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Survey for Pathogens of Phragmites in New York

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PURPOSE: This study surveyed for potential pathogen biological control agents for the management of the nonindigenous *Phragmites australis*.

INTRODUCTION: *Phragmites australis* (Cav.) Trin. ex Steudel (hereafter referred to as phragmites) is a perennial grass that reproduces primarily by vegetative growth although dispersal by seed may occur at relatively low frequencies. It has a cosmopolitan distribution and is commonly found in marsh communities and along the edges of lakes, rivers, and ponds. The fossil record indicates that phragmites was present in the southwestern United States 40,000 years ago (Hansen 1978). Other paleoecological studies have shown the presence of phragmites on both the Atlantic and Pacific coasts for several thousand years (Niering et al. 1976, Goman and Wells 2000, Orson 1999). Botanical records from the 1800's describe the grass as being rare or uncommon (Torrey 1843, Willis 1874, Dame and Collins 1888); however, by the early 1900's it was considered to be quite common and spreading (Saltonstall 2002). Today phragmites exists in all of the mainland United States and southern Canada and it is considered an indicator of wetland disturbance.

Several hypotheses have been suggested to account for phragmites spread after the 1800's including site disturbance, pollution, changes in hydrologic regimes, and increased soil salinity (Marks et al. 1994). Alternatively, Saltonstall (2002) suggested that non-native genotypes may have been introduced into North America. She examined modern populations with historical ones from herbarium specimens and found differences in the DNA that aligned modern populations more closely to Eurasian phragmites than to native North American strains. Particularly disturbing was that native strains were no longer present in many of the sites documented by herbarium samples, but had been replaced with the highly competitive and aggressive Eurasian strain. Because the two strains appear morphologically similar in gross appearance, the presence of the introduced phragmites was in all likelihood overlooked while it was steadily replacing native populations.

Based on sequence data from 283 modern samples and 62 herbarium samples collected before 1910, Saltonstall (2002) identified 27 haplotypes of phragmites. Eleven haplotypes are unique to North America (haplotypes A-H, S, Z, and AA) and are considered to be native. Two of the haplotypes (haplotypes M and I) have a widespread distribution worldwide with haplotype M being the most common. Today, modern populations of phragmites in the United States show a pattern of expansion of haplotype M; it has extended its range into the Southeast where phragmites historically did not occur, and has recently become prevalent in the Midwest. Haplotype I, often referred to as the Gulf Coast phragmites, is found in South America and Asia

and extends from Florida to California in the southern United States. It is unknown how this haplotype arrived and spread into scattered colonies across the South.

The rapid spread of phragmites and the virtual absence of native herbivores in expanding populations have also contributed to the hypothesis that a more aggressive genotype has been introduced (Tewksbury et al. 2002). Literature and limited surveys documented only 26 herbivore species on phragmites in North America (Tewksbury et al. 2002). These included 16 recent introductions, five of unknown status, and only five native species. Of interest is that only two of the native species, a skipper (Lepidoptera: Hesperiidae) and a gall midge (Diptera: Cecidomyiidae) are monophagous on phragmites. The other three insect species have recently expanded their feeding range to include invasive phragmites, indicating the availability of a new host.

Presently, there is some disagreement as to the taxonomic assignment of the native and introduced phragmites. Saltonstall et al. (2004) accepted the name *P. australis* subsp *australis* (per Clayton (1968)) for haplotype M. They formally recognized the name *P. australis* subsp. *americanus* Saltonstall, Peterson and Soreng for the native species. The I haplotype was assigned the name *P. australis* subsp *berlandieri* (E. Fourn.) Saltonstall and Hauber (Saltonstall and Hauber 2007). Ward (2010) recognized only two species of phragmites as occurring in the United States; however, he did not include any native populations in his studies but rather compared morphological characters of phragmites populations found in Florida and Virginia. He concluded the Virginia plants conformed to the known features of introduced phragmites or haplotype M and were *P. australis* subsp *australis*. However, the numerous differences in the morphology between the two populations from Florida and Virginia justified a higher ranking (i.e. that of species) to the haplotype I. Ward accepted the name *P. karka* (Retz) Steud. The Florida plants had similar characteristics to *P. karka* found in Africa, Pakistan, India, Ceylon, Taiwan, Japan, and Australia (Ward 2010).

Due to the rapidly increasing populations of phragmites, biological control research is a worthwhile alternative to conventional means if effective agents can be discovered. Tewksbury et al. (2002) list an abundance of species that exist outside of North America that could potentially be developed as agents. This list includes 22 fungal pathogens, only one of which may be host-specific. Mazurkiewicz-Zapałowicz et al. (2006) found 22 fungi on phragmites in Poland. Of interest is that only one of the species (*Puccinia magnusiana*) listed in the two investigations overlapped. Except for *P. magnusiana*, the rest of the species found in Poland were saprophytes or secondary invaders so none of them would be acceptable as a biocontrol agent in the classical sense. Current quarantine restrictions negate the possibility of importing a microbial agent that is not host-specific. The one potential agent, *Deightoniella arundinacea* (Corda) S. J. Hughes, cited in the Tewksbury et al. (2002) study that may be host-specific also occurs in the United States and has been reported on phragmites in Minnesota, North Dakota, Nebraska, South Dakota, and Wisconsin.

According to the publication, “Fungi on Plants and Plant Products in the United States” (Farr et al. 1989), 31 species of fungi were reported on phragmites prior to 1989. Of these, six have phragmites listed as the only host, therefore *Pseudographis phragmitis* Dearn and House, *D. arundinacea*, *Hendersonia arundinacea* (Desmaz.) Sacc., *Hendersonia grantii* Dearn., *Phragmopeltis phragmitis* Dearn., and *Stagonospora phragmitis* (Oudem.) Leuchtmann would definitely make better biocontrol candidates than fungi with broad host ranges. Rusts are often

studied as biocontrol fungi; however, the two rusts that occur on phragmites in the United States are heteroecious and have alternate hosts. The alternate hosts for the rusts *Puccinia magnusiana* Körn. and *P. phragmitis* (Schumach.) Körn. are *Anemone canadensis* L. (Canada anemone) and several species in the family Polygonaceae, respectively (Arthur 1934). There are also two smuts that have been reported only from phragmites. *Neovassia iowensis* Hume and Hods. and *Ustilago grandis* Fries occur only on inflorescences (Fischer 1953) and could potentially reduce seed production, but since reproduction by rhizomes is much more common, neither would be effective as a biocontrol agent.

The use of indigenous pathogens for biological control is an alternative to a classical biocontrol approach and offers an advantage in that host specificity is not an issue. The indigenous pathogen is developed as a mycoherbicide and registered with the U.S. Environmental Protection Agency (EPA). Any restrictions on its use are written on the label.

The primary purpose of the present study was to survey native and invasive phragmites populations in New York for potential pathogen biological control agents. New York was chosen because access to native populations was provided by Bernd Blossey, Cornell University. Because the invasive phragmites is introduced, it may be more susceptible to pathogenic ingress from agents that are found on native phragmites. Although phragmites is cosmopolitan, occurring on every continent except Antarctica, the range of pathogens that attack the plant may be different depending on the geographic locations of different populations.

MATERIALS AND METHODS: Nineteen stands of phragmites located in and around Syracuse, New York, were surveyed for disease-causing organisms. Four of the nineteen stands were native phragmites. If disease symptoms were observed on plants in a population, a representative sample was collected, placed in a plastic bag, labeled, and kept cool in a refrigerated container. The plants were shipped to the biomanagement laboratory at the U.S. Army Engineer Research and Development Center (ERDC) in Vicksburg, MS for processing.

In the laboratory, each plant sample was carefully scanned for disease symptoms (i.e. leaf spots, chlorosis, streaking), indicating the presence of different pathogens. Small sections were cut from diseased areas, surface sterilized in 10% Chlorox for 1 minute, and placed between slits cut in Martin's agar (MA) plates (H_2O, 1 L; agar, 20 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; peptone, 0.5 g; dextrose, 10 g; yeast extract, 0.5 g; rose Bengal, 0.05 g; streptomycin sulfate, 0.03 g). The plates were incubated in the dark at room temperature (24 °C) and observed daily for fungal colony growth. Each fungal organism that grew from a diseased plant section was carefully picked with a microspatula and placed on a potato dextrose agar (PDA) (Difco, Detroit, MI) slant. An agar slant is simply a test tube placed at an angle during agar cooling to give a large slanted surface for inoculation. The slants from all 19 collections were sorted together into morphospecies based on colony color, texture, and growth pattern. Each morphospecies was given a collection number. The number of times each isolate appeared in each collection and over all the collections was enumerated. This provided a means to determine if there were any differences in the isolates collected from the native versus the invasive phragmites.

For identification purposes, the isolate from each slant was placed on PDA and potato carrot agar (PCA) (Dhingra and Sinclair 1995) petri plates. The plates were incubated at room temperature under a grow lamp set to a 12/12 light/dark photoperiod until fungal colonies developed and they

could be identified. Colonies that did not sporulate were classified as either dematiaceous (dark colored) or moniliaceous (hyaline) Ascomycetes. The colonies were also transferred from the PDA slants to one-half strength corn meal agar (CMA) (Difco, Detroit, MI) slants for longer term storage at 4° C.

RESULTS AND DISCUSSION: Swearingen and Saltonstall (2010) developed a field guide to distinguish native from exotic forms of phragmites in the United States. During the August 2010 surveys, these characteristics were used to great advantage in separating the introduced M haplotype from the native species. Native phragmites tended to occur in low-density stands intermixed with other native plant species. At the time of sampling in August 2010, the lower leaves had senesced and the leaf sheaths had detached from the culm (Figure 1). Very distinguishing characteristics were the smooth shiny culms that were red to chestnut colored (Figure 1). Black spots were often observed on the culms of the native phragmites (Figure 2). These spots were attributed to infection from a native fungus. However, the exact species could not be confirmed because Koch's postulates could not be completed due to lack of live plant material for testing. In contrast, the introduced phragmites often occurred in very dense stands that included both live stems and dead stems from the previous years' growth (Figure 3). The leaf sheaths adhered tightly to the culm (Figure 4). According to Swearingen and Saltonstall (2010), the sheaths persist on the culm as long as it remains standing. As had been reported by Swearingen and Saltonstall (2010), the black spots are absent from the culms of the introduced species.

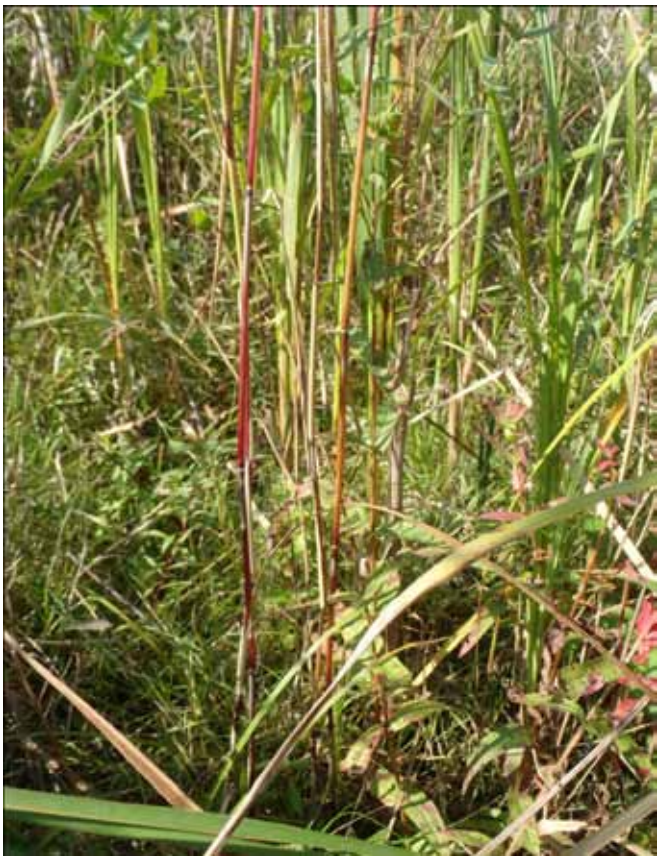

Figure 1. A stand of native phragmites near Syracuse, New York. Note the red culms and the absence of leaf sheaths on the lower stems.

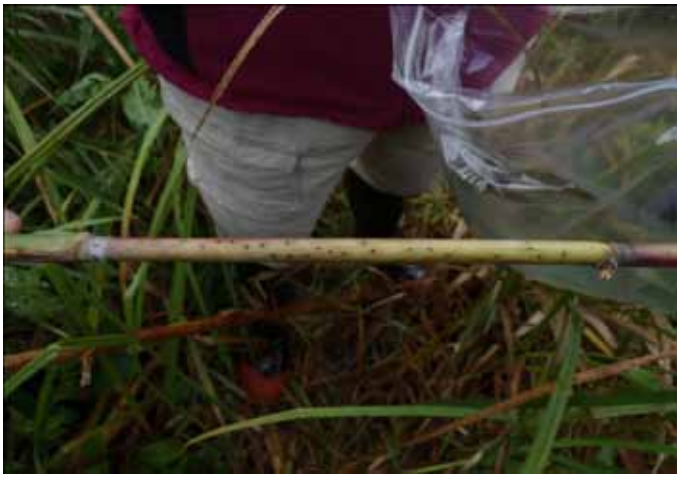

Figure 2. Black spots on culms of native phragmites.

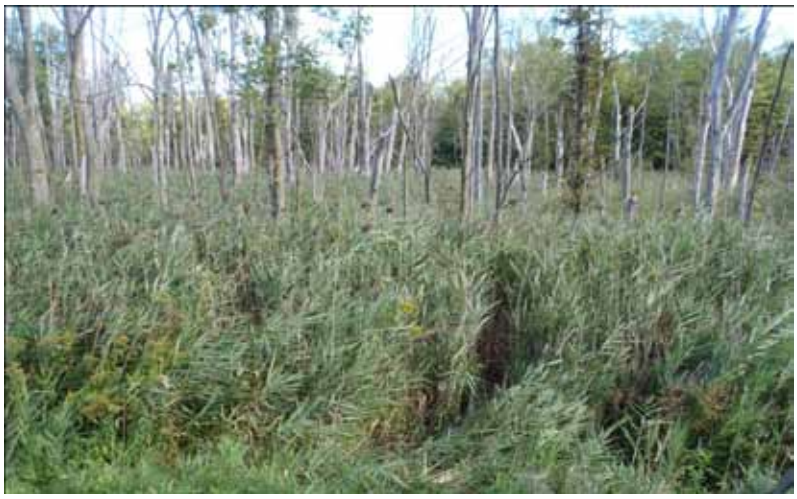

Figure 3. Introduced phragmites often occurs in dense stands.

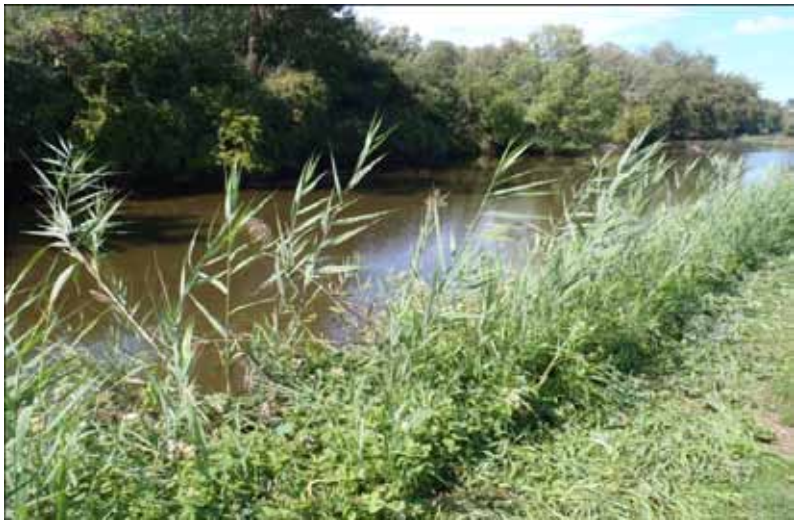

Figure 4. Leaf sheaths remain attached to the culm of introduced phragmites.

Although diseased tissues were found on both native and introduced phragmites leaves and culms, the populations were seemingly unaffected by their presence with the possible exception of the rust *P. magnusiana* on native phragmites at one site. The plants were stunted compared with other sampled populations, reaching only about 1.5 m. An abundance of leaf spots were observed on the introduced haplotype (Figure 5), but there was no evidence that they were causing much impact to the populations. At all sites, the plants appeared extremely robust, reaching heights at times as great as 4 m.

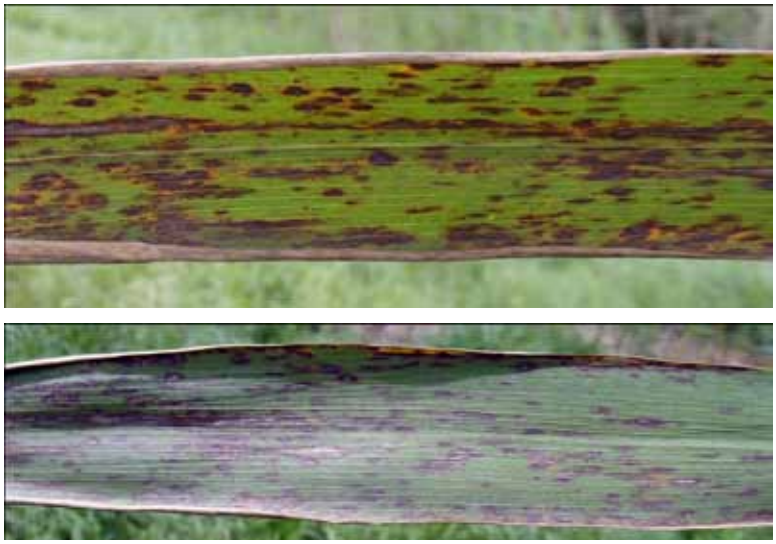

Figure 5. Leaf spots on introduced phragmites.

A total of 30 species of fungi were found on native and introduced phragmites, including the rust *P. magnusiana* (Table 1). Rusts are difficult to culture, therefore isolations were not attempted. Although two spore stages (urediniospores and teliospores) occur on phragmites, the urediniospores were not observed. The urediniospores spread the rust to other phragmites plants during the growing season because they can reinfect a host plant. They are gradually replaced by teliospores (black overwintering spores) in late summer and fall. The teliospores covered approximately 75% of the leaf surface on most plants. As temperatures become favorable in spring, they produce basidiospores that can infect the alternate host, Canada anemone. Since the rust is heteroecious, it would not be considered an acceptable biocontrol agent. The rust has also been reported from Europe, where the alternate hosts are species in the genus *Ranunculus*.

Only two species in Table 1, *Deightoniella arundinacea* and *Stagonospora simplicior*, have been previously documented as occurring on phragmites in the United States (Farr et al. 1989). Both in the United States and Europe, *D. arundinacea* appears to occur only on phragmites. The fungus may be a potential biocontrol candidate if it causes significant damage to the plant and can be formulated into a bioherbicide. *Stagonospora simplicior* has a broad host range, including many native prairie grasses, making it a poor candidate as a biological control agent.

The most commonly isolated fungal species were *Arthrinium phaeospermum, Fusarium nivale, F. stilboides, Hansfordia ovalispora, Alternaria alternata*, a *Phoma* sp., *Pithomyces chartarum*, a moniliaceous Ascomycete 1, and a dematiaceous Ascomycete 1 (Table 1). *Arthrinium phaeospermum* and *A. alternata* are very common cosmopolitan species (Ellis 1971). Often found

Table 1. Fungal species isolated from 19 phragmites sites in New York, August 2010. Fungal species found on native phragmites (N) introduced (I); or both (B).

Species	No. of sites	No. of isolates	Native or introduced
Arthrinium phaeospermum (Corda) M. B. Ellis	15	37	B
Fusarium nivale (Fr.) Ces.	12	40	I
Fusarium stilboides Wollenw.	12	39	B
Hansfordia ovalispora Hughes	9	20	B
Alternaria alternata (Fr.:Fr.) Keisssl.	7	23	B
Moniliaceous Ascomycete 1	7	12	I
Phoma sp. 1	7	32	B
Pithomyces chartarum (Berk. & M. S. Curtis) M. B. Ellis	6	6	I
Dematiaceous Ascomycete 1	3	10	I
Colletotrichum gloeosporioides (Penz.)Penz. & Sacc.	3	3	I
Stagonospora simplicior Sacc. & Briard	2	2	N
Fusarium culmorum (W. G. Smith) Sacc.	2	2	I
Chaetomium globosum Kunze:Fr.	2	2	I
Phoma sp. 2	2	3	I
Deightoniella arundinacea (Corda) S. J. Hughes	1	1	N
Stagonospora cylindrica Cunnel	1	1	I
Sphaerosporium sp.	1	1	I
Sclerostagonospora sp.	1	1	I
Fusarium oxysporum Schlechtend.:Fr.	1	1	I
Cladosporium oxysporum Berk. & M. A. Curtis	1	1	I
Moniliaceous Ascomycete 2	1	2	N
Pestalotiopsis guepinii (Desm.) Stey.	1	1	I
Dematiaceous Coelomycete	1	2	I
Mucor sp	1	1	I
Fusarium solani (Mart.) Sacc.	1	1	N
Septoria sp.	1	4	I
Penicillium raistrickii G. Sm.	1	1	I
Dematiaceous Ascomycete 2	1	1	I
Stagonospora vitensis Unam	1	1	I

[1] N = native, I = introduced, B = both.

on the leaves and culm bases of many species of grasses, neither species is known to cause significant damage and most likely are present as secondary invaders. Although *A. phaeospermum* was not cited by Tewksbury et al. (2002) in their list of potential fungal biocontrol agents, it was reported from Europe by Wirsel et al. (2001) as an endophyte in phragmites root tissues, particularly from plants collected in flooded sites. The fungus was also reported by Mazurkiewicz-Zapałowicz et al. (2006) on phragmites in Poland. Roots were not examined in the present study because many root pathogens have a broad host range and often do not survive in culture on artificial media.

All the *Fusarium* species listed in Table 1 occur on a wide variety of plants. Both *F. nivale* and *F. culmorum* occur on a number of grass species worldwide (Booth 1971). Many of the cereal grasses (wheat, rye, barley, and oats) are particularly susceptible to disease caused by *F. culmorum,* making it an unsuitable candidate for biocontrol of phragmites. The same would be true for *P. chartarum* because it frequently parasitizes fodder grasses. Both *F. oxysporum* and *F. solani* have very broad host ranges, occurring on a variety of plants including grasses.

Although *Stagonospora simplicior* was found only on native phragmites in the present study, it occurs on 13 other grass genera and one sedge genus (Farr et al. 1989); therefore, it is likely to migrate to the introduced phragmites. *Stagonospora cylindrica* was originally described from specimens collected in the United Kingdom (Cunnell 1957). Due to the woody nature of the introduced phragmites and the fact that it persists into subsequent growing seasons, it would provide a suitable substrate for the fungus for an extended period. The host of *Stagonospora vitensis* is in the sedge genus *Carex* (Sutton 1980). Since phragmites and sedges occupy similar habitats, inoculum would be available to infect a new host if it was susceptible to invasion.

Although *Colletotrichum gloeosporioides* was not reported on phragmites by Farr et al. (1989), it has been reported on 470 host genera (Sutton 1980) worldwide. Typically it causes a disease called anthracnose that appears as black, sunken, leaf or stem lesions (Agrios 2005). Symptoms may be mild to severe. In the present study, it was only isolated once from three different sites and there was no evidence that it was severely impacting introduced phragmites.

Five fungal species occurred on both native and introduced phragmites (Table 1). The five species all have wide distributions and broad host spectrums. Four species were found only on the native phragmites. The native populations were very small compared to the introduced species and were somewhat isolated. There is no reason that the four species could not transition to introduced phragmites. Twenty species occurred only on the introduced haplotype. This was most likely an artifact of increased sampling of introduced populations. It is unknown how the introduced phragmites arrived in the United States, but there is some speculation that ballast water containing seed was the source of the introduction. If this is the case, then it would be less likely that the introduced haplotype would have a large assemblage of fungi associated with it at the time of its introduction. However, since it has been in the United States for at least 100 years, fungi could easily establish on it from many sources that have broad host ranges.

Neither of the unidentified moniliaceous and dematiaceous Ascomycetes produced spores on artificial media. Spores, not hyphae, are the preferred inoculum onto a target species. They can be produced in large numbers both on artificial and broth culture media. The fact that the three isolates did not produce spores would reduce their potential for biocontrol agents.

An extremely important consideration for biological control of introduced phragmites would be to thoroughly test any agents on native phragmites. If any agent were to significantly impact the native species, it would be an unacceptable biocontrol agent. As mentioned above, native populations, at least in New York, were few and very limited in size. They are being threatened by encroachment by the introduced species, and any additional pressures could seriously affect their survival.

FUTURE WORK: Additional collections from native phragmites stands from different parts of the country would add new potential biocontrol pathogens for efficacy testing on introduced phragmites.

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