Viability of late Holocene wetland seeds buried beneath legacy sediment from Big Spring Run, Lancaster, PA

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Abstract:

A forefront issue in the realm of environmental protection is restoring the health of our watersheds. Water bodies such as the Chesapeake Bay, for example, have become severely polluted due to high nutrient and sediment loads (Mahaney et al., 2004, Walter et al., 2008). The sources of these nutrients and sediments are thought to be agriculture, sewage treatment facilities, and urban/suburban runoff, among other contributors. Another factor, however, is the loss of wetland function throughout a watershed as a result of wetland drainage and burial. At the study site of Big Spring Run, a headwater tributary to the Susquehanna River in the Chesapeake Bay watershed, a buried wetland containing abundant seeds was identified several years ago. This wetland was buried by historic sediment ("legacy sediment") from upland soil erosion. In an effort to discover whether the seeds found in this wetland are viable, we collected seeds from the youngest (uppermost) layer of the buried wetland. The purpose of this research is to determine whether these seeds could potentially aid in wetland plant reintroduction subsequent to the removal of legacy sediment, as well as to further identify some unidentified seeds. After collecting soil samples, we planted both raw soil samples containing seeds as well as identified seeds and attempted to germinate them under favorable greenhouse conditions. None of the seeds, which were characteristic of a wet meadow wetland, germinated. We conclude that the extent of seed degradation over the period of ~300 years of burial was too great to rely on these extant seed banks as a potential source of wetland plant reintroduction.

Background

For the past few hundred years, humans have greatly altered the biogeomorphology and hydrology of rivers and wetlands in the eastern United States by land use changes and the construction of milldams (Mahaney et al., 2004, Walter et al., 2008). These changes have resulted in the burial, and thus the disappearance of wetlands in many areas, including the study site for this investigation, Big Spring Run (BSR) in southern Lancaster County, PA. Wetlands are a vital aspect of the biosphere as they filter excess nutrients from water, mitigate flooding, and provide habitat for many plant and animal communities unique to these environments.

The BSR tributary flows northward, joining Mill Creek about 1.3 miles downstream from the area that is to undergo restoration (Merritts et al., 2005). The area was formerly a wet meadow wetland but sediment trapping from upland erosion from agriculture and a mill dam downstream from BSR created a slack water environment that trapped sediment. Since the breach of this dam in the early 20th century, a knickpoint propagated upstream, eroding both the legacy sediment and the organic-rich soils of the buried wetland (Walter et al., 2008). The resulting stream channel has been migrating across the valley floor for the past century. Due to this deep incision, the buried wetland soils have been exposed along the base of the stream banks. This has allowed past Franklin & Marshall researchers to remove and examine soil samples of this wetland. Analysis of these soils has revealed that they are abundant in seeds of species that are typically found in wet meadow wetlands (U.S. EPA, 2010). Reintroduction of plants via seeds is less expensive than to collect and cultivate seedlings. Therefore, if the seeds beneath the historical sediment were indeed viable, the costs would be much lower to reintroduce wet meadow wetland-native plants.

The three main objectives of research in this area are to identify the seeds by species, determine whether they can germinate, and decide which plants would be best suited for reintroduction. While there are obvious benefits to identifying plant species which were present in historic wetlands, it can be difficult to positively identify their seeds when they have become degraded over time. Past research on paleo-wetland seeds by other F&M students (Erik Ohlson, Katherine Datin, Mark Voli, and Chris Scheid) has allowed the identification of most but not all of the seeds collected. Even experts in Holocene wetland seeds such as Dr. William Hilgartner, a collaborator at The Johns Hopkins University, are unable to identify some of the many different species of sedge (Carex) seeds that we have extracted. In order to identify the species of seeds which have been unresolved by visual inspection, there have been attempts to grow the seeds. If successful in germinating and growing these seeds, the mature plants would be much more readily identified.

Growing seeds collected from buried wetlands poses several challenges due to the conditions which the seeds were subjected to over the period of time during their burial. The first settlers began transforming the landscape around the late 17th century, providing a rough estimate of how long ago these wetlands were buried by sedimentation. Radiocarbon dating of organic material found among the soil samples containing the seeds we collected has provided an estimate of the age of the uppermost layer of the wetland, as approximately three-hundred years. In addition, this wetland began to form some 4,000 years ago, and in some analogous areas, wetlands can be found to have begun forming some 10,000 years ago. Due to the compaction of the wetland under the legacy overburden, even a narrow horizontal layer of wetland sediment might encompass hundreds of years. To improve the likelihood of identification and viability of the seeds, it is therefore prudent to focus on the uppermost layer of the wetland.

The age of these seeds poses a challenge in attempting to grow them, as many of the biomolecules contained inside the seeds may have been destroyed as a result of hydrolytic and oxidative damage (O'Donoghue et al., 1996, Schlumbaum et al., 2008). Schlumbaum et al., (2008) have shown that optimal conditions for DNA preservation consist of a cool, dry and/or anoxic environment. While it is commonly believed that DNA older than one million years old would be completely degraded, it is less well known what degree of degradation occurs over hundreds of years under diurnally and seasonally variable temperatures as well as varying hydrologic regimes. In addition to DNA degradation, contamination can occur when DNA molecules from adjacent sources become mobilized with water or by bioturbation (Willerslev et al., 2005).

In attempts to grow buried wetland seeds, there are not only constraints due to conditions after burial, but also problems associated with conditions after sample collection. In addition to variables associates with burial conditions and storage conditions, there are also limitations on the growth conditions that are favorable for the seeds to germinate. All of these factors were investigated in order to develop an approach which would have the greatest likelihood of encouraging germination of approximately 300-year old seeds.

A preliminary pilot study was conducted during the Summer of 2009 in order to determine the viability of the buried wetland seeds. Franklin & Marshall students Erik

Ohlson and Katherine Datin stained seeds with a 1% tetrazolium solution and also attempted to germinate them. The stained seeds were not found to be viable. Tetrazolium staining also was done on modern seeds purchased from a supplier for comparison. For the germination tests, the seeds were placed on moist filter paper under petri dishes and exposed to 12 hours of grow lights daily. Another germination trial consisted of spreading bulk aggregate pre-settlement samples over sand in a shallow tray and exposing the seeds to 12 hours of grow lights at a room temperature of 22˚C. None of these seeds germinated under either of these environmental conditions.

In this second effort to grow paleo-wetland seeds, we utilized more sophisticated methods to induce germination. By subjecting the seeds to the most favorable environmental conditions in a greenhouse, we anticipated a greater likelihood of germination. Success in germinating these seeds would help to further identify some of the seeds and provide a framework for the environmental conditions which are necessary to germinate these wetland plant species. This knowledge will contribute to our understanding and ability to properly restore wetlands in this region. As a riparian restoration project is planned to begin in August, 2010, at the study site of BSR, it would be beneficial to know whether the buried paleo-wetland seeds are capable of germinating after removal of the historic sediment.

Methods

Geomorphic Setting of Study Site

Soil samples were collected along stream banks near the location of a trench which was excavated in 2004 to evaluate the soil composition. Both the stream banks and the trench reveal that at a depth of roughly 0.9 m there is a boundary between historic sediment and a buried wetland. The youngest soils of this buried wetland are estimated to be 300 years old, while the oldest soils are approximately 4,000 years old (Merritts et al., 2005). The uppermost boundary of this floodplain is dark brown and consists of organic rich silts, clays, and some sand. The sediment deposited on the floodplain forms a sub planar surface and is referred to as the valley flat henceforth.

Figure 1. Satellite image of BSR sampling sites. Samples collected on 2/5/10 were along a stream bank across the transect of the valley. Samples collected on 9/25/09 were along a stream bank parallel to the valley.

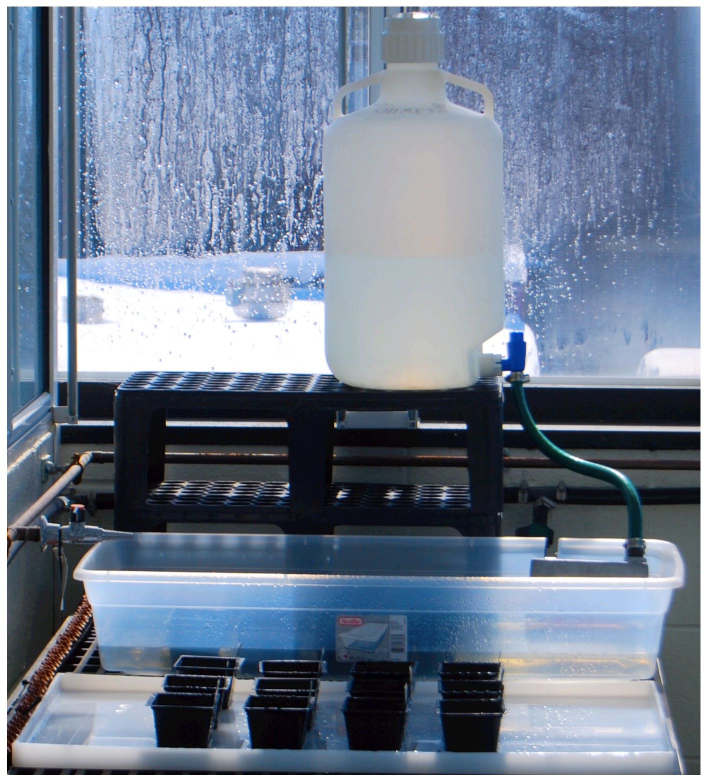

Figure 2. Plant Growth Facility bench set-up. The tray in the foreground was used to simulate facultative wetland conditions while the container in the background simulated obligate wetland conditions . The water level was maintained with a float valve pump attached to a carboy.

We planted three separate groups of seeds, each under two different treatments. The groups consisted of: the top layer of wetland raw sediment with seeds, a set of individual seeds which had been extracted from the top layer of wetland sediments, and a third set of modern seeds purchased from a supplier.

The Plant Growth Facility module was regulated at conditions reflecting that of springtime. Daytime and nighttime temperatures were maintained between 75-80˚C and 65-70˚C, respectively. Daytime and nighttime humidity were maintained at 50-70%. The setups received full sunlight and were not treated with artificial lighting. The two treatments consisted of one group of submerged seeds, maintained under a constant depth of ~2 cm, while the other group of seeds was kept moist by daily watering (Figure 2). For the submerged treatment, the pots were kept in a container in which the water level was maintained by a Trough-O'-Matic auto float valve. For each of the treatments, the bottoms of the pots were covered with a layer of sterilized sand. Sterilized vermiculite was used as a germination medium. One control pot with no seeds was placed under each treatment to address the potential of seed contamination in the greenhouse. The pots were monitored for seedling emergence during the growing period. The approach described here was adopted from Laura Burbage, Environmental Scientist for Camp, Dresser, & McKee Inc.

Seeds Collected from the Field and Kept in Sediment

Soil samples were collected from a stream bank at BSR on February 5, 2010. We chose three sampling sites along a stream bank that was oriented perpendicularly to the down-valley direction (Figure 1). The soil samples collected at this site provided a cross-sectional view of the composition of seeds in the buried wetland. The uppermost layer of the buried wetland sediment was sampled just beneath historic sediment. Some mixing of the wetland layer and the legacy sediment occurred along this contact, which may be attributed to bioturbation from worms and other burrowing organisms. The soil samples were organic-rich, with occasional gravel. Two 2-cm thicknesses of horizontal layers were separated from a depth of 109-120 cm from the valley flat to the top of pre-settlement soil. The samples were frozen upon returning to the laboratory.

Each field sample—which represented a 2-cm layer of wetland soil--was split into two sub-samples: one sub-sample to be submerged and another to be kept under moist conditions. Each of the six sub-samples of soil was homogenized in a large bowl by mixing the soil thoroughly with approximately 100 mL of water. For the planting of these samples, a thicker layer of sand was placed on the bottom of the pots to prevent mud from passing through the bottom. The muddy samples were poured over a thin layer of vermiculite and covered with additional vermiculite. These sampling locations were randomized in each of the two treatments by using a randomization generator in Excel.

Seeds Extracted from Sediment

Our first set of seeds was collected as 4-cm thick whole soil samples at a site where the stream flows parallel to the valley. The soil samples were frozen upon returning to the laboratory. After defrosting the horizontal layer, 4-cm layers were homogenized with 40 mL of water. The seeds then were extracted from the soil using a dissection microscope and subsequently photographed and identified to the level of genus, and further to species if possible. These seeds, which were collected September 25, 2009, were not planted until February 2nd 2010. These seeds included *Carex comosa* (bristly sedge), *Carex stricta* (common tussock sedge), *Carex stipata (awlfruit sedge),* *Carex volpinoidea* (fox sedge), *Naja flexilis* (waternymph) *Polygonum punctatum* *(*smartweed*), Tulipa spp. (*tulip*), Rubus spp. (berry), Eleocharis* spp. (blunt spike rush), *Scirpus cyperinus* (woolgrass), *Scirpus spp.(spikerush), Cyperus spp.* (flat sedge), *Liriodendrons spp.* (tuliptree), and several unidentifiable seeds (Table 1). Seeds of identical species or genus were planted together, whereas unknown species of seeds were planted separately. Seeds were planted according to the aforementioned potting design and subjected to the two treatments.

Wetland Plant Species (or genera)	Common Names	Growth Environment	Cold stratification required
Carex comosa	bristly sedge	OBL	NO
Carex stricta	tussock sedge	OBL	NO
Carex stipata	awlfruit sedge	OBL	NO
Carex vulpinoidea	fox sedge	OBL	NO
Eleocharis spp.	spikerush	Data NA	Data NA
Scirpus cyperinus	woolgrass	FACW, OBL	NO
Najas flexilis	nodding water nymph	OBL	Yes
Tulipa spp.	tulip	Data NA	Data NA
Rubus spp.	berry	Data NA	Data NA
Cyperus spp.	flat sedge	Data NA	Data NA
Liriodendron spp.	tuliptree	Data NA	Data NA
Polygonum puncatatum(modern)	smartweed	FACW, OBL	NO
Eleocharis obtusa (modern)	blunt spike rush	OBL	NO

Table 1. Wetland plant species or genera names (if species identification was not possible) of buried wetland seeds and modern seeds (specified in first column). Common names are also provided, however for seeds identified by genus, the common name is less specific. The natural habitat of each species or genera is denoted by OBL (obligate wetland) and/or FACW (facultative wetland). The requirement of cold stratification for germination is also shown. Data NA (not available) applies to cases where the lack of species identification limited this portion of data.

Modern Seeds from Seed Supplier

In order to determine a baseline timeframe for germination of some of the wetland plant species, we ordered modern seeds of *Carex comosa* (Bristy sedge), *Eleocharis obtusa* (Blunt Spike Rush), *Polygonum punctatum* (Smartweed), and *Carex stricta* (Common Tussock Sedge) from the Prairie Moon nursery. Approximately twenty seeds from each species were planted on February 15, 2010, according to the aforementioned potting design under the two treatment conditions.

Results & Discussion

Of the collected seeds we planted, both those kept in raw soil samples as well as those that were extracted from the buried wetland soil samples and identified, no seedlings emerged. This included seeds of *Carex comosa, Carex stipata, Carex stricta, Carex vulpinoidea, Cyperus spp., Eleocharis spp., Liriodendrons spp., Najas flexilis, Polygonum punctatum, Rubus spp., Scirpus cyperinus, Scirpus spp.* in addition to many unidentifiable seeds. Of the modern seeds purchased from a supplier, germination occurred in *Carex comosa*, *Carex stricta*, and *Polygonum punctatum* in both the facultative and obligate wetland conditions. The only modern seedlings that did not emerge were those of *Eleocharis obtusa.*

Several factors may have negatively affected the viability of the approximately 300-yr old buried wetland seeds we collected. These include conditions in which the seeds were subjected to in situ for several centuries, storage conditions in the laboratory for up to several months, and the growth conditions in the facility.

Past research demonstrates that several carices sustain seed viability longest when the seeds are stored under wet and cold conditions (Budelsky et al., 1999) However, even under these conditions Budelsky et al. found that after 2.5 years of storage, only 46% of the planted seeds germinated. We speculate that if the potential for germination continues to decrease after years of storage even under optimal conditions, then after a period of three-hundred years it is unlikely any seeds would remain viable. Also, since the seeds that we collected were among the youngest relative to seeds located in deeper layers of the buried wetland, we assume that the older and deeper the seeds are, the more highly degraded they will have become.

One reason we considered the possibility that there might be viable seeds is the sheer abundance of seeds as well as the fact that the burial conditions of the seeds may have been favorable for preservation. Compaction of the buried wetland due to the overburden of several feet thick legacy sediment as well as the submersion of the wetland beneath water, are the likely reasons why it was anoxic. Although seeds tend to remain viable longer in anoxic conditions, like those of the compacted buried wetland from which they were collected, seasonal changes in temperature and humidity could have had a negative impact on long-term viability (Schlumbaum et al., 2008). Bioturbation by ground-dwelling organisms such as worms and moles may have allowed oxygen to reach some areas of the buried wetland soil, perhaps negatively impacting preservation of the seed banks (Jensen et al., 2004).

Another factor which is known to influence the viability of seeds is the conditions under which they are stored after they have been collected from the field. The best conditions for long-term storage of several species in the Cyperaceae (sedge) family seem to be wet or moist and cold conditions (van der Valk et al., 1999, Budelsky et al., 1999). Our storage conditions therefore should have been appropriate and should not have been a factor that negatively affected the viability of the seeds that we collected.

The possibility of germination of the buried wetland seeds might be highly dependent upon the growth conditions we employed. Although it has been established by Gillespie (1989) that *Carex stricta* will germinate under greenhouse conditions as long as the temperature is above 10˚C, many other aspects of the conditions that must be addressed include media, hydrologic regime, and light (van der Valk et al., 1999). Jensen et al. (2004) note that for many small-seeded fen wetland species, greatest germination rates occur in light. One research study shows that maximum germination rates (78%) occur in *Carex comosa* when the seeds are kept under conditions with light and diurnal temperature fluctuations of 25/20˚C (day/night) after a period of 10 months of wet-cold storage (Budelsky et al., 1999). For *Carex stricta,* highest germination rates (46%) also occurred under conditions with light and diurnal temperature fluctuations, although for this species highest rates were achieved at temperatures of 20/15˚C and in fresh seeds that were never stored. Another study found that maximum germination of *Carex comosa* and *Carex stricta* occurred under conditions with light and diurnal temperature fluctuations of 35/20˚C, with rates of 39% and 61%, respectively (Baskin et al., 1996). Both studies found that the germination rates of Carex stricta are not affected very much by soil moistures. Our growth conditions had diurnally fluctuating temperatures of 23/20˚C, so we believe that these conditions should have been favorable for germination to occur. This is further substantiated by the fact that three of the four species of the modern seeds did germinate under identical growth conditions.

For the modern seeds, it is possible that *Eleocharis obtusa* would have germinated if the seeds had been subjected to the sixty day cold stratification that helps break seed dormancy (Prairie Moon Nursery). It was not possible to do this cold stratification due to time constraints on the project. It is apparent that for plant reintroduction to be successful the seeds must be sown during either the late summer or fall, or treated to cold stratification in a laboratory. It has been noted by Jones et al. (2004) that efforts must be made to prevent the risk of the sown seeds being washed away by spring floods or becoming buried too deeply to germinate after flood events. All of these issues must be taken into account to achieve a successful restoration project.

Conclusion

Although the project was unsuccessful in germinating the seeds that were collected from the buried wetland at BSR, we still gained valuable information that will be useful towards restoration efforts. An important conclusion is that over the period of time subsequent to the burial of these wetland seed banks, the seed coats likely began to degrade with time, allowing the tissues to decompose and DNA hydrolysis to occur. This would explain why the seeds did not germinate under favorable storage and germination conditions. We conclude that since none of the seeds were capable of germinating under favorable greenhouse conditions, it is very unlikely that there would be any germination of seeds in situ, or that the germination rates would be so low that it would not be a sufficient method of plant reintroduction.

This research has better informed us on the best approach to wetland restoration and may be valuable to other researchers or organizations with an interest in restoring a wetland by allowing extant seed banks to germinate. Since many analogous wetlands were buried at the time that settlers began construction of milldams and farming, we can presume that the seed banks at these locations have most likely become degraded due to hundreds of years of seasonal and diurnal temperature changes. Perhaps in areas where wetlands were buried during the last few decades it would be feasible to rely on extant seed banks for reintroduction of wetland plant species.

The “seed banks” which have been discovered by Dr. Dorothy Merritts, Dr. Robert Walter and Mark Voli in the buried wetland at BSR may be more appropriately referred to as a “seed library”. Though the seed bank reserves no longer serve the purpose of long-term storage of the genetic material, they do serve the function of providing an extensive history of the composition of different plant species which would have been found in these wetlands. Determining which plants would have existed in these buried wetlands is not only useful for determining which type of wetland may have existed, but also exactly which species would be best for reintroduction during restoration efforts.

One of the implications of this research is that more money will be necessary for plant reintroduction during a restoration. Planting of seeds may be the most cost effective method of reintroduction. As a result, seeds will need to be either cold-stratified in a laboratory setting or planted sometime during fall the year prior to restoration to allow stratification to occur during the winter.

In the future, as additional buried wetlands are discovered, scientists will have a better idea of whether or not extant seed banks could be relied upon based on the period of time during which the wetland had been buried. The overarching objective of research in the area of wetland restoration is to locate where wetlands previously thrived in an attempt to restore them to their full ecological potential. It is prudent that we restore as many wetlands as possible and try to connect wetland systems throughout the Chesapeake Watershed in order to restore their functions of mitigating floods, providing habitat to plant and animal communities, and providing cleaner water which will benefit the Chesapeake Bay ecosystems as well as the ecosystems of the entire watershed.

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