 2017) in R (R Core Team 2019) and considered $P < 0.05$ statistically significant. We conducted type III analysis of variance (ANOVA) and Wald X^2 tests using the car package. We estimated marginal means and pairwise comparisons using Tukey's test with the eemeans package (Lenth 2022). We backward-eliminated non-significant terms via loglikelihood tests. We estimated conditional and marginal R^2 with MuMIn package (Bartoń 2020) estimated predictions with the ggeffects package (Lüdecke 2018) and checked model assumptions using the DHARMa package (Hartig 2020).

We fitted GLMM and LMM independent models to evaluate effects of *Phragmites* origin, year and their interaction on *Phragmites* survival and growth. All models included crossed random effects (intercept) for planting location in the common garden and for site and population (native or non-native) within site. We modeled survival through 2015 with a GLMM with binomial errors, biomass with a LMM (square-root transformed) and stem number with a GLMM with negative binomial errors. We only included non-zero values for biomass and stem number analysis. We calculated annual rate of spread until 2010 and evaluated differences as a function of *Phragmites* origin with an LMM with Gamma distribution.

Common garden - results

Survival of *Phragmites* varied by origin (Estimate SE: 0.50 ± 1.25; $X^2_{df=1}$=0.16; P=0.69), year (-1.60 ± 0.18; $X^2_{df=1}$=82.00, P<0.001), and their interaction (1.70 ± 0.2; $X^2_{df=1}$=54.54; P<0.001). While survival of non-native *P. australis australis* was high throughout the study period (99%), survival of native *P. australis americanus* significantly decreased over time, dropping to < 0.1% by 2015 (Fig. 11). In addition, only 6 of 13 native *P. australis americanus* populations survived until 2015, whereas all non-native populations survived.

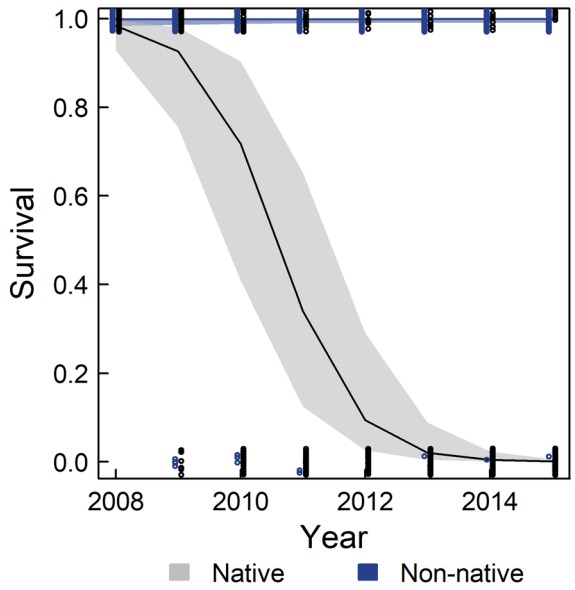


Fig. 11. Survival of native *P. australis americanus* and non-native *P. australis australis* grown in a common garden in Ithaca, New York from 2008 to 2015 (N=130; 13 paired populations of native and non-native *Phragmites*; 10 planting locations per pair). Data show predictions and 95% CI from a Generalized Linear Mixed Model with binomial errors. Model included crossed random effects for planting location and for population nested within site. Points depict observations at each planting location.

Annual rate of spread (m/year), number of stems and biomass of *Phragmites* were significantly higher for non-native than native populations (Figs 12-13). In 2010, two years after planting, spread rate of non-native *P. australis australis* was significantly higher (estimate ± 1SE: 1.16 ± 0.16; $X^2_{df=1}$=49.61; P<0.001; 95% CI marginal mean: 2.61-3.52 m/year) than spread rate of native *P. australis americanus* (95% CI marginal mean: 0.69-1.29 m/year; marginal R^2=0.66; conditional R^2=0.78; Fig. 12).

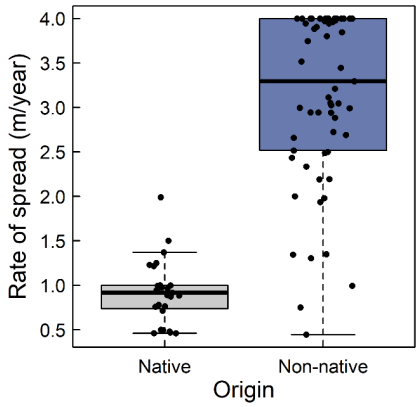


Fig 12. Rate of spread (m/year) two years after planting (2010) of native *P. australis americanus* and non-native *P. australis australis* in a common garden in Ithaca, New York (N=130; 13 paired populations of native and non-native *P. australis*, 10 planting locations per pair). Points depict replicate observations.

Table 6. Effects of origin and year on (A) number of stems and (B) biomass (square-root g) of *Phragmites* in a common garden in Ithaca New York from 2008-2015 (N=130; 13 paired native and non-native populations; 10 planting locations per pair) according to Generalized Linear Mixed Models with negative binomial (number of stems) and normal (biomass) errors and Type III Wald X^2 tests for the fixed effects. Models included crossed random effects (intercept) for planting location in the common garden and for population nested within site.

(A) Number of stems

	Estimate	Std. Error	z value	Pr(>|z|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	1.88	0.26	7.11	<0.001	50.51	<0.001
Origin (non-native)	-1.31	0.29	-4.50	<0.001	20.28	<0.001
Year	1.75	0.15	11.52	<0.001	132.66	<0.001
Year ^2	-0.24	0.03	-9.18	<0.001	84.22	<0.001
Origin x Year	1.21	0.17	6.95	<0.001	48.28	<0.001
Origin x Year^2	-0.13	0.03	-4.61	<0.001	22.21	<0.001

(B) Biomass (g)

	Estimate	Std. Error	df	t value	Pr(>|t|)	$X^2_{df=1}$	Pr(>Chisq)
Intercept	-0.81	1.44	110.48	-0.56	0.58	0.32	0.57
Origin (non-native)	-1.97	1.65	127.14	-1.19	0.24	1.42	0.23
Year	5.35	0.79	634.80	6.79	<0.001	46.06	<0.001
Year ^2	-0.54	0.09	624.89	-5.65	<0.001	31.96	<0.001
Origin x Year	3.50	0.91	630.64	3.86	<0.001	14.89	<0.001
Origin x Year^2	-0.13	0.11	622.52	-1.19	0.24	1.41	0.24

We found significant effects of year (polynomial), origin and their interaction on *P. australis* number of stems and biomass (Table 6). Stem number peaked in 2010 for both native and non-native populations and, non-native *P. australis australis* had significantly higher stem numbers than native *P. australis americanus* in all years (Fig. 13; marginal R^2=66; conditional R^2=0.89). Biomass of native and non-native *Phragmites* increased until 2011, when non-native

P. australis australis biomass plateaued while native *P. australis americanus* biomass declined in the few trenches (6 in 2015) where populations continued to exist (Fig. 13, marginal R^2=0.67; conditional R^2=0.77).

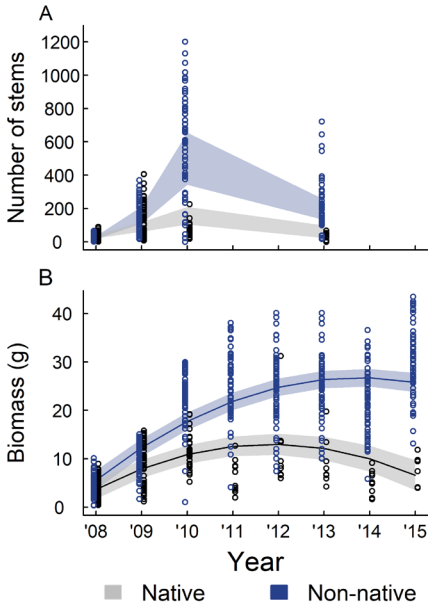


Fig. 13. (A) Number of stems and (B) biomass (square-root transformed; g) of native *P. australis americanus* and non-native *P. australis australis* in a common garden in Ithaca New York from 2008 to 2015 (N=130; 13 paired native and non-native populations; 10 planting locations per pair). Data show predictions and 95% CI from a Mixed Model. Model included random effects for planting location and for population nested within pair. Points depict observations at each planting location. Only surviving populations were included in the analysis (N=6 and 13, for native and non-native populations by 2013).

Our work on spread and competitive interaction under standardized conditions n revealed the superior competitive ability of introduced *P. australis australis*. This pattern held regardless of source location for native *P. australis americanus* genotypes collected across North America. But we also found a distinctive temporal component that short-term experiments are unable to reveal. Survival and biomass allocation as well as number and height of stems (not shown) in the first few growing seasons are broadly similar between native and introduced genotypes, but then the patterns are diverging. While many native genotypes die out over time, there is basically no death of the introduced genotypes. We do not yet understand the mechanisms behind these patterns, but suspect that both direct competition (when native and introduced clones started to interact in the trenches by occupying the same space) as well as potential negative soil feedback (PSF) (van der Putten *et al.* 2013; Crocker, Nelson & Blossey 2017) may play important roles. These processes play out over time and why introduced *Phragmites* may be less vulnerable to PSF or be stronger competitor will require additional mechanistic work. But it is also clear that annual spread rates, at least in the trenches, is significantly faster for introduced *Phragmites*, at least in the absence of other plant competitors. Our experiment is unable to address how different moisture or salinity conditions, different climates or soil fertility may affect the outcome of the interaction of native and introduced lineages, but long-term observations and the ability of introduced *Phragmites* to thrive in extremely different environments offer little hope for the native species to be able to thrive, unless the initial introduced of introduced genotypes is prevented (see section 5a, b above).

Overview of our approach to developing and refining our demographic model

Developing a demographic model of a perennial, clonal plant species is uniquely challenging. The role of clonal reproduction—such as by vegetative reproduction by *Phragmites*—often plays a critical role in the population growth and spread of invasive species. At the same time, the demographic role of vegetative reproduction is highly context-dependent and greatly understudied, especially within invasion processes (Arroyo-Cosultchi *et al.* 2022). This, in part, is because observing how clonal plants are connected belowground via rhizomes is at best logistically infeasible and at worst impossible in field populations, especially without destroying the very plants we are trying to observe. To overcome this obstacle, a common approach is to treat individual stems as unique 'individuals', irrespective of whether those stems are connected belowground via rhizomes or in actuality are truly distinct individuals with independent root systems (Fig. 14).

This approach comes with its own additional challenges, as it requires tracking survival and performance of individual stems through time. Thus, we must be able to find, identify, and repeatedly sample the same stems through time—often within very dense stands of *Phragmites* that are difficult to navigate and to see through. Through trial and error, we have found that colored twist ties or pipe cleaners can be used to mark unique stems such that they can be identified by a unique combination of colored ties and can thus be more easily monitored repeatedly time (Fig. 15). Using cut PVC rings marked with the unique stem identifier using a sharpie also works well—we have developed this approach for monitoring individual stems of Japanese knotweeds (Fig. 15). Further, understanding how demographic rates contribute not only to population growth rates but to population spread is one way that demographic models may be especially useful for informing invasive species management.

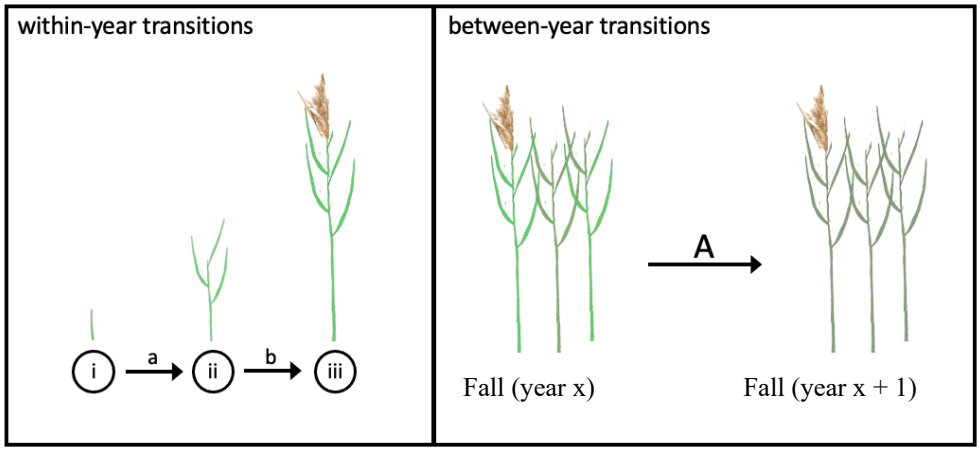


Fig 14. Our field and common garden experiments have allowed us to collect data that informs two different sets of demographic parameters: those that that govern within-year transitions between *Phragmites* stems of different size classes within a growing season (left panel; i: 'asparagus-like shoots', non-flowering stems, and flowering stems) and those that govern how the number of *Phragmites* stems produced by the end of the fall in one year predicts the number of stems that will be produced by the end of the fall in the following year. Although within-year transitions may be particularly important for understanding how herbivory, climate, competition, and other variables influence seed production (and thus dispersal events), between-year transitions appear more critical for understanding local growth and spread of *Phragmites* populations.

Fig. 15. Using colored pipe cleaners (upper left) or twist ties can be a useful way of marking individual stems in the field—we have used up to four different bands as unique identifiers (e.g., blue-blue-blue-blue vs. blue-blue-blue-yellow vs. blue-blue-blue-red). We have also used numbered PVC rings to track individual stems, as shown for tracking emerging Japanese knotweed stems (upper right). We also experimented with different methods to make demographic parameters of individual *Phragmites* stems spatially explicit. For example, we tried using labeled golf tees to photograph the exact location of cut *Phragmites* stems within m^2-quadrats in the field, which we then imported into ImageJ to correlate the relative locations of *Phragmites* stems within a stand to metrics of individual stem performance. This approach, however, was logistically impractical, as it required cleaning the leaf litter well enough to be able to capture all of the golf tees representing cut *Phragmites* stems within a photograph (bottom photograph: see how the white golf tees can easily blend in and be hidden by the stem litter).

Constructing spatially explicit demographic models for *Phragmites* would therefore be particularly beneficial but would require mapping the location of individual stems in relation to each other at least once a growing season. We were able to develop such a method for common gardens planted at our outdoor research facility at Cornell University but so far have been unable to develop a method for collecting spatially explicit stem data within naturally occurring field populations (Fig. 15).

We are currently collaborating with Dr. Jennifer Price Tack, a decision-scientist and the large carnivore and elk research scientist in the Wisconsin Department of Natural Resources, to now integrate stem-level and $1m^2$-quadrat-level demographic parameters into an ISR approach to a state-space model (Fig. 16). This approach will allow us to model changes in *Phragmites* stem density—and its impact on community metrics such as plant diversity—through space and time. We are developing this model to account for how changes in stem density over time are mediated by the ancestral origin of *Phragmites* (i.e., *P. australis australis* vs. *P. australis americanus*), population, climate of origin, intraspecific competition, insect feeding damage—especially by *Archanara* spp., and other ecologically important variables. Thus, this model will be informed by the demographic parameters that we have been accumulating over the last several decades across field and common garden experiments (Table 6). We already envision this being an important future aspect of the technology transfer (Task 9) of this research, as we have already created the framework for a ShinyApp that will be accessible online to local practitioners,

scientists, or other interested members of the general public. This App will allow individuals to input *Phragmites* stem densities for however many years data they have available, and will then allow them to run a gridded cell simulation of how *Phragmites* stem densities (and its impact on plant diversity) is predicted to change over time in response to variables such as the arrival of the biocontrol agents or other important ecological factors.

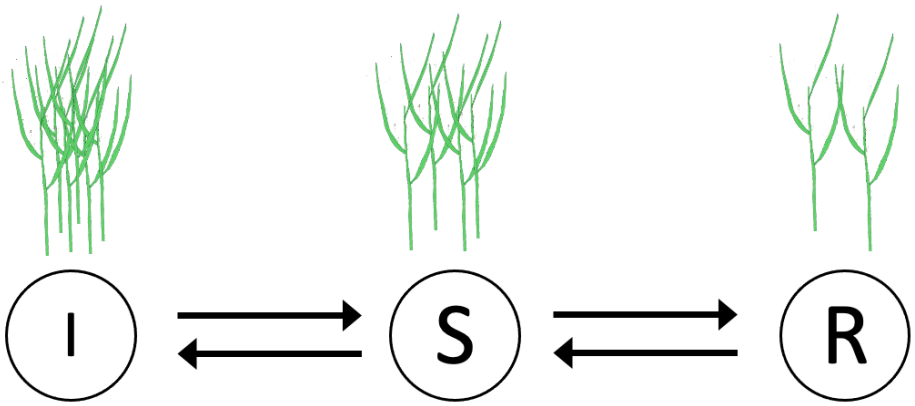


Fig. 16. An oversimplified conceptual model of an ISR state-space model for *Phragmites*, in that *Phragmites* populations can shift along the gradient from 'invasion' (I) to' stabilization' (S) to 'recovery' (R) through space and time. Which 'space' a patch of *Phragmites* falls into depends on the stem density within that patch—specifically, whether *Phragmites* stem densities are high enough to drive declines in local metrics of plant diversity ('I'), high enough to drive recovery of plant diversity ('R'), or somewhere in between ('S). Arrows represent the probability of annual transitions between states.

In support of demographic modelling efforts, we tested larval dispersal of *A. neurica*, in a field experiment in Switzerland since we are unable to do such experiments in the US. It would be important to know, how far larvae can disperse to attack new shoots and how that may affect discrimination between native and introduced *Phragmites*. This investigation involved placing eggs on old *Phragmites* stems into the field right before expected larval hatch in spring, or releasing newly hatched larvae. We attempted these experiments in 2017, 2018 and again in 2019 and planned to check for distances of infested shoots from release points as indication of potential larval dispersal from oviposition sites. Unfortunately, extensive cold snaps repeatedly killed all larvae. Large temperature fluctuations in early spring with early plant growth and then killing frosts have become more common. While plants were able to regrow from below ground rhizomes, insects were killed without the ability to regenerate within the same season.

We did not attempt to repeat this experiment in spring 2020, but based on 2017 and 2018 experiences, we made contingency plans storing eggs in a fridge, which allowed us to set up the experiment again at three plots where healthy *Phragmites* shoots survived. Survival or larval fitness or hatch rates, based on our recovery of infested stems, may have been very low. This is often the case when *Archanara* eggs are cold stored over extended periods. After six weeks, we were able to locate six damaged stems, all within 1-meter distance of the release point, indicating very limited larval dispersal. Three of the stems had been damaged by first and three by third instars. It will be necessary to repeat experiments, ideally without a cold snap or under more controlled conditions at the natural hatching time of eggs, to measure larval dispersal capacity.

We had planned to conduct some of these experiments at Cornell in quarantine (see 2019 Annual Report for details). We applied for and received permission to import *Archanara neurica* and *A. geminipuncta* into the Cornell quarantine and we received 500 eggs of each from CABI in early November. These eggs were stored in a refrigerator under appropriate conditions, but eggs desiccated, and we lost the colony. We would have been unable to raise insects or conduct our experiments due to Covid-10 restrictions. We now plan to do these experiments and work with our Canadian collaborators at their release locations. Our demographic model will not be fully complete due to some lack of these detailed data, but where appropriate we will model and then "fine-tune" the model as new information becomes available. But the basic structure will be retained.

Table 7. Summary of types of data that have been collected across the last two decades that will be used to inform our models. Note that we have been waiting for critical information about how *Archanara* spp. will disperse and impact *Phragmites* within North America but expect to have this information from the sites being monitored in Ontario, Canada by Winter 2022. Such models will need to be further fine-tuned as dynamics may shift once large, outbreaking *Archanara* populations develop.

Already collected evidence for informing demographic models of *Phragmites*

parameter	data collected from…
Phragmites expansion rates	10+ *P. australis* and *P. australis americanus* populations grown in both field and common garden scenarios in North America (common garden also provides data on how expansion rates vary under competition vs. no competition scenarios between *Phragmites* subspecies)
Phragmites stem phenology	four *P. australis* and four *P. australis americanus* populations grown in a common garden in North America
annual transitions in *Phragmites* stem densities	five *P. australis* and four *P. australis americanus* populations in New York State
Archanara spp. demographic parameters	egg production, hatch rates, and transition rates between life stages for lab and field-grown (in Europe) insects

incoming evidence to inform demographic models of *Phragmites*

parameter	data is being collected from…
overwintering success of *Archanara* spp. in North America	11 *P. australis* populations in Ontario, Canada; anticipated to have reliable estimates by the end of 2022
average within-season dispersal of *Archanara* spp.	11 *P. australis* populations in Ontario, Canada; anticipated to have reliable estimates by the end of 2022
impact of *Archanara* spp. on *Phragmites* stem survival and stem density	at least four *P. australis* populations in Ontario, Canada; anticipated to have reliable estimates by the end of 2022

Subtask 5d: Monitor *Phragmites* Herbivores and other Invertebrates at Field Sites

With the planned arrival of stem boring moths as biocontrol agents (either via eventual approved for field releases in the U.S. or dispersal of adults across the Canada-NY border), we anticipate changes in competitive ability of introduced *P. australis australis* that should result in increases in diversity of abundance of native plant species, as well as changes in invertebrate communities that use native plants and wetlands and other habitats under risk of *P. australis* invasion, or already invaded. Appropriately documenting these changes long-term is a challenge, not only logistically, but also due to lack of standardized methodology. We are experimenting with various new methods (not all funded under this contract) using indicator plants and animals, and these explorations are not restricted to *P. australis*. In the previous section, I detailed results of approaches we have taken to document changes associated with *Phragmites* (both native and introduced) clonal expansion in the field and under standardized common garden conditions. The following sections document our approaches to developing baseline information about animal communities and how *Phragmites* may affect their abundance.

We initially anticipated focusing exclusively on invertebrates, as for those organisms we could rely on existing sampling protocols. Furthermore, we had existing long-term data on *Phragmites* herbivore assemblages from Europe and North America collected since 1998 but not yet fully analyzed or published. We used the 2020 season to further advance some of these analyses since we were unable to do field work due to Covid restrictions. In addition, through a collaboration with the Conservation Bioacoustics lab at the Cornell Laboratory of Ornithology, and with additional exploratory funding from the US Fish and Wildlife Service, the NY Invasive Species Research Institute and a USDA Hatch grant, we added foci on sentinel monitoring, and acoustic monitoring using stationary recorders and machine learning processes to these assessments (discussed in more detail in Task 9). We summarize our efforts in these endeavors, as these provide complimentary information to what has been funded by this grant in support of the overall goals of the *Phragmites* biocontrol work program.

Insect herbivores- European vs. US insect communities in Phragmites

We have made important contributions towards better understanding specialized insect herbivores within both native and introduced *Phragmites*. Dr. Dávalos and Dr. Endriss, in collaboration with Dr. Häfliger, continue to work on analyzing an extensive existing database of stem dissections to determine how herbivores interact with each other and with their *P. australis* host. This data was previously collected running transects through more than 100 *Phragmites* stands across both North America (stands of endemic North American as well as introduced European *Phragmites*) and central Europe (stands of European origin only) (Fig. 17). Stems were harvested from multiple quadrats ($1m^2$ in North America; $0.16m^2$ in Europe) within each transect. These stems were then dissected to compare insect herbivore community composition between native and introduced *P. australis* stands in both North America and central Europe.

Dr. Dávalos and Dr. Endriss developed statistical models to investigate metrics of herbivore community composition as well as metrics of herbivore preference, with our goal of publication in late 2022. We highlight some of the more relevant results below, particularly of

metrics of diversity and of relationships among pairs of insects. These pairwise interactions help refine understanding of whether insects will compete with each other for space and resources in *Phragmites* and whether release or spread of the two *Archanara* spp. will influence herbivore community interactions in predictable ways, which may include tri-trophic interactions. Similarly, we also report whether specific species of insect herbivores are more likely to attack stems of certain diameters than by random chance alone. With the discovery of accidental introduction of multiple European herbivores (but not necessarily their natural enemies), our datasets offer potentially new insights into how host plants and different trophic levels may interact on two continents. The following therefore are meant to showcase the types of understandings we have been able to produce from these analyses rather than providing a comprehensive review.

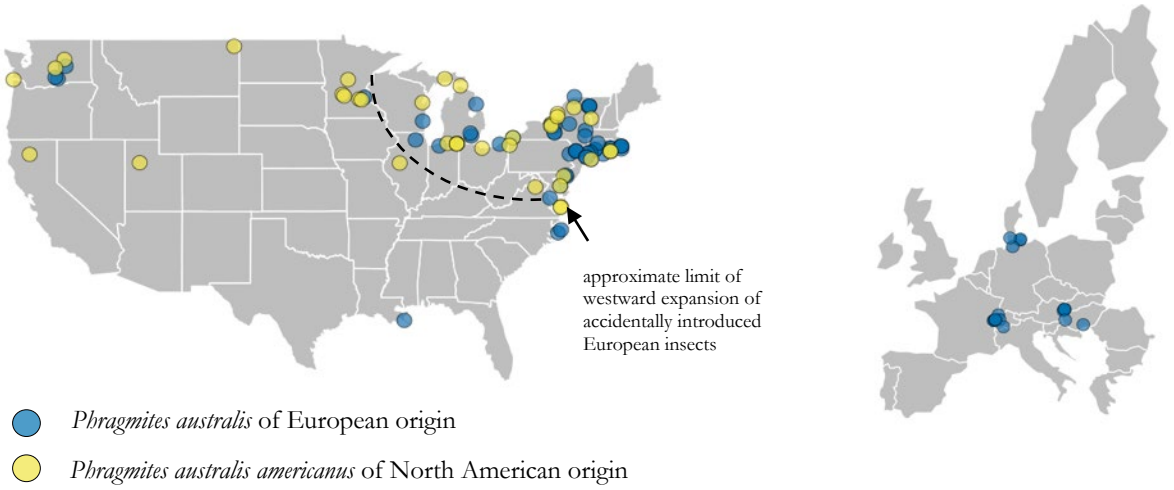


Fig. 17. Sites in North America and Europe where *P. australis* stems were collected for dissections. Yellow circles represent native *P. australis americanus* stands while blue circles represent *P. australis* of European origin.

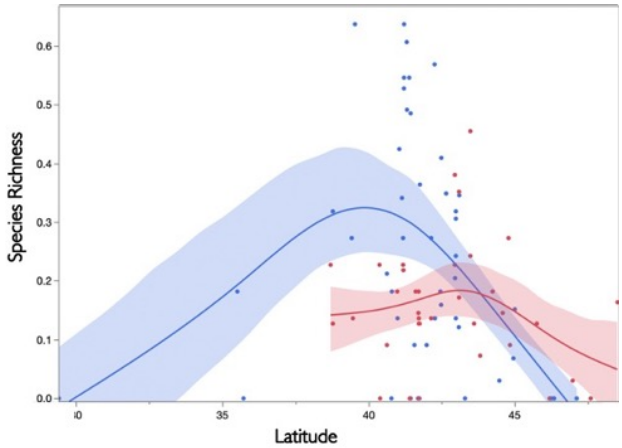


Fig. 18. Insect species richness as a function of latitude in North America. Data represent the number of species/m^2 with each point representing the site average for species diversity. Lines of best fit with 95% confidence intervals in blue represent introduced European genotypes *P. australis australis* and red lines represent native North American genotypes. *P. australis americanus*.

Even prior to the release of the two *Archanara* spp., our dissection results (largely evaluating herbivores residing within stems), indicate that herbivore diversity is higher in introduced than in native genotypes of *Phragmites* in North America (Fig. 18). These herbivores are largely introduced species that, at least at present, show a limited spatial (longitudinal and latitudinal) distribution in North America (Figs. 18-20). We anticipate that over time, these insects will continue to follow their host plant that has colonized much of North America but will likely be restricted based on their own climate adaptations.

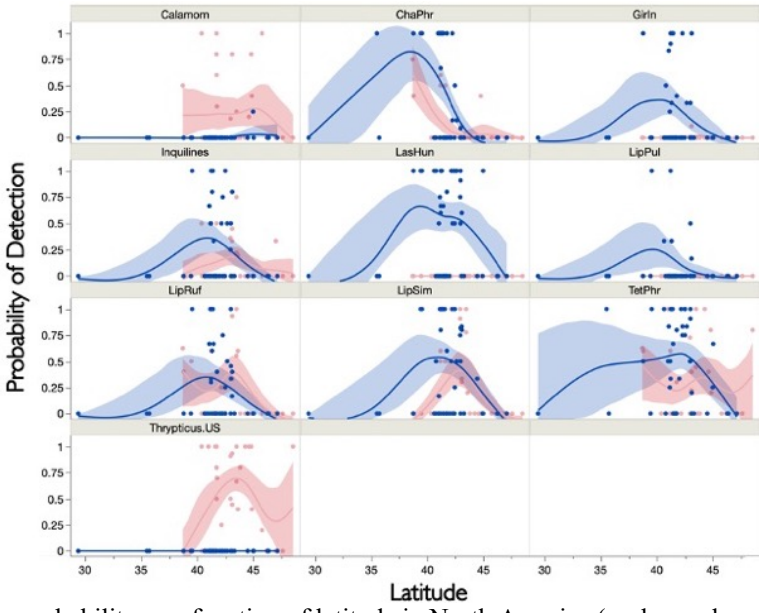


Fig. 19. Insect detection probability as a function of latitude in North America (each panel represents a different species). Lines of best fit with 95% confidence intervals in blue represent introduced European genotypes *P. australis australis* and red lines represent native North American genotypes. *P. australis americanus*.

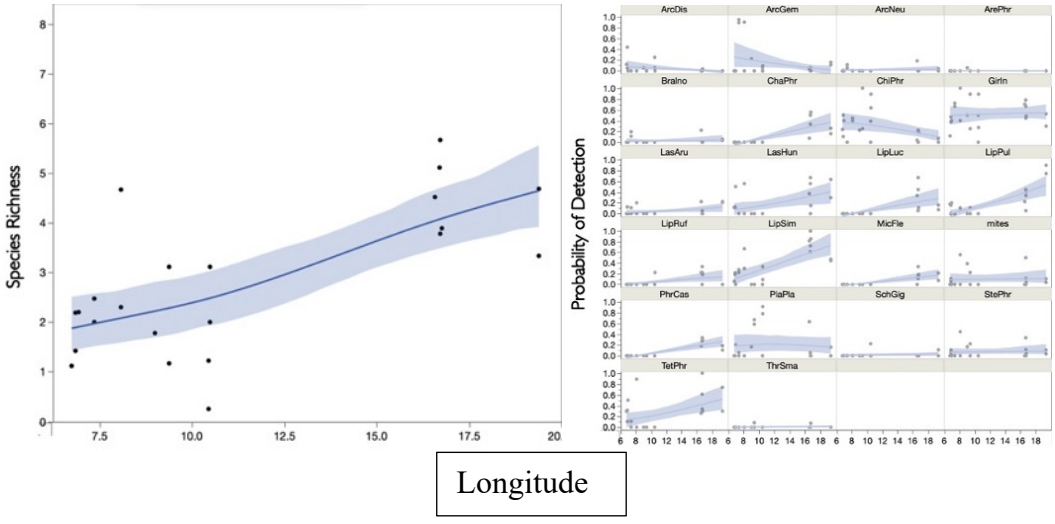
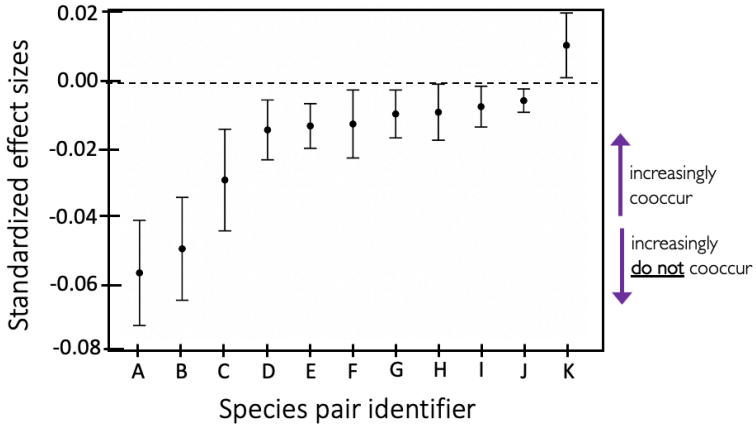


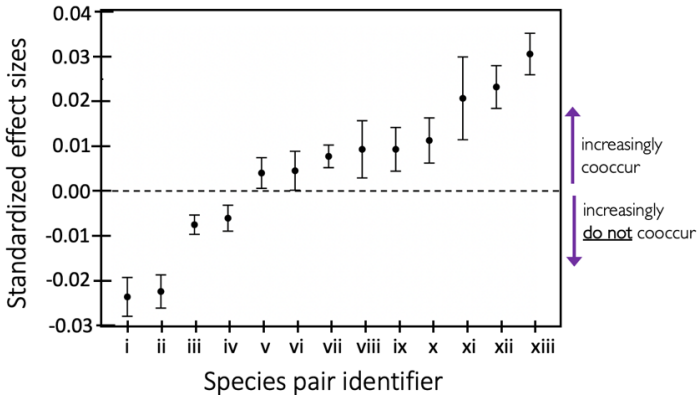
Fig. 20. *Phragmites* insect species richness (left) and insect detection probability (right) as a function of longitude in Europe (see also Fig. 13). On left the line of best fit with 95% confidence intervals represent data of the number of species/m^2 with each point representing the site average for species diversity. On right panel lines of best fit with 95% confidence intervals for the same data but each panel represents a different species. More easterly sites are on the right side of individual panels.

We used the cooccur package (Griffith, Veech & Marsh 2016) to fit a probabilistic model of species co-occurrence (Veech 2013; Veech 2014) to presence/absence data of insect herbivores within each sampled quadrat and within each stem. Specifically, we used pairwise species comparisons to evaluate whether specific species of insect herbivores were more or less likely than expected by random chance alone to occur with each of the other insect herbivore species identified in a specific quadrat or stem. For each quadrat, we then extracted standardized effect sizes for all pairwise interactions, which we fed into in downstream analyses to see if the likelihood of cooccurrence (i.e., the effect sizes) were influenced by latitude, among other predictor variables of interest. Since latitude was not a significant driver of these pairwise interactions, we reverted to our previous approach of averaging our effect sizes by range (North America and Europe) and subspecies (*P. australis australis* and *P. australis americanus*). Using this approach for introduced *P. australis australis* in North America, we found that with the exception of the scale *Chaetococcus phragmites* and *Lipara similis*, most of the major herbivore species selected for this comparison, which are accidentally introduced to North America, species were less likely to occur in the same stem than expected by random chance (Fig. 21). However, the same pattern did not appear when we analyzed the herbivores in native *P. australis americanus* where most species were more likely than not to occur in the same stem (Fig. 22).



Species pair id	Species 1	Species 2
A	*Lipara rufitarsis*	*Lipara similis*
B	*Lipara pullitarsis*	*Lipara similis*
C	*Lipara similis*	*Giraudiella inclusa*
D	*Lasioptera hungarica*	*Giraudiella inclusa*
E	*Lipara rufitarsis*	*Lasioptera hungarica*
F	*Lipara rufitarsis*	*Giraudiella inclusa*
G	*Chaetococcus phragmitis*	*Lipara rufitarsis*
H	*Chaetococcus phragmitis*	*Lasioptera hungarica*
I	*Tetramesa*	*Lipara similis*
J	*Tetramesa*	*Lipara rufitarsis*
K	*Chaetococcus phragmitis*	*Lipara similis*

Fig. 21. A subset of pairwise interactions (on a per stem basis) reflecting spatial dynamics of specific insects within introduced *P. australis australis* populations in North America. Bars represent 95% confidence intervals around mean effect size for each unique species-species pairwise interaction. Effect sizes were calculated from community analyses conducted for each of 352 unique $1m^2$ quadrats. An effect size of 0 is represented by the dashed, purple, horizontal line, and means that the distribution of two species represented by the pairwise interaction does not differ from the null hypothesis that these two species are randomly distributed across stems. As the effect size increases from zero, the species represented by the pairwise interaction are more likely to co-occur within a stem than if they were randomly distributed across stems. In contrast, as the effect size decreases from zero, the species represented by the pairwise interaction are less likely to co-occur within the same stem than if they were randomly distributed across stems. Species pairs are listed in full in the table to the right of the plot.



Species pair id	Species 1	Species 2
i	*Lipara rufitarsis*	*Calamomyia phragmites*
ii	*Lipara rufitarsis*	*Lipara similis*
iii	inquilines	*Calamomyia phragmites*
iv	*Lipara similis*	*Calamomyia phragmites*
v	*Tetramesa*	*Lipara similis*
vi	*Tetramesa*	inquilines
vii	inquilines	*Thrypticus williston*i
viii	*Tetramesa*	*Chaetococcus phragmitis*
ix	*Lipara similis*	inquilines
x	*Calamomyia phragmites*	*Thrypticus williston*i
xi	*Thrypticus williston*i	*Lipara similis*
xii	*Lipara rufitarsis*	*Thrypticus williston*i
xiii	*Lipara rufitarsis*	inquilines

Fig. 22. A subset of pairwise interactions (on a per stem basis) reflecting spatial dynamics of specific insects within native *P. australis americanus* populations in North America. Bars represent 95% confidence intervals around mean effect size for each unique species-species pairwise interaction. Effect sizes were calculated from community analyses conducted for each of 352 unique 1m^2 quadrats. An effect size of 0 is represented by the dashed, purple, horizontal line, and means that the distribution of two species represented by the pairwise interaction does not differ from the null hypothesis that these two species are randomly distributed across stems. As the effect size increases from zero, the species represented by the pairwise interaction are more likely to co-occur within a stem than if they were randomly distributed across stems. In contrast, as the effect size decreases from zero, the species represented by the pairwise interaction are less likely to co-occur within the same stem than if they were randomly distributed across stems. Species pairs are listed in full in the table to the right of the plot.

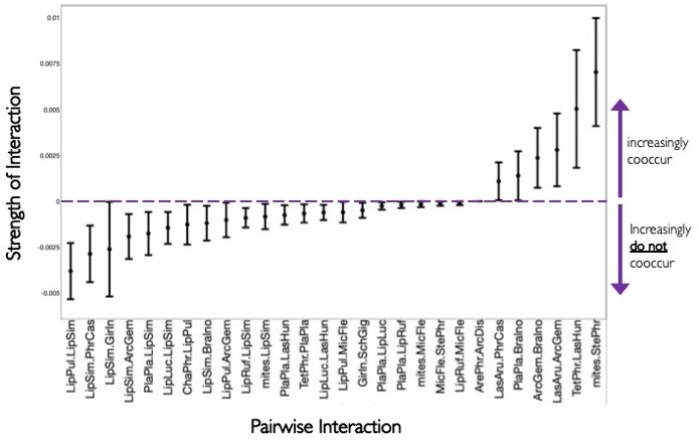
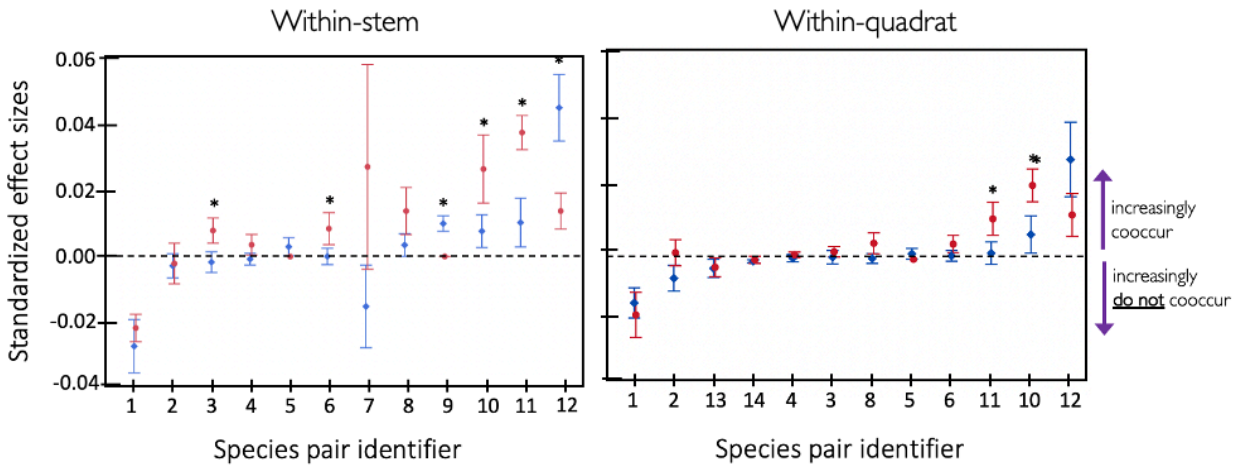


Fig. 23. A subset of pairwise interactions reflecting spatial dynamics of specific insects within *Phragmites* populations in Europe. Bars represent 95% confidence intervals around mean effect size for each unique species-species pairwise interaction. Effect sizes were calculated from community analyses conducted for each of 353 unique 0.16m^2 quadrats. An effect size of 0 is represented by the dashed horizontal line, and means that the distribution of two species represented by the pairwise interaction does not differ from the null hypothesis that these two species are randomly distributed across stems. As the effect size increases from zero, the species represented by the pairwise interaction are more likely to co-occur within a stem than if they were randomly distributed across stems. In contrast, as the effect size decreases from zero, the species represented by the pairwise interaction are more likely not to co-occur within the same stem than if they were randomly distributed across stems.

Similar analyses of the data collected by Patrick Häfliger in Europe, while representing different species pairings, show interesting responses. First, insect diversity appears to increase from West to East (Fig. 20), while there is no clear pattern in the probability to detect different species according to longitude (Fig. 20). As in North America, certain pairs appear more or less likely to occupy the same sampling quadrat (please note that sample quadrat size is significantly

smaller in Europe). Interestingly, *Lipara* spp. once again show high negative interactions and avoidance of each other (Fig. 23), which is no surprise because each attacks the growing point leaving room for only a single occupant. Further, the direction of species interactions (i.e., negative vs. positive) appears to fairly consistent across scales, although the strength of species appears stronger at the within-stem vs. within-quadrat level (Fig. 24).



Species pair id	Species 1	Species 2
1	*Lipara rufitarsis*	*Lipara similis*
2	*Chaetococcus phragmitis*	*Lipara rufitarsis*
3	*Tetramesa*	*Lipara similis*
4	*Tetramesa*	*Lipara rufitarsis*
5	*Tetramesa*	*Giraudiella inclusa*
6	*Tetramesa*	inquilines
7	*Tetramesa*	*mites*
8	*Tetramesa*	*Chaetococcus phragmitis*
9	*Chaetococcus phragmitis*	inquilines
10	*Chaetococcus phragmitis*	*Lipara similis*
11	*Lipara rufitarsis*	inquilines
12	*Lipara similis*	inquilines
13	mites	*Lipara similis*
14	mites	*Lipara rufitarsis*

Fig. 24. A subset of pairwise interactions reflecting spatial dynamics of specific insects at the within-stem and within-m^2-quadrat scale for *Phragmites* populations in North America. Blue bars and points represent *P. australis* and red bars represent *P. australis americanus.* Bars represent 95% confidence intervals around mean effect size for each unique species-species pairwise interaction. Effect sizes were calculated from community analyses conducted for each of 353 unique $1m^2$ quadrats. An effect size of 0 is represented by the dashed horizontal line and means that the distribution of two species represented by the pairwise interaction does not differ from the null hypothesis that these two species are randomly distributed across stems or quadrats. As the effect size increases from zero, the species represented by the pairwise interaction are more likely to co-occur within a stem than if they were randomly distributed across stems. In contrast, as the effect size decreases from zero, the species represented by the pairwise interaction are less likely to co-occur within the same stem or quadrat than if they were randomly distributed across stems. Species pairs are listed in full in the table below the plots.

We found that for many species occurrences within a sampling quadrat appear correlated (Fig 24), but there are only several species pairs that express strong positive or negative relationships. For example, the gall midge *Calamomyia phragmitis* and the fly *Thrypticus willestonii* often occur together, which is not a surprise given that both species are native specialists on native *P. australis americanus*. But the introduced gall fly *Lipara rufitarsis* also commonly co-occurs with the native *Thrypticus*, and this can only occur on native *P. australis americanus*. This interaction of a native host plant and one native and one introduced herbivore represents an entire new eco-evolutionary experiment, but one that does not disadvantage the native herbivore, at present. The opposite pattern emerges for the relationship of *L. rufitarsis* and *C. phragmitis*, another introduced-native pair where the interaction occurs on native *P. australis americanus* (Fig. 24), but the two species appear to avoid each other. That the multiple *Lipara* spp. compete for resources is not surprise given that there is only one growing point/stem and there can never be two individuals completing their development in a single stem, although our evaluation is on spatial use per square meter, not on a per stem basis.

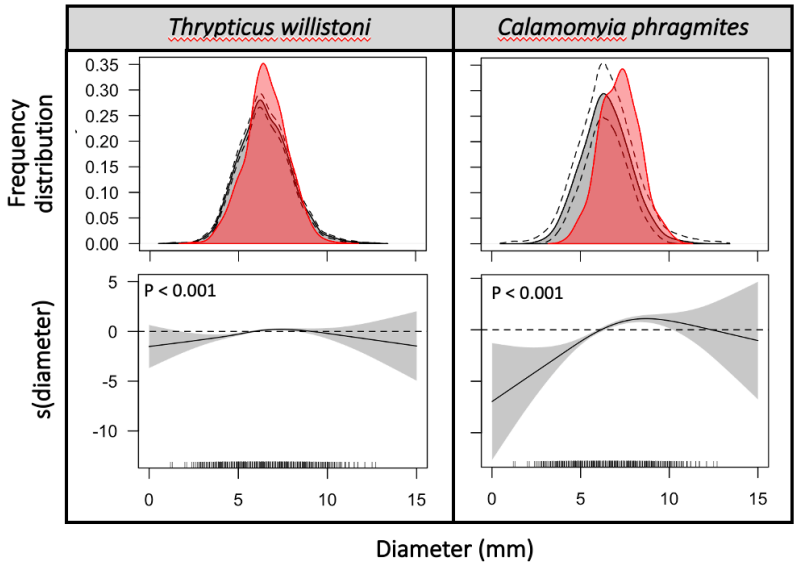


Fig. 25. The top row illustrates two overlaid relative frequency distributions: one (in red) is the distribution of diameters for *Phragmites* stems that were attacked by the insect herbivore in question (*T. willistoni* on left, *C. phragmites* on right), and one (in gray) is the distribution of diameters for *Phragmites* stems that were not attacked by these insect herbivores. There were always more stems that were not attacked than attacked, so we used a bootstrapping approach (n=1000 iterations) to calculate the mean (solid black line) and 95% confidence intervals (dashed black lines) for 1000 random 'draws' of stems that were not attacked, such that each draw standardized the number of samples compared between stems that were attacked vs. not attacked. The bottom row illustrates the corresponding derivative curve (i.e., GAMM spline) for the models evaluating how attack rates vary across *Phragmites*' stem diameters. The solid black line is the model estimate for the slope of the relationship between diameter and attack rates and the gray shading represents the 95% credible intervals around this estimate—attack rates are predicted to increase with stem diameter when $y > 0$ and are predicted to decrease with stem diameter when $y < 0$. Estimates are not significant if the 95% credible intervals do overlap with the x-axis (i.e., $y \neq 0$; depicted by 'NS').

We were curious whether certain plant traits can explain some of these observed pairwise species interactions. We therefore also explored how different species make determinations to avoid each other or use different plant traits (for example stem height or stem diameters) or microenvironments (plant densities or water levels). For example, we investigated whether

individual insect species differ in the likelihood they will attack stems of certain diameters. Here, we highlight a few interesting results. For example, analyses of common insect herbivores of introduced *P. australis australis* in North America revealed that several herbivore species are more likely to occur in stems of certain stem densities than by random chance alone. For example, both *Thrypticus willistoni*, a species of long-legged fly in the Dolichopodidae family, and *Calamomyia phragmites*, a gall midge in the Cecidomyiidae family, are less likely to attack small-diameter native *Phragmites* stems, but more likely to attack large-diameter stems than by random chance alone (Fig. 25).

We also find evidence that the mechanisms that govern why some species of insect herbivores are more likely to attack *Phragmites* stems of certain diameters are similar between the introduced North American and native European range of *P. australis*. For example, *Giraudiella inclusa*, a gall midge, occurs on *P. australis australis* in both ranges—and in both ranges, *G. inclusa* is less likely to occur on stems less than 5mm in diameter and more likely to occur on stems greater than 5mm in diameter than by random chance alone (Fig. 26).

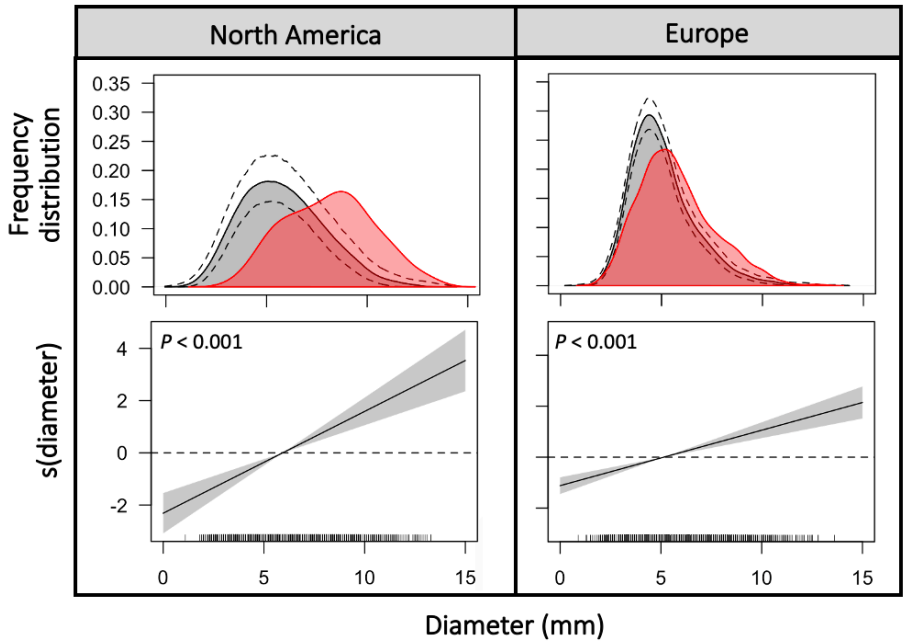


Fig. 26. The top row illustrates two overlaid relative frequency distributions: one (in red) is the distribution of diameters for stems that were attacked by *Giraudiella inclusa*, and one (in gray) is the distribution of diameters for stems that were not attacked by *G. inclusa*. There were always more stems that were not attacked than attacked, so we used a bootstrapping approach (n=1000 iterations) to calculate the mean (solid black line) and 95% confidence intervals (dashed black lines) for 1000 random 'draws' of stems that were not attacked, such that each draw standardized the number of samples compared between stems that were attacked vs. not attacked. The bottom row illustrates the corresponding derivative curve (i.e., GAMM spline) for the models evaluating how attack rates vary across *Phragmites*' stem diameters. The solid black line is the model estimate for the slope of the relationship between diameter and attack rates and the gray shading represents the 95% credible intervals around this estimate—attack rates are predicted to increase with stem diameter when $y > 0$ and are predicted to decrease with stem diameter when $y < 0$. Estimates are not significant if the 95% credible intervals do overlap with the x-axis (i.e., $y \neq 0$; depicted by 'NS').

In contrast, the mechanisms governing which diameter stems herbivores attack appear to differ when insects occur in introduced *P. australis australis* vs. native *P. australis americanus*

in North America. Thus, for insect herbivores that occur in both subspecies of *Phragmites*, these herbivores typically attack *Phragmites* stems of similar diameters in both the introduced North American and native European range. However, these same herbivores often attack stems of different diameters when they occur on *P. australis australis* vs. *P. australis americanus* in North America. For example, stem diameter does not appear correlated with which *P. australis americanus* stems are attacked by the mealybug *Chaetococcus phragmitis* in North America (Fig. 27). Yet *C. phragmitis* is less likely to attack small-diameter *P. australis australis* stems and more likely to attack large-diameter *P. australis australis* stems than by random chance alone, irrespective of whether *P. australis australis* occurs within its native European or its introduced North American range (Fig. 27). We find a similar pattern for *L. similis*, in that this galling fly appears to attack *P. australis* stems of similar diameters in Europe vs. North America, but attacks stems of different diameters when attacking *P. australis australis* vs. *P. australis americanus* (Fig. 28). These results support previous findings that host-specificity of insects that feed on *Phragmites* is governed at the subspecies level (Casagrande *et al.* 2018), which further supports that biocontrol of introduced *P. australis australis* in North America has low risk of negatively impacting the demography of cooccurring native populations of *P. australis americanus*.

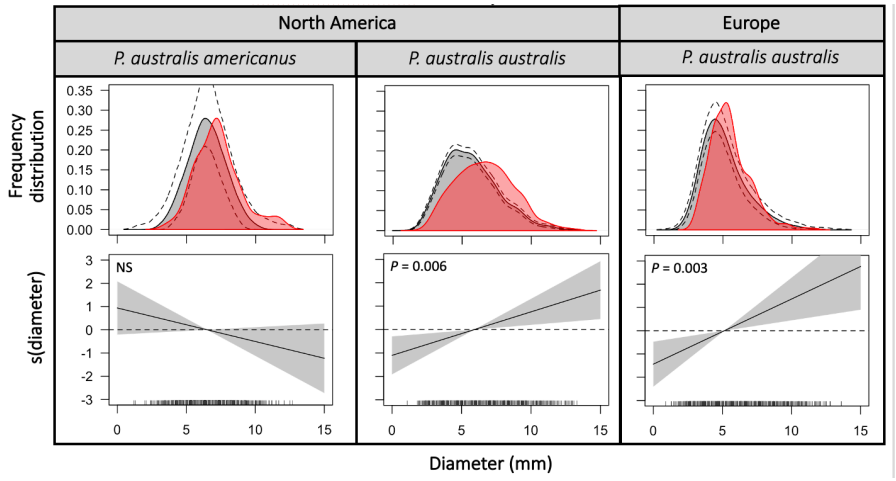


Fig. 27. The top row illustrates two overlaid relative frequency distributions: one (in red) is the distribution of diameters for stems that were attacked by *Chaetococcus phragmitis*, and one (in gray) is the distribution of diameters of stems that were not attacked by *C. phragmitis*. There were always more stems that were not attacked than attacked, so we used a bootstrapping approach (n=1000 iterations) to calculate the mean (solid black line) and 95% confidence intervals (dashed black lines) for 1000 random 'draws' of stems that were not attacked, such that each draw standardized the number of samples compared between stems that were attacked vs. not attacked. The bottom row illustrates the corresponding derivative curve (i.e., GAMM spline) for the models evaluating how attack rates vary across *Phragmites*' stem diameters. The solid black line is the model estimate for the slope of the relationship between diameter and attack rates and the gray shading represents the 95% credible intervals around this estimate—attack rates are predicted to increase with stem diameter when $y > 0$ and are predicted to decrease with stem diameter when $y < 0$. Estimates are not significant if the 95% credible intervals do overlap with the x-axis (i.e., $y \neq 0$; depicted by 'NS').

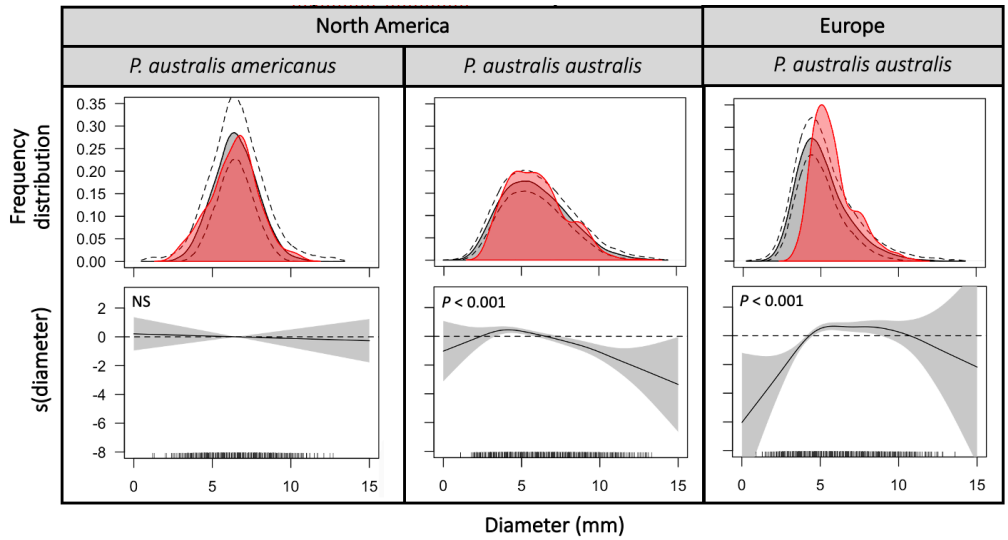


Fig. 28. The top row illustrates two overlaid relative frequency distributions: one (in red) is the distribution of diameters for stems that were attacked by *L. similis*, and one (in gray) is the distribution of diameters of stems that were not attacked by *L. similis*. There were always more stems that were not attacked than attacked, so we used a bootstrapping approach (n=1000 iterations) to calculate the mean (solid black line) and 95% confidence intervals (dashed black lines) for 1000 random 'draws' of stems that were not attacked, such that each draw standardized the number of samples compared between stems that were attacked vs. not attacked. The bottom row illustrates the corresponding derivative curve (i.e., GAMM spline) for the models evaluating how attack rates vary across *Phragmites*' stem diameters. The solid black line is the model estimate for the slope of the relationship between diameter and attack rates and the gray shading represents the 95% credible intervals around this estimate—attack rates are predicted to increase with stem diameter when $y > 0$ and are predicted to decrease with stem diameter when $y < 0$. Estimates are not significant if the 95% credible intervals do overlap with the x-axis (i.e., $y \neq 0$; depicted by 'NS').

Another interesting result is that in North America, *Tetramesa*—a phytophagous wasp—is less likely to occur in small-diameter *P. australis americanus* stems and more likely to occur in large-diameter *P. australis americanus* stems than by random chance alone. In contrast, *Tetramesa* are equally likely to attack *P. australis australis* stems regardless of their diameter or range of origin (Fig. 29). Taken together, these results may, again, simply reflect that even insect herbivores that occur on both subspecies of *Phragmites* still make different decisions when feeding on *P. australis australis* vs. *P. australis americanus*. However, this observed pattern may also reflect that more than one species of *Tetramesa* occurs on *Phragmites* than previously assumed. The past several years we have been collaborating with Dr. Jason Dombrowski, the manager of the University Insect Collection and the coordinator of the Insect Diagnostic Lab at Cornell University to confirm species-level identifications for insects found in *Phragmites* using a DNA-barcoding approach. Although most of our insect herbivores were not found within the DNA-barcoding database, this approach did confirm that at least two *Tetramesa* species occur within *P. australis* within North America. Further taxonomic resolution will be necessary to put species names to these species and we may also deal with previously undescribed species. Dr. Stefan Vidal is currently revising this genus on a worldwide basis, and he has agreed to help us work though *Tetramesa* spp. in native and introduced *Phragmites* in North America.

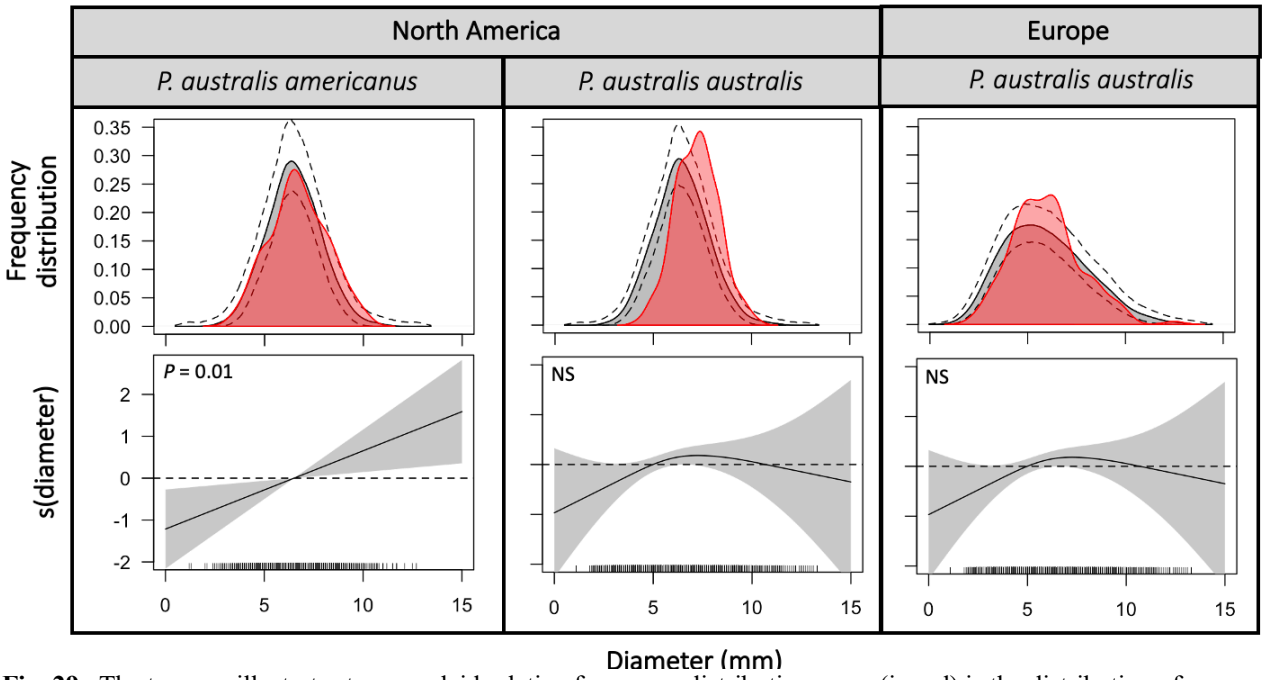


Fig. 29. The top row illustrates two overlaid relative frequency distributions: one (in red) is the distribution of diameters for stems that were attacked by *Tetramesa*, and one (in gray) is the distribution of diameters of stems that were not attacked by *Tetramesa*. There were always more stems that were not attacked than attacked, so we used a bootstrapping approach (n=1000 iterations) to calculate the mean (solid black line) and 95% confidence intervals (dashed black lines) for 1000 random 'draws' of stems that were not attacked, such that each draw standardized the number of samples compared between stems that were attacked vs. not attacked. The bottom row illustrates the corresponding derivative curve (i.e., GAMM spline) for the models evaluating how attack rates vary across *Phragmites*' stem diameters. The solid black line is the model estimate for the slope of the relationship between diameter and attack rates and the gray shading represents the 95% credible intervals around this estimate—attack rates are predicted to increase with stem diameter when $y > 0$ and are predicted to decrease with stem diameter when $y < 0$. Estimates are not significant if the 95% credible intervals do overlap with the x-axis (i.e., $y \neq 0$; depicted by 'NS').

Insect herbivores - Spatial dynamics in native P. australis americanus

In addition to the general distribution of specialized native and accidentally introduced herbivores and their natural enemies on a site-by-site basis and across North America, a 2003 honors student research project jointly supervised by A. Dávalos and B. Blossey, investigated local distribution patterns of herbivores in the Northern Montezuma Wetlands Complex in upstate New York. The honors thesis revealed some interesting small-scale distribution patterns among species attacking native *P. australis americanus*. We decided to re-do this analysis using an undergraduate student cohort from SUNY Cortland supervised by A. Dávalos. In November 2018, we resampled our site and the existing native *P. australis* using an identical sampling design (Fig. 30). A winter collection captures the vast majority of herbivores since most of them overwinter in the stems. Students measured and dissected all stems and allowed insects to emerge in small vials.

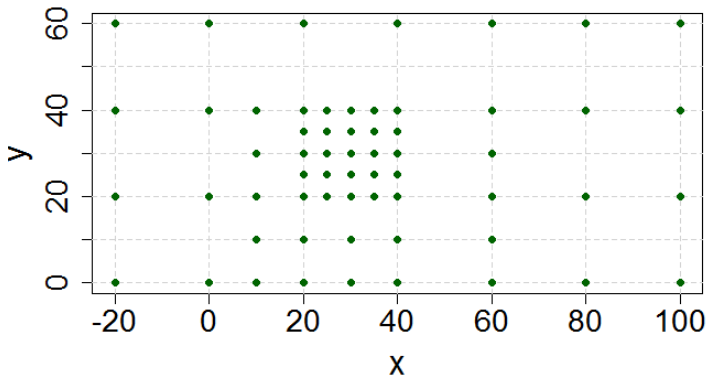


Fig. 30. The spatial arrangement of the 60 1m^2 sampling quadrats in a 100x60m rectangle established in fall 2018 within a stand of native *P. australis americanus* at Carncross in the Northern Montezuma Wetland Complex.

Table 8. Name, trophic position and distribution information for taxa submitted and identified via barcoding. ID's were returned at various levels of resolution. Insects for submission emerged from native *Phragmites* stems collected at Carncross.

Taxon		**Trophic position**	**New to North America**	**Know from Phragmites in Europe**
Genus	species			
Cryptonerva	*diadema*	inquiline in *Lipara* galls	yes	yes
Tetramesa	sp. 1	herbivore	?	?
Tetramesa	sp. 2	herbivore	?	?
Tetramesa	sp. 3	herbivore	?	?
Agrothereutina (subtribe)	sp. 1	parasitoid	?	?
Endromopoda	sp.1	parasitoid	?	?
Endromopoda	*phragmitidis*	parasitoid on *Tetramesa*	Yes, 1 record from Canada	Yes
Pteromalidae (family)	multiple spp.	parasitoids	?	?

We allowed insects to emerge, separated them by morphospecies and then worked with Dr. Dombrowski to submit them for identification and barcoding. Data entry and insect emergence are complete but full data analyses are still pending due to delays in insect identification. We just received (July 2022) further barcoding information from Dr. Dombrowski and we find an interesting and surprising addition to the fauna of native *Phragmites* and North America (Table 8). For many species there is no reference material in BOLD (the barcoding site we used) but barcoding information allows to separate taxa. We may need to add 5-10 new species to the existing fauna of North America, some of these are clearly introduced, others may be new to science (Table 8). The species span different trophic levels from herbivores, to inquilines, to parasitoids.

We also documented other interesting patterns, for example, how *Phragmites* stem densities affect the distribution and abundance of an unidentified fungal species (Fig. 31). We will produce similar analyses and graphs for the remaining insects and their response to each other and stem densities. In part we were awaiting species identification for different predators and parasitoids (Fig. 32) we recorded to complete these analyses.

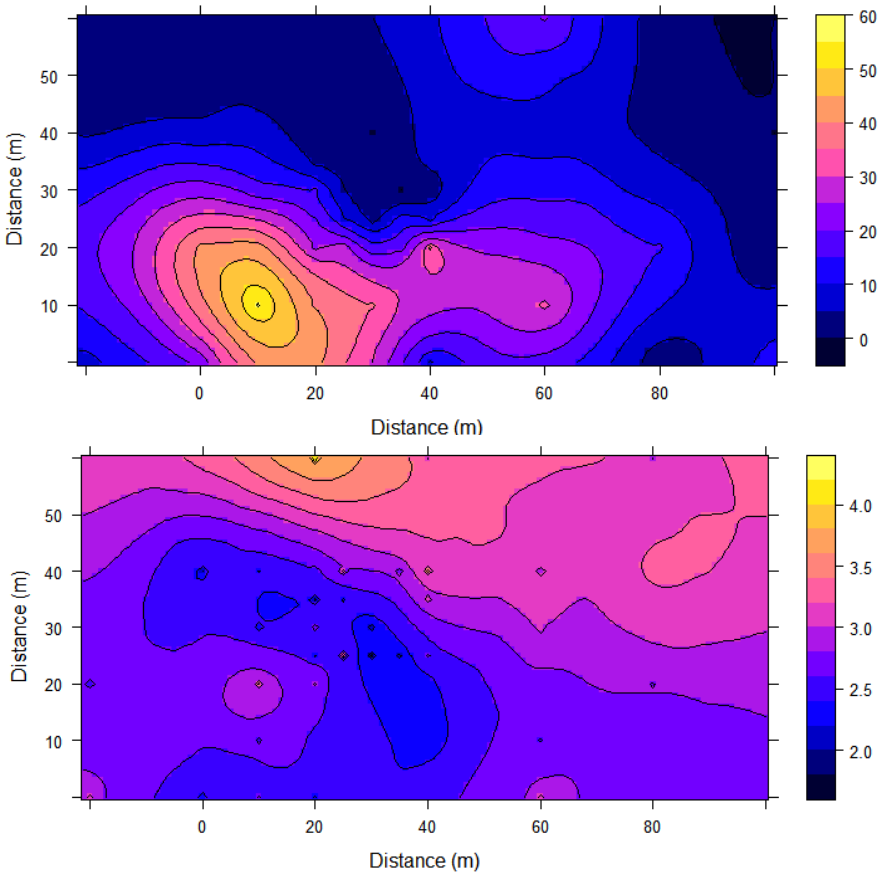


Fig. 31. Interpolated surfaces from ordinary kriging showing high abundance of *P. australis americanus* in the lower left section of the plot (top panel) and abundance of a fungal spot (bottom panel) at Carncross in winter 2018/19.

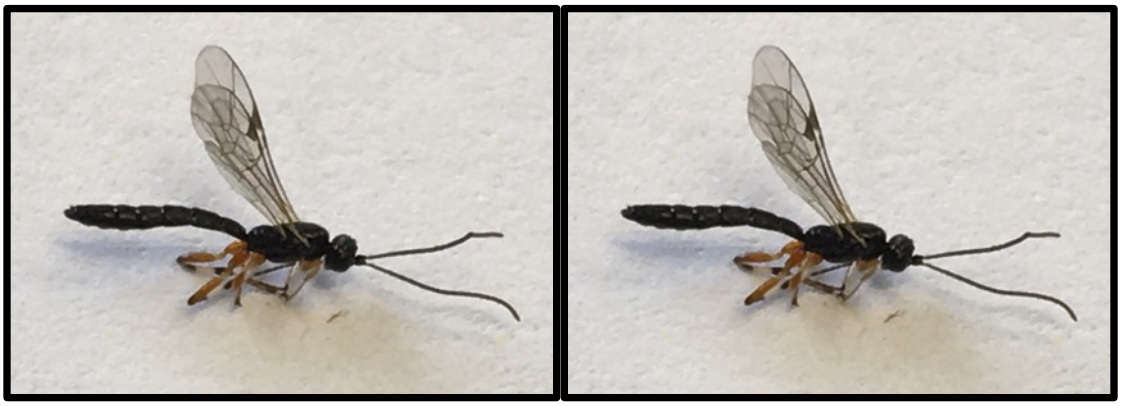


Fig. 32. *Endromopoda phragmitidis* male (left) and female ichneumonid wasps reared from the herbivore *Tetramesa* sp. which is a specialized internal stem miner on *Phragmites*.

Insect herbivores - monitoring Phragmites to assess the possibility of additional new introductions

We decided to redo our analyses of insect communities in the eastern US following the same protocol we used previously (with some slight modifications). The last of our earlier insect community sampling occurred nearly a decade ago, and not only do we anticipate that insects have dispersed further, we could also be missing newly arrived species, or species that initially were in low abundance or had a limited distribution. But with the anticipated introduction of biocontrol agents, we felt it was appropriate to have an updated survey for the insect communities currently utilizing *Phragmites* in North America. Furthermore, we plan for our revised protocol to important in a standardized monitoring protocol we are developing for managers to assess the outcome of biocontrol introductions. This work focused on coastal habitats in eastern North America where introduced *P. australis australis* has the longest residence time and our previous sampling showed the highest diversity in accidentally introduced insect herbivores (Tewksbury *et al.* 2002).

Table 9. 2019-2020 introduced *Phragmites australis australis* sampling sites along the Atlantic Coast. Locations are selected near previously sampled *P. australis australis* stands to obtain an update on potential insect community changes.

Site	2019-2020 sampling date	Town	State	Latitude	Longitude	Status	Previous sampling year (s)
Galilee (RI03)	10/25/2019	Narragansett	RI	41.37967	-71.50809	Introduced	2003, 2010
Worden's Pond (RI02)	11/22/2019	South Kingstown	RI	41.42941	-71.56799	Introduced	2003
Charlestown Beach	1/14/2020	Charlestown	RI	41.36380	-71.62614	Introduced	2011
Jamestown Mackerel Cove	2/3/2020	Jamestown	RI	41.48908	-71.38128	Introduced	2011
Barn Island Boat Launch	3/11/2020	Stonington	CT	41.33708	-71.87657	Introduced	

Between October 2019 and March 2020, personnel at URI initiated sampling in Rhode Island and Connecticut (Table 9, Fig. 33). We collect data by running transects through *P. australis australis* stands and harvest stems within a $1m^2$ quadrat spaced at 1 m intervals (Fig. 34). A minimum of 100 stems are harvested at each site, transported to URI and stored in a polyethylene-covered storage shed that experiences temperatures similar to ambient outdoor conditions. We then examined stems externally for stem characteristics and signs of external herbivory, before dissecting them to identify internal herbivores and their respective predators/parasitoids (Fig. 34). We saved representative specimens of all insects found during dissections, which are stored in sealed cups at room temperature to capture emerging adults to confirm identification (Fig. 34). If no emergence occurs during this time, cups are placed at 5ºC for 3 - 4 months and then returned to room temperature to break potential dormancy. All successfully identified (based on characteristic feeding damage) or reared insects were saved to confirm identification after adult emergence.

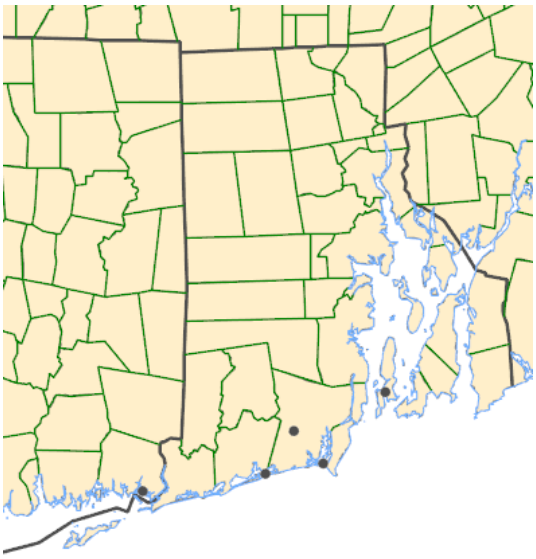


Fig. 33. URI *Phragmites* sampling locations for 2019-20.

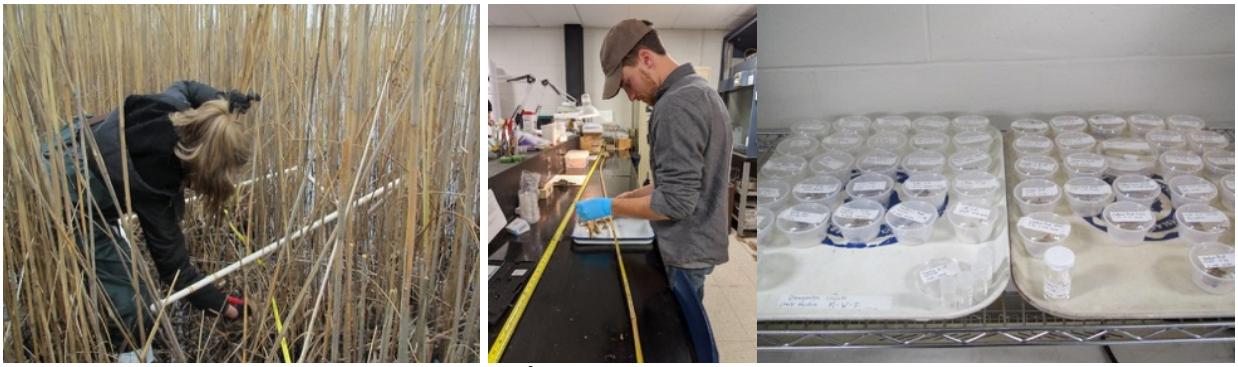


Fig. 34. Stems collection in the field within a 1m^2 quadrat (left), stem measurements and examination (center) and rearing cups with moist filter paper and host plant material (right).

Average stem density among sites varied from 16 to 69 stems, with the lowest density at Galilee and highest at Barn Island (Table 10). At Galilee and Worden's pond, more than five quadrats were needed to reach the minimum of 100 stems. Average stem height varied between 147.5 cm (Galilee) and 508.4 cm (Worden's Pond). Average stem diameter varied between 4.4 mm (Barn Island) and 9.2 mm (Worden's Pond).

Table 10. Location of *Phragmites* sampling, number of stems dissection, stem densities, height and diamters.

Location	Number of quadrats	Total number of stems	Average stem density (# stems/ m^2)	Average stem height (cm)[a]	Average stem diameter (mm)
Galilee	7	109	16	147.5	4.96
Worden's Pond	6	110	18	508.4	9.2
Charlestown Beach	5	131	26	200.6	5.3
Jamestown	5	148	30	183.5	5.01
Barn Island	5	345	69	214.5	4.44

[a] Stems with broken tips or bottoms omitted.

We recorded *Chaetococcus phragmitis, Lasioptera hungarica, Lipara rufitarsis* and *Lipara similis* at 4 of 5 sites with Warden Pond showing the lowest diversity. All our sites were introduced *P. australis australis*, and we did not find any of the native herbivores, such a *T. willestoni* or *C. phragmites*, that are common and widespread in native stands. We found the rice grain gall midge, *Giraudiella inclusa* at 2 of our sampling locations, and the species remains restricted to the easternmost sampling location in the Northeast. For now, we see differences in herbivore assemblages from a decade ago, but apparently no new arrivals. There are a number of unknown species, some of which may be *Cryptonerva diadema*, most likely inquilines that use galls as shelter or generalist native species that are only occasionally recorded. What is apparent for many species is the development of tri-trophic interactions, i.e. parasitoids are frequently recorded (Fig. 35), although their species identify awaits confirmation, even after barcoding. We did not encounter novel important herbivore species but confirmed existing records.

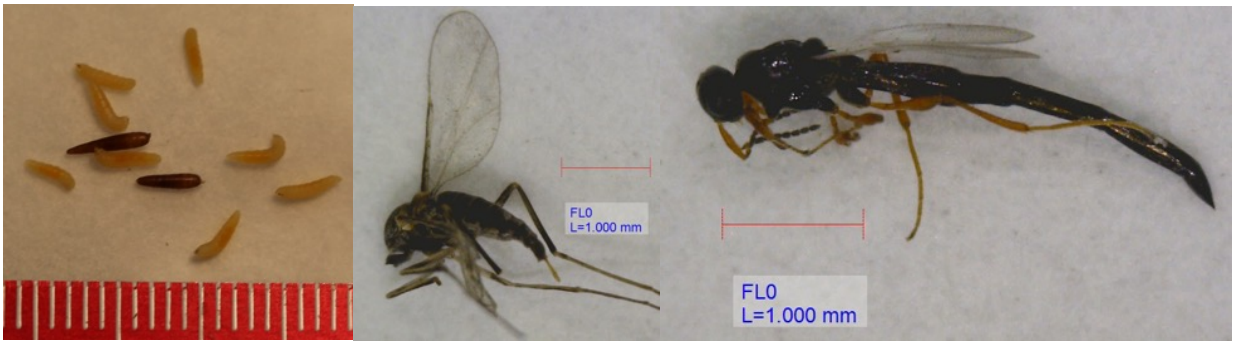


Fig. 35. *Lasioptera hungarica* larvae (yellow on left), parasitoid pupae (black on left), *Lasioptera* adult midge (center) and adult parasitoid (right).

TASK 6: MAINTAIN OR EXPAND THE CAPTIVE INSECT COLONY AND SUPPLY EGGS TO US FOR RESEARCH AND/OR RELEASE.

The purpose of this task was to increase availability both for research purposes in North America and in preparation for anticipated field releases and high demand after USDA/APHIS approval is obtained. Work at CABI, in addition to some support work for insect dispersal and demography model development continued to focus on maintaining and increasing the captive colony (see also Task 7 where I detail artificial rearing techniques that were developed jointly with Robert Bourchier from Canada). We terminated rearing at URI in 2018 since all host specificity testing had been completed, but we may assist in mass production technique development at the Cornell quarantine in the future. Over the years, CABI personnel had some early set-backs and lately some encouraging improvements in developing the ability to transition from the successful but work intensive hand rearing of *Archanara* larvae.

For our conventional rearing on *Phragmites* stems, we individually transfer freshly hatched *A. neurica* and *A. geminipuncta* larvae with a paint brush into cut *Phragmites* stems (one larva per stem for *A. neurica*, in general two larvae for *A. geminipuncta*). A maximum of 12 stems are inserted in moist horticulture foam wrapped in plastic foil and placed in plastic cylinders (diameter 10 cm, height 37 cm), covered with a gauze lid (Fig. 36, left). We check cylinders daily and as soon as a larva left its shoot it was transferred onto a new shoot section. Thus, three to five stems are needed per larva until pupation. In case larvae re-entered stems, we dissected stems, and the larva removed in order to avoid cannibalism. Each year, we collect a few larvae of each species at a nearby field site to avoid inbreeding depression. Once larvae pupated, we removed pupae from stems, sexed them, and placed five pupae together on a layer of vermiculite in a plastic cup (diameter 5.5-6.5 cm, height 8 cm). We add wet cotton pads to avoid desiccation of pupae. After four weeks, emergence of adult moths was checked daily.

Fig. 36. Set-up of moth rearing in cylinders with *Phragmites* stem sections (left) and mating cages (right).

We held 1-3 pairs of newly emerged moths for mating and oviposition in wooden cages (40 x 40 x 65 cm) under outdoor conditions (Fig. 36, right). We provided 6 *Phragmites* shoots with intact leaf sheaths as oviposition sites. We replaced shoots once after 3-4 days and emptied cages after females have died (usually after 7-10 days). We subsequently check leaf sheaths for

eggs, which are kept for overwintering in Petri dishes (5 mm in diameter; maximum 300 eggs per dish) placed in a styrofoam box and stored outdoors in a wooden hut at ambient temperatures (minimum average at night -10°C, maximum average at day 30°C).

In general, rearing *A. neurica* was more successful than rearing *A. geminipuncta* (Table 11). Except for 2019, usually, 20-30% of *A. neurica* larvae developed into adults and we were able to produce several thousand eggs every year. In contrast, we faced quite variable results with *A. geminipuncta*, leading to a nearly complete crash of the colony with only 2% of larvae successfully completing development in 2016. Inbreeding or diseases are the most probable reasons for the rearing issues with *A. geminipuncta.* Thanks to additional field collections of pupae, we were able to rebuild the rearing colony. In addition, by investing more care in respect to hygiene of rearing containers and avoiding cannibalism and mating of descendants from the same female, we were able to once again increase rearing success of *A. geminipuncta*.

Table 11. Summary for rearing of *Archanara neurica* and *A. geminipuncta* on stem sections from 2016-2020 at CABI Switzerland.

Year	No. larvae set up	No. pupae developed	% larvae pupated	No. adults emerged	% adults emerged[a]	No. eggs/female	No. eggs produced
A. neurica							
2016	462	145	31.4	93	20.1	88.3	2700
2017	732	344	47.0	98[b]	23.7	79.0	>3000
2018	717	294	41.0	208	29.0	88.5	>4000
2019	323[c]	59	18.3	33	10.2	78.4	1000
2020	24[c]	9	37.5	6	25.0	151.8	700[d]
A. geminipuncta							
2016	491	42	8.6	31	6.3	75.0	700
2017	684	177	25.9	47[b]	18.1	69.0	700
2018	516	98	19.0	42	8.1	81.7	>1000
2019	1006	191	19.0	121	12.0	103.3	>5000
2020	1548	327	21.1	>200	>13	124.8	>10000

[a], based on the number of larvae set up. In case pupae were removed for shipment, numbers were adjusted.
[b],150 pupae of *A. neurica* and 110 of *A. geminipuncta* were shipped to University of Rhode Island and AAFC Lethbridge, reducing the number of adults emerged.
[c], fewer larvae than in previous years were set up using stem sections, because more were set up on artificial diet.
[d], including eggs originating from larvae collected in the field.

The existing mass production techniques using stem sections are a safe procedure, albeit a time and resource extensive operation requiring good care for several months during the larval development period each summer. But this is an important fall-back option to at least supply thousands of eggs annually to collaborators once field release permits are granted. A major breakthrough was the development of rearing trials by Dr. Bourchier at Lethbridge using the McMarron diet for rearing *A. neurica.* There are some remaining challenges for *A. geminipuncta* and work for this species and improvements continue (for details see Task 7). The most successful approach at present is to combine artificial diet for the early stages with feeding later instars shoots that facility pupation.

TASK 7: DEVELOP MASS PRODUCTION TECHNIQUES

We experimented with improvements in the available care of the existing larval rearing techniques developed in Switzerland using cut shoots (Fig. 35). By intensifying care, basically through hiring summer students, we were able to improve rearing success and larval survival. This is still an elaborate process requiring constant vigilance and supervision of conditions with frequent food changes, but we know this can be successful in producing thousands of eggs and hundreds of adults for field release. This has now worked well at CABI and at URI, but it will require large amounts of manual labour. After early disappointing trial runs with semi-artificial diets at CABI and URI, Dr. Bourchier (Agriculture and Agri-Food Canada, Lethbridge) using independent funding has found the ability to rear at least one of the species successfully (*A. neurica*), and personnel at CABI started experimenting with the McMarron diet, and different diet formulations.

We obtained ingredients to prepare the McMorran diet from the Insect Production Services (Natural Resources Canada). The diet was prepared in 1-litre batches. The standard recipe was used for *A. neurica*. Since newly hatched *A. geminipuncta* larvae did not accept the plain diet, we experimented by adding 100 g of ground *P. australis* (central parts of the stem about 2 cm below to 10 cm above the meristem). In 2020, we added an additional treatment by supplementing the diet with 160 g of *P. australis*. We offered diet cubes (0.5 - 1cm^2, Fig. 36) to single larvae in round jars (diameter 45mm, height 30mm) and replaced diet once/week. Finally, we also transferred about 100 older larvae of *A. geminipuncta* (third instars) reared on *P. australis australis* stems to McMorran diet to assess whether older larvae are able to complete development on artificial diet.

Fig. 37. Set-up of *A. geminipuncta* rearing with (green) and without (yellow) addition of fresh *Phragmites* (left); mature larva feeding on a cube of McMorran diet including fresh *Phragmites* stems (center); and successful pupation (right).

Our first trials to rear *A. neurica* on artificial diet at CABI in 2019 worked well. Larvae accepted the diet well, going through five instars (one additional instar than observed on *P. australis* stems). Out of 930 larvae transferred individually, 48% pupated, but only 14% successfully emerged (Table 12). This is less than the usual 20-30% reached on stem sections, but slightly more successful than in our conventional rearing on cut stems of that year, where only 10% of adults successfully emerged from 323 larvae initially set up (Table 11). Considering that rearing on artificial diet involves less work, this is great progress with regard to future mass

rearing. Rearing of *A. neurica* on McMorran diet worked less well in 2020, with only 11.5% of larvae pupating and 7.7% adults emerging (Table 12). The subsequent number of eggs produced by emerging females, and egg quality (i.e. larval hatch the following year) also seemed to be reduced for moths reared on artificial diet.

Table 12. Summary for rearing of *Archanara neurica* and *A. geminipuncta* on artificial diet in 2019 and 2020.

	Year	#larvae set up	# pupae developed	% larvae pupated	# adults emerged	% adults emerged[a]	#eggs/ female	# eggs produced
A. neurica								
	2019	930	449	48.3	133	14.3	48.2	2100
	2020	542	63	11.5	42	7.7	50.9	1000
A. geminipuncta								
	2019	140	0	0	0	0		
	2020	1500	0	0	0	0		

[a], based on the number of larvae set up

More work is needed until we can successfully rear *A. geminipuncta* on artificial diet. Most of the larvae reared on pure McMorran diet died within three weeks, without even advancing to the next instar. Adding freshly ground *P. australis australis* stems significantly increased larval survival and about 70% of the larvae survived and developed longer than three weeks in 2019. However, none successfully pupated. In 2020, we used a third diet with larger amounts of *P. australis australis*, and we also tried moving larvae back to the pure McMorran diet, once they had established on the diet including *P. australis australis*. None of the treatments seemed to help to improve rearing success of *A. geminipuncta*. Although many larvae were able to molt up to six times and survive for 5-6 weeks on the diet, all died before being ready for pupation. We had some issues with development of fungi, but larval death seemed often independent of contamination.

However, seven of the larvae that were first reared on stem sections and transferred as third instar larvae to the three diets managed to pupate successfully (Fig. 36, right). These were performing much better and grew much faster (especially on plain diet) than larvae reared on diet from the beginning. The higher larval mortality for *A. neurica* observed on artificial diet in 2020 compared to 2019, could be due to the fact that larvae in 2020 originated from eggs produced by females already reared on diet in 2019. Rearing the moth for several generations on artificial diet might reduce their fitness. Covid related work problem prevented much for the needed "detective" work in Canada to improve diet formulations, and greatly limited work at CABI. But some work continued.

Unfortunately, *A. geminipuncta* remains difficult to rear on artificial diet. Originally, we thought that missing vertical structures will prevent pupation, but our recent experiences rather indicate that some nutritional element is missing from the artificial diet, and that pupation outside of stems is possible. One avenue to further pursue may be to rear larvae first on stem sections and only then transfer them onto diet. Of 930 larvae transferred individually on cubes of pure McMorran diet, 48% pupated, but only 14% successfully emerged. Nevertheless, despite this high mortality, this is slightly more successful than our conventional rearing on cut stems, where from 323 larvae 10% successfully emerged. Considering that rearing on artificial diet

involves much less work, this is a great progress with regard to future mass rearing. Several 1000 eggs of *A. neurica* were obtained from adults reared on this artificial diet.

To initiate feeding larvae in the field typically sever the growing point and then consume the slowly decaying materials – this may be an adaptation to avoid potentially toxic substances *P. australis* produces, but nothing is known about such secondary compounds at the present time. But these observations will inform our rearing strategies in the upcoming years. For now, a combination of hand rearing and artificial diet development has assured the availability of thousands of eggs at CABI for work in quarantine or potential field releases. Lately, we mixed artificial with rearing on cut stems for part of the larval period. This greatly improved rearing success and a mixed methods approach may be needed unless we are able to formulate a more specific diet that allows full larval development and pupation into healthy adults. Maintaining high insect quality will be an important consideration, particularly when rearing on artificial diet.

TASK 8: IMPORT, RELEASE AND STUDY THE EFFECT OF INSECTS AT RELEASE SITES

Archanara geminipuncta and *A. neurica* have thus far only been approved for field release in Canada, not the U.S. Dr. Bourchier's group first released *A. geminipuncta* and *A. neurica* in Canada in 2019, but these releases were limited in scope and location due to Covid-19 restrictions. Spring 2021 was therefore the first time biocontrol agents were released under ideal conditions following a scientifically rigorous design. Consequently, this year (2022) represents the first opportunity to collect meaningful information about overwintering success, establishment, and initial impact of these biocontrol agents.

Dr. Endriss travelled to Canada this June to visit one of the 13 monitored release sites in Southern Ontario. Both *A. geminipuncta* and *A. neurica* were released at this site in experimental treatments (*i.e.*, species released singly vs. together, as eggs vs. pupae, in shade vs. sun, and in various treatments combinations). This visit was an opportunity to discuss initial release and monitoring protocols with collaborators as well as observe and create a photo-library of insect damage (Fig. 38). This information is critical for both understanding potential of these insects to control *P. australis australis* more generally and for being able to detect when these species disperse across the Canada-NY border. In addition to our lab, collaborators in Dr. Bourchier's group, the U.S. Army Corps of Engineers as well as Dr. Rebecca Rooney's group at the University of Waterloo were present for this field visit.

Fig. 38. Dr. Michael McTavish, a postdoctoral research associate in Dr. Bourchier's group, explains the current release and monitoring protocols for insects in Southern Ontario (top left). *Archanara* spp. cause dieback of *Phragmites* stems throughout the growing season. Early damage causes stem dieback when they are still small 'asparagus-like' shoots (bottom left), which can be difficult to detect in the field. Later in the season, robust, taller stems still die back, gradually browning starting from the top of the stem (bottom right, dying stem shown by white arrow), often eventually resulting in the complete senescence of the stem (top right, again shown by white arrow).

Fig. 39. Visible signs of *A. geminipuncta* damage. From left to right: (1) larval emergence hole upon switching shoots, 'browning damage' inside (2) stem adjacent to the emergence hole, and (3) on the outside of the stem, and (4) frass and typical *Archanara* spp. feeding damage on the inside of the stem. The side branches visible in the middle two photos is very distinctive to *Archanara*. While *Phragmites* also branches in response to attack by *Lipara* spp., these branches usually occur on the top half of the stem. In contrast, branching by *Archanara* seems to occur on nearly every node of the stem, even nodes very close to the ground. Note: we can often distinguish damage by *A. geminipuncta* vs. *A. neurica*. The 'browning' damage observed in the second to left photo is reported to be more circular when caused by *A. neurica*. Further, if frass is present above the emergence hole this indicates damage by *A. geminipuncta*, as this species pupates head up while *A. neurica* pupates head down.

While we have been limited in our ability to assess release procedures, establishment and impacts of *A. geminipuncta* and *A. neurica* in the US due to lack of USDA/APHIS approval, so far, releases in Canada appear promising. Damage by *Archanara* spp. is distinctive: they bore very unique holes into and out of *Phragmites* stems when developing as larvae (Fig. 39), and the resulting impact of herbivory on stem performance looks very different from damage caused by other insects that currently occur in *Phragmites* stands in North America (Fig. 38). Most of the data we have thus far is on larval releases—each winter, Patrick Häfliger has shipped eggs from Switzerland to Canada, which Dr. Bourchier's group then places in an outdoor shed until insects emerge as first-instar larvae. Larvae are then placed within cut *Phragmites* stems (three larvae per stem for *A. geminipuncta*; one larva per stem for *A. neurica*, as larvae of these species are cannibalistic). Cut stems are typically ~30cm tall, with the first internode hollowed out before inoculating with larvae. These stems are then placed in the field in moistened floral foam for two weeks (Fig. 40). Within this time, larvae typically bore out of the cut stems and (ideally)

select a new stem to continue development. Collecting inoculated stems after this period provides an estimate of efficacy of this method—the number of bore holes provides an estimate of how many larvae successfully emerged from inoculated stems.

Fig. 40. Leftover floral foam blocks used to hold cut *Phragmites* stems inoculated with *Archanara* spp. larvae (left; note slits in plastic to hold individual *Phragmites* stems). Closed (middle) and open (right) egg release containers. Containers are closed in the field to protect against predation. However, the open container on the right illustrates the nested container design—eggs were placed into a PVC cup with an open top (missing in this photo), which was held inside of the larger container by the black ziptie (shown by white arrow). Once larvae emerged from eggs, they climbed over the top of the inner PCV cup, then fell through the mesh bottom of the outer cup into the *Phragmites* stand.

Overall, *A. geminipuncta* may attack an additional three stems per growing season after they leave the inoculated stems, with *A. neurica* attacking slightly fewer stems. In general, the evidence suggests *Archanara* spp. larvae disperse two meters or less from their initial release point. Further, within a 30 cm radius of the release point, up to 50–60% of *Phragmites* stems were damaged this growing season, with an average of ~30% of stems damaged within this 30 cm radius across all 13 monitored sites. When adjacent stems were searched just outside of this radius for an additional three minutes, an average of one to two attacked stems were detected per minute. Thus, even when larvae are released at low numbers, they appear to successfully establish and have a significant, though highly localized, impact on invasive *P. australis australis*.

Egg releases may have similarly high—or even higher potential—than larval releases. However, overwintering survival not been confirmed for *Archanara* released as eggs, as eggs were not viable in 2021—they were exposed to too many cold and hot fluctuations in 2021 due to Covid-19 protocols. But this year (2022), Dr. Bourchier's group refined the protocol for egg releases by creating a nested container design with a mesh bottom to protect against predation (Fig. 40). Specifically, 60 eggs were placed into a PVC cup with an open top, which was then placed inside of a container with a mesh bottom. Once larvae emerged from eggs, they climbed over the top of the inner PCV cup, then fell through the mesh bottom of the outer cup into the *Phragmites* stand. This approach worked well and Dr. Bourchier's group is looking to advance this protocol forward next year by assessing whether *Archanara* establish better from eggs overwintered in the lab and put out in the spring vs. put out in the field as eggs at the beginning

of the winter. One of the biggest challenges with insect releases will be correctly synchronizing the insects with the *Phragmites* growing in the field—once the protocol for egg releases is further refined, this synchrony may be more effectively achieved with egg rather than larval releases.

In terms of long-term monitoring, our collaborators in Canada are focusing on two monitoring windows: one in early summer to detect insect establishment and spread, and one in the fall to monitor impact of insect herbivory. What they have cautioned so far is that the summer window for successfully detecting *Archanara* spp. is quite narrow—once stems get too tall and dense it becomes too difficult to detect larval damage. Further, as the *Phragmites* stems grow, the leaves often grow and shift, often obscuring the distinctive entrance and emergence holes within a few short weeks. They also report that the holes themselves can quickly degrade. In contrast, going out too early in the summer runs the risk of not detecting damage, as fewer stems will be attacked, and damage will thus be less visible.

Another challenge for monitoring is that egg laying by these species appear very patchy. While current protocols appear very effective at detecting spread of larvae within several meters of their release points, trying to detect the presence of *Archanara* within larger *Phragmites* stands after adults have a change to disperse may therefore require an unreasonably large amount of person hours. Given these monitoring challenges, developing pheromone traps may be especially promising for detecting dispersal of *Archanara* where they have not been explicitly released. Our collaborators in Canada are working on this approach. We also still believe that detecting the presence of insect damage by visually scanning *Phragmites* populations is promising, as *Archanara* spp. appear to trigger a specific type of branching that is easily distinguishable from branching caused in response to damage by other insects, particularly *Lipara* spp. within *Phragmites* stands.

Other long-term goals include greater clarity about how establishment success of *Archanara* spp. is affected by the number of eggs, pupae, or even adults that are released within the *Phragmites* stand. For example, next year Dr. Bourchier's group hopes to give mating pairs of adult insects 24 hours in cages in the lab before releasing them in the field. This approach has worked well for Patrick Häfliger in Europe. We are also working on refining how to rear large numbers of insects in the lab more generally. This research is currently largely occurring in Canada given that the Canadian authorities approved field releases. However, we plan on establishing a rearing colony at Cornell once approval for field release is granted in the U.S. (or when insects naturally disperse over the Canada-NY border).

At present our active involvement in this task is restricted by the pending field release approval. But we are closely working with our Canadian collaborators and will greatly benefit from their experiences and experiments. They purposefully scattered field releases close to the NY border and we are actively assessing dispersal. The specific branching pattern will be a great help for early detection even if populations are low. Some of the selected Canadian release sites are also offering the opportunity to assess the selectivity of the *Archanara* species because both native and introduced *Phragmites* is present at some sites. This will enable us to fulfill further requirements to obtain a US field release permit (see Task 2).

TASK 9: TECHNOLOGY TRANSFER TO AGENCIES AND STAKEHOLDER GROUPS

As with Task 8, we have been limited in the scope of work we can complete under this task, as a lack of approval for field release of biocontrol agents in the U.S. combined with Covid-19 restrictions have delayed or made impossible much of the work initially proposed under this task. We have given many presentations and webinars, including to NYSDOT audiences in and via webinars including on biocontrol development, monitoring or morphological distinction between introduced and native *Phragmites*. We have published multiple papers and additional ones are ready to be submitted this fall. We have decided to try, as much as possible, to make papers Open Access. We are continuing our outreach activities through webinars, talks and meetings with wetland managers. Various additional products are under development but are not in a form that we can share at this point, which includes the monitoring protocol since we are lacking critical field information to fully assess the utility of what we are proposing, specifically presence of biocontrol insects in the field in the US. We need to field test these approaches in the presence of biocontrol agents before we can be confident that they will serve wetland and land managers as expected. And that the assessment methods we propose can establish the link between management, such as release of biocontrol agents and changes in *Phragmites* abundance and response of other wetland biota.

Here, we therefore summarize our progress on efforts to use stationary recorders to assess impacts of *Phragmites* invasions, and sentinel plantings after *Phragmites* herbicide management to showcase how we would approach technology transfer to agencies and stakeholder groups—and to highlight the promising utility of these approaches. We also outline how what we have learned from our Canadian colleagues and the 2022 field visit to a *Archanara* release site will inform assessing biocontrol agent establishment, dispersal and impact on *Phragmites*. We also outline some work we are advancing for other taxa that may become important tools for long-term assessments as we are moving the biocontrol program forward.

Summary of using stationary recorders to assess the impacts of *Phragmites* invasions

Declining population growth rates of native *P. australis americanus* is only one of the negative impacts of introduced *P. australis australis*. Another goal of management is to mitigate negative impacts of introduced *Phragmites* on other native species, including birds, bats or anurans. Thanks to the collaboration, guidance, and support of many USFWS and DEC biologists as well as private landowners, we were able to conduct a pilot project (funded by USFWS) in which we evaluated whether use of autonomous passive-acoustic monitoring devices (Fig. 41) could deliver information about avian and anuran communities in 11 wetlands of the Northeast (two locations in New York and one each in Delaware and Maryland), including in *Phragmites*-invaded habitats during the 2019 field season. Ideally, we tried to group sites within locations so that each sampled region had three recorders, one each in: an introduced stand of *Phragmites*, a native stand of *Phragmites*, and a reference marsh that represents ideal marsh bird habitat. We first deployed recorders between late April and mid-May 2019, depending on the timing of logistics and site permissions. We allowed our recorders to record continuously (i.e., 24 hours a day for 7 days a week) until final retrieval in early August 2019. We are in the process of further training a convolutional neural network (CNN) called BirdNET (https://birdnet.cornell.edu/) to

automatically identify marsh birds and anurans. Birds and anurans have species-specific vocalizations that can be identified by analyzing the duration, frequency, and patterns of their notes and songs or calls and we add this information to the almost 3000 of the most common species of North America and Europe that have already been incorporated into this machine-learning algorithm, which is constantly being updated.

We have made good progress towards attaining this goal but have not yet fully completed this objective. Obtaining a species list through BirdNET only takes as long as it takes to upload the files but preparing a subset of audio files for fine-tuning and validating our final analyses is incredibly time consuming. The time put into this preparation is vital for understanding the utility of this approach, and for streamlining these analyses moving forward. Dr. Endriss, who is heading up the data processing and analyses, sustained a spinal injury during a car accident in September 2019, which—combined with COVID restrictions that created obstacles to collaboration—caused a significant delay in advancing the analyses of the recordings. This year, however, we recruited a bird specialist, Alec Hopping, to finish annotating and validating the necessary files for these analyses. We are therefore still finishing identifying each bird call/song to species within a subset of our audio files (Fig. 42). Once we fine-tune and validate the utility of this approach, using BirdNET to analyze future datasets has the potential to happen on a much faster timescale.

Fig 41. Our reference marsh (Guy's marsh) for Montezuma, NY consisted largely of cattails (left). We tried to obscure the recorder from view from nearby trails by placing the recorder within a stand of old cattail stems, but also used zipties to secure dead cattails stems to the outside of the recorder and t-post to better camouflage the recorder (center). Early in the season, we secured bundles of dead cattail or *Phragmites* stems atop of recorders to prevent overheating (right).

Thus, we are still refining how stationary recorders can be used directly by managers, but we already clearly find that stationary recorders facilitate data collection and have strong potential to enable communities, NGO's agencies or individual landowners without specialized knowledge to collect this information and better monitor the recovery (or lack of recovery) of biotic communities in response to management actions. Specifically, our recordings were able to capture clear vocalizations from many bird species, including both generalist species as well as specialists that are of greater conservation concern (Table 13).

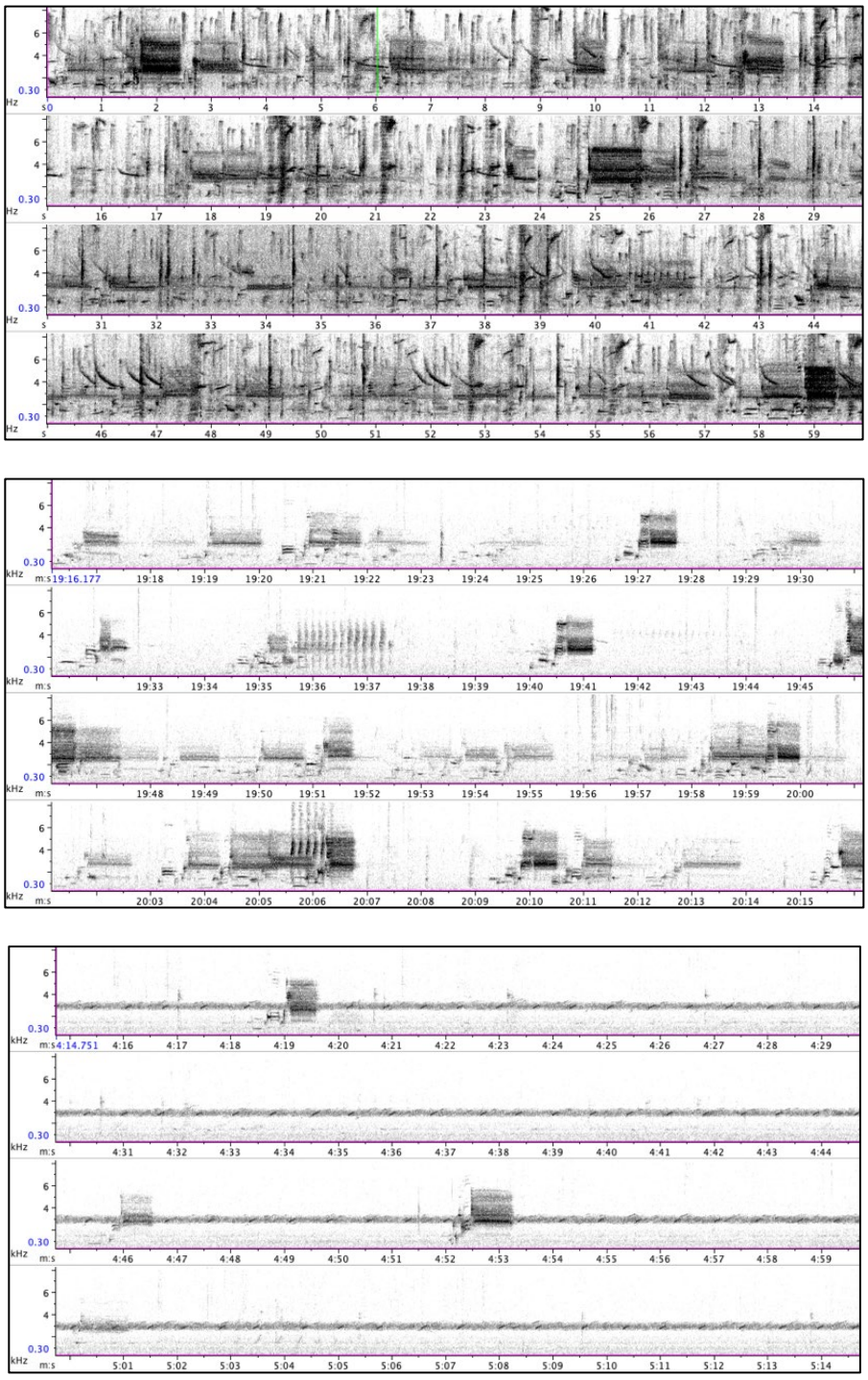


Fig. 42. For each recorder location, we randomly selected one hour-long audio file from each of the following categories: dawn during peak birding season, dawn during the end of birding season, and dusk at during the end of birding season. Each category was meant to represent high, medium, and low call frequency, respectively, providing us a diverse set of bird calls and bird call frequencies to annotate to validate BirdNET selection identifications. Above, for example, are 60 second snippets of spectrograms representing the high (top), medium (middle), and low (bottom) call frequencies from the recorder placed in introduced *Phragmites* at Iroquois NWR.

Table 13. Bird species detections that BirdNET was already able to identify with high confidence across a subset of 33 hours of audio files in 2020. For over 30 hours of audio files from 2018 used to validate this data, 'high confidence' BirdNET identifications were correct >95% of the time. BirdNET was able to identify calls and songs of 41 species with high confidence.

Bird Species	# of detections		Bird Species	# of detections
Red-winged blackbird	573		Purple finch	3
Swamp sparrow	404		Wood duck	3
Common grackle	343		Chipping sparrow	2
Common Yellowthroat	329		Common redpoll	2
Virginia rail	110		Northern waterthrush	2
Tree swallow	81		Red-tailed hawk	2
Canada goose	77		Rose-breasted grosbeak	2
Eastern wood-pewee	50		Cedar waxwing	1
American goldfinch	32		Downy woodpecker	1
Eastern kingbird	30		Gray catbird	1
Willow flycatcher	22		Great blue heron	1
American robin	20		Great crested flycatcher	1
European starling	18		Green-winged teal	1
Barn swallow	15		Herring gull	1
Brown-headed cowbird	5		Indigo bunting	1
Rusty blackbird	5		Northern flicker	1
Snow goose	4		Northern rough-winged swallow	1
Blue jay	3		Ovenbird	1
Chimney swift	3		Song sparrow	1
Eastern meadowlark	3		Spotted sandpiper	1
Mallard	3			

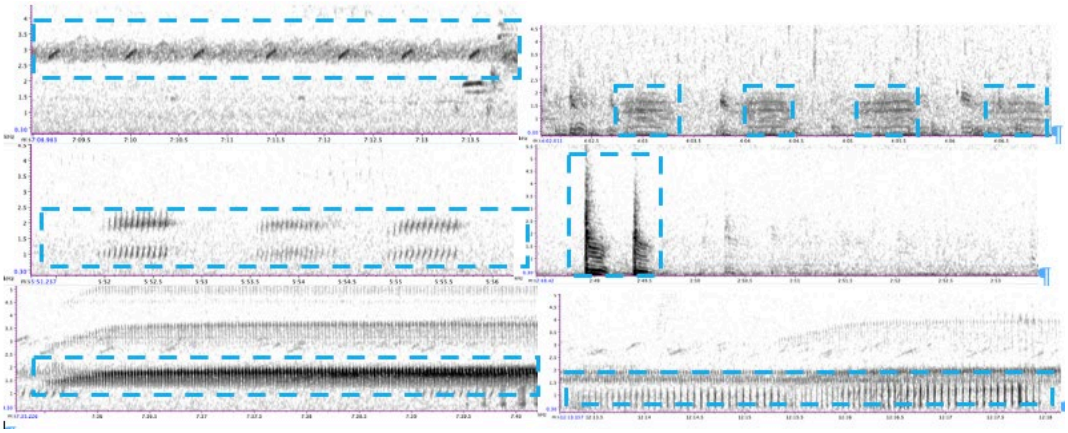


Fig. 43. Common anurans in wetlands in the eastern U.S. produce unique spectrograms that allow recordings to be used to easily differentiate between different anuran species vocalizing within stands of *Phragmites.* Above, we depict spectrograms of five second snippets of spring peeper (*Pseudacris crucifer*; top left), American bullfrog (*Lithobates catesbeianus*; top right), grey treefrog (*Hyla versicolor*; center left), green frog (*Lithobates clamitans*; center right), American toad (*Anaxyrus americanus*, bottom left—note that this series of fast-pasted ticks also resonates more faintly at higher frequencies), and Northern Leopard frog (*Lithobates pipiens*, bottom right) from audio files representing different levels of loudness and clarity (dashed blue boxes indicate calls by the specific frog species).

We were also able to clearly distinguish between different anuran (Fig. 43), insect (Fig. 44), and even mammal species (Fig. 44). We were also able to detect biotic differences between different habitat types (e.g., Table 14), including in local species assemblages (Fig. 45) and species phenology (Fig. 46). This was particularly true for anurans, where our analyses are most advanced due to the limited diversity compared to birds. Stationary recorders therefore represent a potential breakthrough in providing continuous data capturing bird, anuran and potentially other vocalization by bats and insects simultaneously.

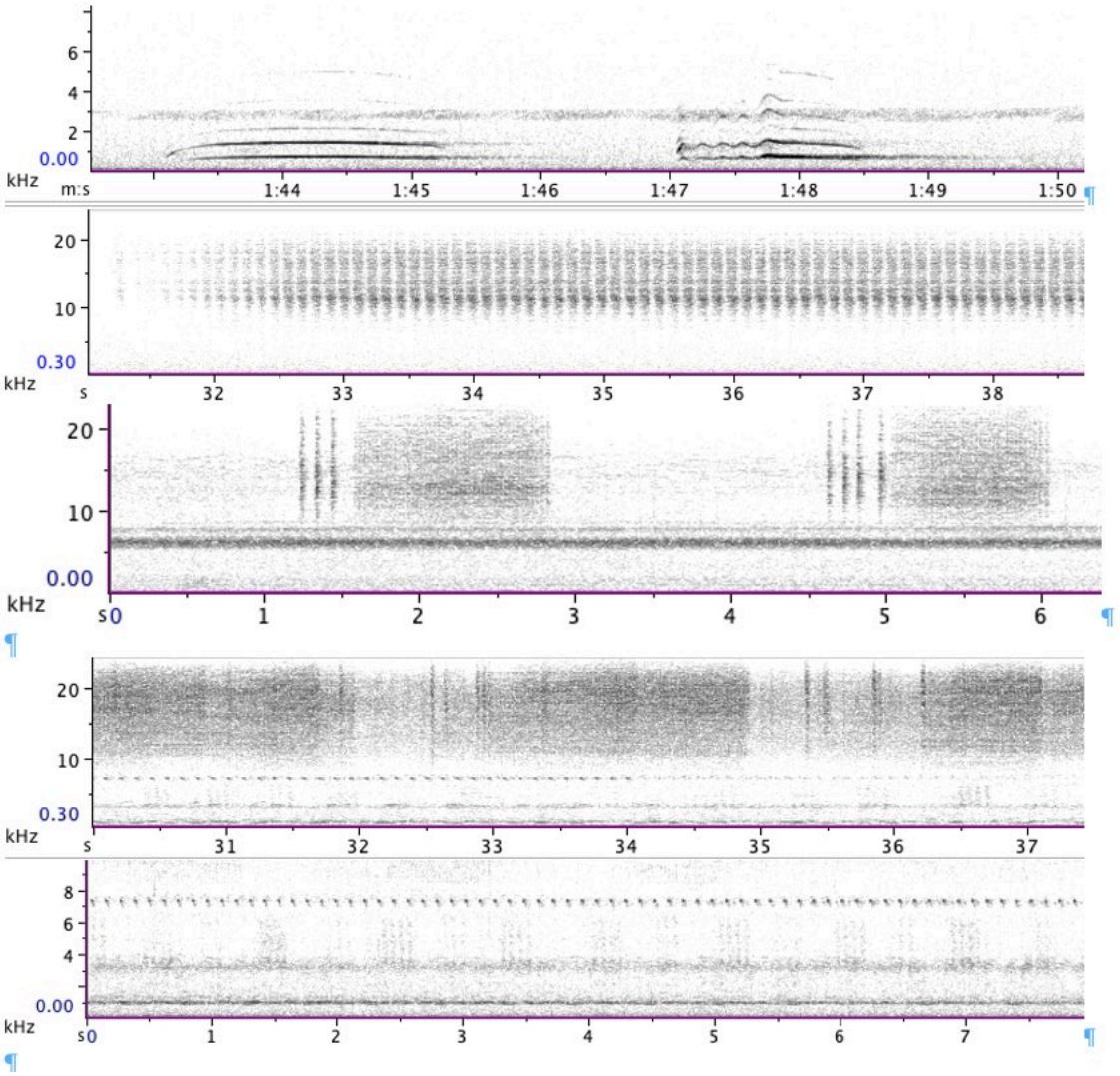


Fig. 44. Spectrograms clear coyote calls (top panel; note the yips shown by the ripples around 1:47-1:48), while the lower spectrograms show clear spectrograms of sounds made by different insect species that were commonly found across our audio recordings. The spectrogram in the second row, for example, likely shows sounds made by a Saltmarsh Meadow Katydid (*Conocephalus spartinae*), while the spectrogram in the third row between 10 and 20kHz shows sounds made by an *Orchelimum sp.*, likely either the Handsome Meadow Katydid (*Orchelimum pulchellum*) or the Black-legged Meadow Katydid (*Orchelimum nigripes*). The spectrogram in the fourth row between 10 to 25 kHz shows sounds made by a *Conocephalus sp.*, likely either a Slender Meadow Katydid (*Conocephalus fasciatus*) or a Short-winged Meadow Katydid (*Conocephalus dorsalis*). Finally, the bottom row shows sounds made by an Allard's Ground Cricket (*Allonemobius allardi*; see ticks around 7 kHz).

Some additional work is required to scale up from our pilot project to full field implementation or before interested individuals or organization may upload their recordings for full analyses by BirdNet. As part of this endeavor, we are working towards expanding BirdNET even further by adding anuran species to the training dataset, but also potentially vocal mammal and insect species. Further, obtaining more accurate information about habitat suitability as *Phragmites* is invading or being controlled will be critical for using this data to assess how biotic communities are responding to *Phragmites* invasion or management.

Table 14. Below, we showcase common species found within introduced *Phragmites*, native *Phragmites*, and reference marshes. Bird species—and their respective number of detections (# det.)—that BirdNET identified as the top ten most frequently detected calls and songs within introduced *Phragmites*, native *Phragmites*, and reference marshes across a subset of 33 hours of audio files. Please note that some of these need to be verified to assure accurate ID, as these identifications include all species identifications, not just those identified by BirdNET with high confidence.

Introduced *Phragmites*			**Native *Phragmites***			**Reference marsh**	
Bird species	**# det.**		**Bird species**	**# det.**		**Bird species**	**# det.**
Red-winged blackbird	1204		Swamp sparrow	317		Red-winged blackbird	521
Common grackle	590		Common Yellowthroat	233		Swamp sparrow	292
Common Yellowthroat	575		Red-winged blackbird	204		Virginia rail	229
Swamp sparrow	532		American robin	66		Canada goose	165
Virginia rail	289		Northern waterthrush	59		Tree swallow	100
Rusty blackbird	128		American goldfinch	47		Common Yellowthroat	95
Willow flycatcher	126		Willow flycatcher	34		Eastern kingbird	43
American goldfinch	113		Rusty blackbird	28		Savannah sparrow	31
European starling	108		Virginia rail	24		Willow flycatcher	33
American robin	103		Tree swallow	19		Killdeer	28

These tools will only become more powerful and accessible with time. When we began this work in 2018, BirdNET was only trained to identify about 150 of the most common bird species in North America. Yet, within the past three and a half years BirdNET has now expanded to identify almost 3000 of the most common species in not just North America, but also Europe. Moving forward, we will work towards expanding BirdNET even further by adding anuran species to the training dataset, but also potentially vocal mammal and insect species as well. Further, in addition to its online platform (https://birdnet.cornell.edu/), BirdNET has already been integrated into the Merlin Bird ID app (freely available) to help the public identify birds by sound as well as sight. As we increase the capabilities and reliability of bird, anuran and other vocal biota's identification through BirdNet, there is likely a "tipping point" where logistics, expertise, financial and other considerations will favor automated analyses and replace (some) field work. This will be equally applicable whether the goals are simple inventories, or more detailed assessments of abundance or response of biotic communities to invasion by *Phragmites* (or by other species) as well as to management activities meant to mitigate the negative impacts of these invasions.

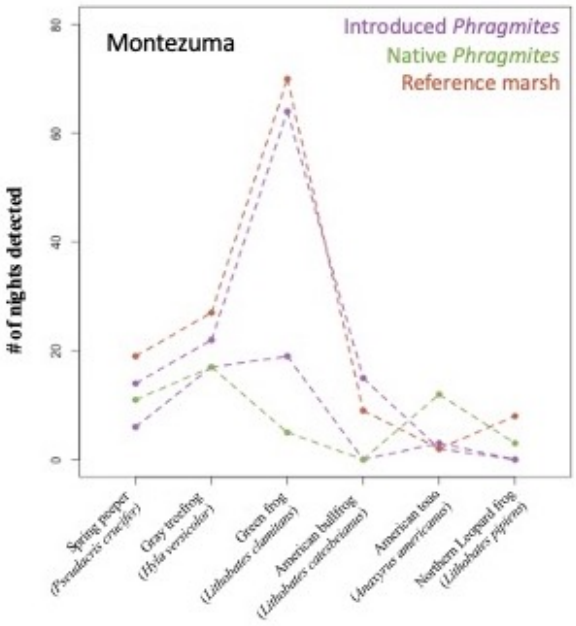


Fig. 45. Differences in the number of nights with vocalizations of six common anuran species over 80 days of recording at four recording sites (as determined by a 5-minute subset of audio recorded near midnight of each day), two with introduced *Phragmites* (purple), one with native *Phragmites* (green) and an uninvaded reference marsh (brown) near Montezuma, NY. Thus, we found that common anuran diversity was highest at our reference marsh (six of the common anuran species were detected), next highest at our two sites with introduced *Phragmites* (five and four of the common anuran species were detected, respectively), and lowest at our site with native *Phragmites* (four of the common anuran species were detected)

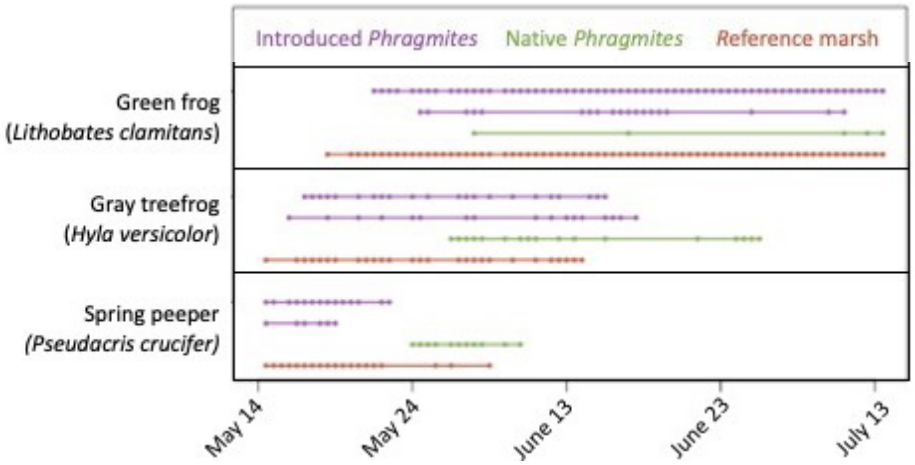


Fig. 46. Differences in phenology of vocalizations of three most common anuran species in spring/summer 2019 from four recorder locations with different vegetation communities near Montezuma, NY. Each dot represents a day on which the species was recorded as present based on our analyses of a 5-minute snippet of spectrograms recorded near midnight of that day. Each box for each species can potentially have 4 lines (see green frog and grey treefrog as examples). The top two purple lines represent the two locations with introduced *Phragmites*, the green line represents native *Phragmites*, while the bottom brown line represents the reference marsh. Note: this figure depicts that common anuran species typically begin (and stop) vocalizing earlier in stands of introduced *Phragmites* than in stands of native *Phragmites*.

Plant sentinels to assess impact of *Phragmites* management

Understanding the impact of *Phragmites* management (using biocontrol or otherwise) requires assessments of the outcome of the applied techniques. But such assessment are rarely applied (Martin & Blossey 2013; Hazelton *et al.* 2014), nor are they standardized, creating problems of accountability. Part of the problem is lack of acceptable and easy to apply methods that do not require an advanced degree and intimate knowledge of the local flora or fauna (see previous section). If the interest is in assessing the response of associated plants, it appears that the typically applied standardized vegetation assessments using permanent quadrats and recording of presence and cover of all species does not necessarily capture important long-term dynamics, or rare species (see also task 5a, b for effort required and duration to capture changes). For years we have tried to assess the suitability of using sentinel plantings (indicator species) to monitor outcome of management interventions (Blossey *et al.* 2019a). This may allow standardization of assessment protocols and comparison across diverse and different plant assemblages and habitats. Our own work on *Phragmites* has shown the uniqueness of different plant assemblages at each study location making cross-site comparisons beyond diversity indices difficult.

Fig. 47. Locations in the Adirondack Park showing return of plant species after suppression of introduced *P. australis australis*. Picture on the right shows sentinel planting location marked by tall PVC stakes.

We used the availability of USDA/Hatch funding, an extensive network of *Phragmites* herbicide control sites (Quirion *et al.* 2018) in the Adirondack Park, and the interests of a MS student (Audrey Bowe) to explore if sentinel plantings are able to assess potential long-term negative (or beneficial) effects of single or repeated herbicide use to control introduced *P. australis australis*. We propagated and then planted three different wetland species (sensitive fern, *Onoclea sensibilis*, swamp milkweed, *Asclepias incarnata*, and white meadowsweet, *Spirea alba*) into sites with a different *Phragmites* herbicide treatment history and adjacent untreated control sites (no *Phragmites* presence) in summer 2019 (Figs. 47, 48). Audrey followed the establishment and growth of these individuals through the summer of 2021 (data collection for 2022 is pending).

Individuals for all species that we planted were able to establish, but we found significant differences in survival rates between sites with a previous *Phragmites* occupation and herbicide treatment history, compared to untreated adjacent reference wetlands (Fig. 49). While overall survival was high (>50%), all three species had better survival in areas that had never been invaded by *P. australis australis* and sprayed. In addition to improved survival in control sites, plant growth (height) was also superior in reference wetlands (data not shown).

Fig. 48. Container propagated seedlings to assess outcome of *Phragmites* management (top row) and follow-up measurements of survival, growth, and reproduction (middle row) in a reference (lower left) and *Phragmites* treatment location (lower right).

We know from previous work that *Phragmites* presence may condition soil in ways that reduce seedling survival for other wetland plant species (Crocker, Nelson & Blossey 2017), so it remains difficult to ascertain, at the present time, whether herbicide treatments alone, or long-term legacy effects of previous *Phragmites* presence, are responsible for the reduced sentinel plant survival. Further analyses evaluating whether intensity of herbicide management (number

of years treated) or size of the *Phragmites* patch may influence survival and growth rates. But findings of negative long-term effects of herbicide management are frequently reported for management of other introduced plant species (Kettenring & Adams 2011) and this raises important question of the utility to use such treatments to enhance conservation efforts.

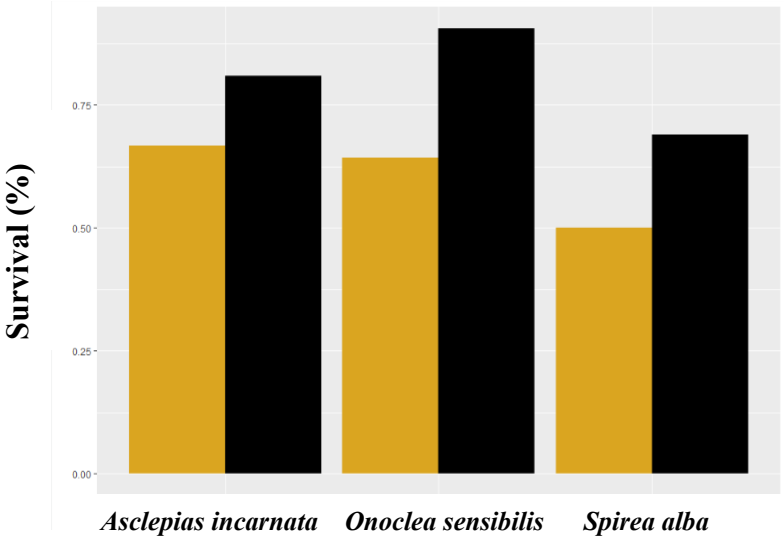


Fig. 49. Survival rates (%) over 2 growing of three wetland plant species transplanted into areas previously treated with herbicide to control introduced *P. australis australis* (goldenrod) and the uninvaded and unsprayed adjacent wetland matrix (black).

But no matter the mechanisms responsible for the reduced survival of our sentinel plants, we consider use of indicator species that are planted into treatment or assessment areas a useful tool for managers. Audrey is preparing a separate publication on the usefulness of this technique as part of her MS thesis. Sentinel plantings allow standardization, avoid the problems associated with differences in plant communities, and allow assessment of core species of interest (for example those we aim to manage for). We will be advancing this line of inquiry (funded independently by the NYISRI) at the Montezuma wetlands following a large scale *Phragmites* removal and marsh restoration and pair this with an assessment of the impacts of deer herbivory on wetland plant recruitment.

Assessment methods for other taxa

We have focused here on two methods capturing impacts on plants via purposeful sentinel plantings and a technique, bioacoustics, with the latter able to capture impacts on a variety of vocalizing taxa (birds, amphibians, insects, mammals). In our reporting for the long-term vegetation monitoring, we need to acknowledge the effort and duration required to embark on a full plant community assessment, albeit in the absence of management interventions. Ultimately, the purpose of any assessments and of a monitoring protocol, is widespread implementation, to follow outcomes of management interventions (or impacts of an invasive species), while delivering reliable information without "breaking the bank" or running into major logistical difficulties.

While there is an enormous variety of other methods to census other taxa, many of them require too much taxonomic or other expertise to allow for widespread implementation. For example, we initially proposed using malaise traps to assess flying insect biomass, but we ultimately decided that the effort required to set traps, sort and determine species and difficulties linking what we capture to local conditions (malaise traps capture flying insects that may have come from far away) was financially and logistically difficult during covid restrictions. And, most importantly, it was also problematic to make a convincing link to impacts of *Phragmites* or its management given that captures would consist of local species and long-distance dispersers. So we abandoned those efforts. The typical habitat *Phragmites* occupies (seasonally, temporarily, or permanently flooded, or even tidal) represent further challenges as both terrestrial, moist soil and aquatic environments may need to be assessed. All of these require different assessment methods, so we will likely need a portfolio of methods allowing managers to choose the appropriate ones for their circumstances.

For example, we have experimented with assessing impacts of different native and introduced plant species on the larval phases of amphibians (Fig. 50). We are advancing similar efforts to assess the success of restoration efforts after *P. australis australis* management and reseeding on the terrestrial phase of recently metamorphosed frogs at the Montezuma Wetlands area. Here we will cage juveniles in arenas for several months and assess their survival and growth while we also manipulate deer access. We will include plant sentinel monitoring efforts as well. We are also using a new small grant to develop assessment techniques for aquatic environments (using water chestnut, *Trapa n*atans as the main invader) to develop truly aquatic assessments using fish, amphibians and invertebrates such as crayfish as sentinel species. Collectively this information will be very useful in fine-tuning assessment and monitoring methods for *Phragmites*.

Fig. 50. Partially submersed reptarium cage to assess impact of different emergent plant species, including of *P. australis australis*, on tadpole survival and development. Details are reported elsewhere (Cohen, Maerz & Blossey 2012).

Assessing biocontrol agent presence, abundance, dispersal and impact on *Phragmites*

At present, we have very limited ability to propose release methods or ways to assess whether the biocontrol agents, once released, have established, how they are dispersing through a site, or regionally to non-release sites and their impact on *Phragmites* or associated wetland biota. As we have reported in the previous section, our collaborators in Canada are making progress on assessing different release methods, and they are beginning to understand how larvae are dispersing, and how to recognize impacts on *Phragmites* itself. But populations are still at a very

low levels, even at release sites, and these methods may shift dramatically, once populations increase and impacts are easier to recognize. We anticipate using combinations of searches for signs of larval attack, specifically of the increased stem branching pattern (Fig. 51) that will be evident over the winter, with adult insect survey methods (blacklight traps) and stem dissection to confirm existence and abundance of local biocontrol agents. The detailed census methods will need to be developed as *Archanara* populations increase and spread.

Fig. 51. In response to *Archana* larval attack that destroys the growing point, main stem growth is eliminated, and in response side shoots develop. These shoots will grow throughout the season and at various heights. This developing side branch has just emerged (photo taken in mid June 2022) and will continue to increase in size (photo credit: Ian Knight, USACE).

Draft outline of a monitoring protocol

While we will need to wait for insects to disperse to the US or releases be permitted before fully implementing a recommended monitoring protocol, we can sketch the outline of this protocol given what we have learned already. The first importance is the habitat type (fully aquatic, temporarily flooded, or fully terrestrial) that will determine what methods can be used. We will recommend permanent quadrats similar to the ones we used for our vegetation monitoring to be installed before any releases (or other treatments) begin. This will provide baseline information - ideally a few years before releases are made. And may also allow those wanting to do a full community assess to do so. At a minimum, stem counts will need to be made and potentially heights be assessed. *Archanara* impact is expected to reduce stem numbers and average height. Either in standardized quadrats, or in transects, the number of stems showing signs of attack should be recorded. This activity can be conducted at the end of a growing season once leaves are off the stem facilitating discovery of branching. It will be important to distinguish branching due to *Archanara* attack from branching caused by *Lipara* spp. We will provide full guidance on that once we have better pictures. Discovery of dispersing adults using light traps requires expert taxonomists, but can be conducted by researchers. Itwill be an option but it will not be available to every land manager.

Which assessment methods to deploy to assess change over time as biocontrol agents impact introduced *Phragmites* stands will depend on habitats, and logistical and financial opportunities. We will outline updates on our sentinel methods, which is a quick and cheap way to assess outcomes and can basically applied by almost any managers. Seedlings for outplantings can either be locally raised or be purchased. This low-cost method requires little

scientific expertise. We will outline other options, specifically the bioacoustic method. Costs of the recorders, batteries and SD cards are low (a few $100/unit) and the Cornell Lab of Ornithology will update the ability for external users to submit samples for identification. This is a rapidly advancing field, and we expect additional breakthroughs annually. Which other methods we may recommend will, in part, depend on the outcome of our work with juvenile amphibians and in water chestnut. Working with vertebrates is more complex and will require additional state or university permits but may offer important insights into new relationships that develop as biocontrol agents (hopefully) reduce the competitive ability of *P. australis australis*. But we will incorporate these possibilities into our monitoring protocol as opportunities.

OUTLOOK

The past 5 years have seen slow but persistent progress towards biocontrol of *P. australis australis*. While it has been disappointing that after TAG approval USDA/APHIS did not (yet) grant field release permits, both *Archanara* geminipuncta and *A. neurica* are now established in North America because Canadian authorities granted field releases (based on the same evidence we provided). We have detailed what USDA/APHIS now requires to amend the petition, i.e. a national survey to assess potential benefits of introduced *Phragmites*, and additional field evidence for the safety of native *P. australis americanus*. We are well positioned to embark on both projects if additional funding becomes available. It will not guarantee a positive decision by USDA/APHIS because of the political pressure and issues with the MS Delta. But dispersal of both moth species from Canada is inevitable if populations continue to grow. Once they disperse into the US and New York we will be enabled to work with them and further distribute them. This is only a question of time, and we are assessing dispersal and are preparing for this event. What the future impact of these stem mining moths is on *Phragmites* and associated wetland biota will need to be assessed in the next years and decades. Successful biological control takes time, and patience, as we have demonstrated in the purple loosestrife program (Endriss, Nuzzo & Blossey 2022). But as we have also demonstrated in our vegetation and acoustic monitoring, co-existence with *P. australis australis* is possible - eradication is not necessary - as long as stem densities do not become too high. This is exactly the impact we expect from both moth species once higher abundances develop (Häfliger, Schwarzlaender & Blossey 2006).

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