ERDC/TN SAV-09-1
August 2009

Production and Field Planting of Vegetative Propagules for Restoration of Redhead Grass and Sago Pondweed in Chesapeake Bay

by Laura Murray, W. Michael Kemp, Deborah Hinkle, and Deborah Shafer

BACKGROUND: During the last several decades, seagrasses and related submerged aquatic vegetation (SAV) have been lost from shallow waters of Chesapeake Bay (Orth and Moore 1983) and other coastal ecosystems worldwide (Short and Wyllie-Echeverria 1996). Losses of SAV beds are of particular concern because these plants tend to create rich habitat and food for animals, supporting growth of diverse fish, invertebrate and waterfowl populations (e.g., Kemp et al. 1984; Orth and van Montfrans 1990; Heck et al. 1995). In the mesohaline portion of Chesapeake Bay, historical SAV abundance, which had been decimated by the late 1970s, has gradually increased from the mid-1980s to present levels. Compared to historical SAV communities in this region, however, the number of recovering plant species has remained depressed, with one species, *Ruppia maritima* (widgeon grass), predominating throughout (Orth et al. 1997). This "pioneer" SAV species is an annual plant with prolific production of viable seeds and high growth potential (Silberhorn et al. 1996; Stevenson et al. 1993; Kautsky 1988). More stable species, like *Potamogeton perfoliatus* (redhead grass) and *Stuckenia pectinata* (sago pondweed), that had previously represented a large component of the SAV community in mesohaline areas of Chesapeake Bay (Stevenson and Confer 1978), are presently scarce in this region (Moore et al. 2000; Orth et al. 1997).

Most SAV species reproduce both sexually (flower, pollen, fruit, and seed) and asexually (clonally) for population expansion and long-term survival (Philbrick and Les 1996). Their reproductive cycle involves several distinct stages (Hutchinson 1975): (1) formation of propagules, including seeds, winter buds, tubers, and foliar fragments, (2) propagule horizontal dispersal and vertical placement on/in sediments, (3) propagule dormancy and exposure to dormancy-breaking conditions (e.g., scarification, anoxia, and low temperature), (4) germination/sprouting, (5) plant growth and development, and (6) formation of new viable propagules. The potential for success in SAV large-scale restoration efforts can be greatly enhanced by applying knowledge of natural reproductive cycles to develop effective protocols for field application.

Although both *S. pectinata* and *P. perfoliatus* produce abundant seeds (e.g., Yeo 1966; Ailstock and Shafer 2004), seed viability and germination success tend to be relatively low under most estuarine conditions (Yeo 1965; Stevenson and Staver 1989). These species may also reproduce vegetatively by fragmentation (e.g., Rybicki et al. 2001), but in many areas there are few local source populations to generate plant fragment propagules. The production and distribution of over-wintering, below-ground propagules may represent an alternative approach for successful restoration of these species in mesohaline reaches of estuaries like Chesapeake Bay.

PURPOSE: This technical note describes techniques for restoring *P. perfoliatus* and *S. pectinata* in the mesohaline region of Chesapeake Bay through the use of over-wintering subterranean propagules (buds and tubers). Results of four related experiments are described including: 1) the natural production of propagules and their viability, 2) the effects of salinity and cold storage duration on propagule viability and production, 3) the effectiveness of artificially induced propagule production, and 4) the success of alternative propagule planting methods.

METHODS

Natural propagule production and viability. In situ rates of plant and propagule production and condition were assessed in estuarine ponds every two months over an annual cycle (April 2006 through February 2007). Triplicate samples of *P. perfoliatus* buds and *S. pectinata* tubers (Figure 1) were collected from plastic trays (25 cm x 34 cm) containing pond sediments (10-cm depth). Trays had been deployed in experimental estuarine ponds (salinity range 9-12) at Horn Point Laboratory (HPL) for 12 months prior to initial sampling. These HPL ponds were established in 1978 for SAV research, and since then they have maintained a healthy population of SAV. Pond sediments, which were used in all experiments reported here, consisted of sand (43 percent), clay (14 percent), and silt (43 percent) mix with a 4.0-percent organic content. At each sampling, above-ground plant material growing in each collected tray was clipped at the sediment surface, rinsed, dried, and weighed. Sediments in each tray were rinsed and sieved to collect the below-ground roots, rhizomes, and tubers. Above- and below-ground biomass was dried (60°C) to a constant weight and reported as grams dry weight (dw) per square meter (g dw/m^{-2}). A random sampling of the tubers (n = 30) were dried and weighed. The average tuber weight was applied to the total number of tubers collected in each replicate sample and added back to the total below-ground biomass.

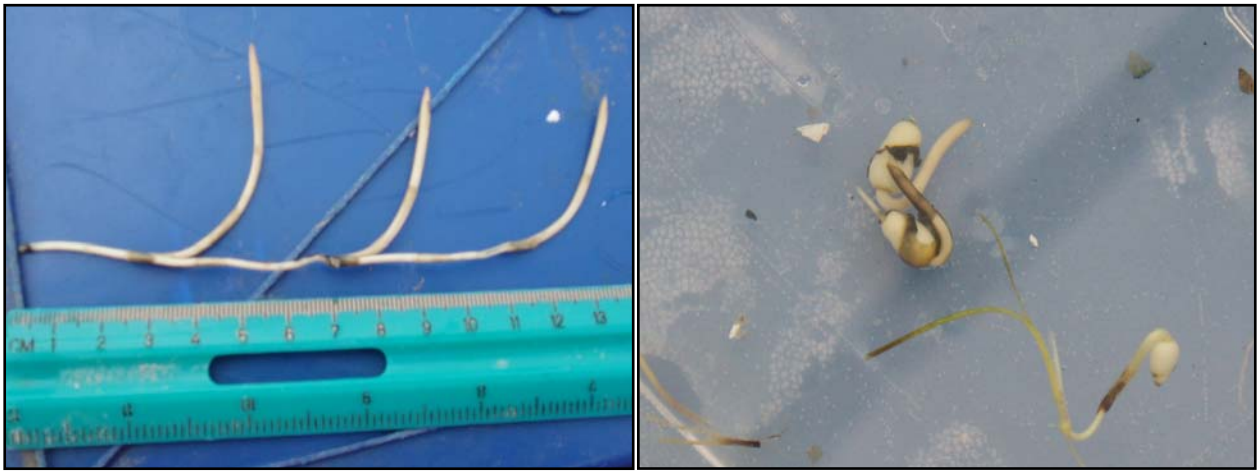

Figure 1. *P. perfoliatus* buds (left) and *S. pectinata* tubers (right).

Tubers and buds of both species were separated from the roots and rhizomes, counted and placed into reclosable plastic bags containing HPL pond water. To address the question of condition and growth potential, propagules were either planted immediately (within one day of collection), or placed into cold storage (4°C) for 6 weeks prior to planting. *Viability* was assessed as the percent of planted propagules producing vertical shoots emerging from sediments in one week. At longer

time scales, plant *growth* was assessed as the number of shoots produced in six weeks per propagule planted.

For both species in the first three sampling periods (April, June, and August) three replicate trays (25 cm x 34 cm) of five propagules each for each species were planted immediately (within one day of collection) into experimental sediments. The same procedure was followed for tubers which had been stored at 4°C (refrigerator) for 6 weeks (cold treatment). The number of planted tubers was expanded in the colder months (October, December, February) to 10 tubers per tray due to the higher numbers of tubers collected. Planted propagules were placed into greenhouse tanks with a mixture of ambient estuarine water from the Choptank River (a tributary of Chesapeake Bay) and freshwater (tap) needed to maintain a salinity of 7-9. Temperature (range 20-27°C) was maintained by placing aquarium heaters in the mesocosms during the colder months. A 12-hr day-night photoperiod was maintained during winter months using artificial lighting. Tank walls and in-tank circulating water pump filters were cleaned weekly.

To help associate shoots emerging from sediments with specific propagules planted in those sediments, individual over-wintering buds (*P. perfoliatus*) and tubers (*S. pectinata*) were planted in a uniform grid pattern in experimental trays. Daily observations revealed that, for all experimental planting units, no more than one shoot appeared at any grid point after 1 week. Subsequent experiments compared shoot emergence from propagules planted in parallel sets of containers with either sediments and overlying water or water only. These studies demonstrated that, after 1 week, patterns of shoot emergence were identical for tubers/buds in containers with sediments-plus-water and with water-only, and that no branching (more than one shoot growing from a single propagule) had occurred.

Together, these observations support the idea that shoot emergence from sediments after 1 week provided a dependable index of tuber/bud viability. Thus, propagule *viability* was calculated as the percentage of planted propagules with shoots emerging from sediments at one week after planting. Subsequent plant *growth* from these propagules was monitored weekly for 6 weeks by counting the total number of shoots in each replicate tray. Values for growth are reported as the average number of shoots per propagule planted at the end of the 6-week experimental period.

Effects of salinity and cold storage duration. Effects of a 6-week cold treatment on viability of propagules collected from estuarine populations of these plants (see previous section) were assessed and separate experiments were conducted to test the effects of *cold storage duration* and *salinity* on propagule viability and growth. In mid-March, 80 propagules of both SAV species were collected from field sites. Half were placed into plastic bags containing filtered (1μm) tap water (salinity = 0), and the other half were placed in bags with filtered water at salinity = 12 (~ mean salinity of field sites at time of collection). These bags were stored at a cold temperature (4°C) until planting.

Effects of cold-storage duration were tested by comparing propagule viability after 4, 8, 12, and 16 weeks with the following approach. Once per month, beginning in April and for three subsequent months, five propagules of both SAV species from both salinity treatments were taken from cold storage and hand-planted (in a fixed grid pattern) into replicate plastic trays containing HPL pond sediment (7-cm depth). Separate trays containing both species were then placed into

each of four greenhouse tanks, two tanks contained fresh tap water, and two were maintained at a salinity = 12 throughout the experiment by addition of freshwater or salt (Instant Ocean, Spectrum Brands Inc., Atlanta, GA). Tank walls and in-tank circulating pump filters were cleaned at weekly intervals. Shoot emergence and growth were monitored weekly as described above.

Artificially induced propagule production. The feasibility of inducing mature plants to produce viable tubers during the summer months was tested by placing trays (three each) with mature plants of both species (*P. perfoliatus* and *S. pectinata)* into four separate fiberglass tanks in an indoor mesocosm facility. Mature plants for *P. perfoliatus* were produced by planting cuttings in trays containing pond sediments and allowing the plants to grow in greenhouse conditions to reach maturity (approximately 4 months). Mature plants for *S. pectinata* were dug from the HPL estuarine ponds (see above). The mesocosms were filled with a mixture of freshwater and ambient estuarine water (to maintain a salinity of 10) that was circulated through a closed-loop recirculation system (Figure 2). Water temperature was cooled incrementally over three days from an initial temperature of 21°C, and maintained at a final temperature of 5°C. Two mesocosms received a longer (12-hr) daylight cycle and two received a shorter (6-hr) daylight cycle. Control groups for each plant species were kept in the greenhouse under natural light and temperature (24-26°C) conditions. The total number of propagules produced in each treatment was counted after 3 and 6 weeks of treatment.

Figure 2. Trays of *P. perfoliatus* and *S. pectinata* in mesocosm tanks.

To evaluate tuber/bud viability after treatment, propagules from each treatment for both species were planted both immediately (directly) and following 6 weeks of cold storage (cold treatment, at 4°C) into pond sediment in each of three plastic trays and distributed randomly among three greenhouse tanks. Artificial light was provided over each tank to simulate the light regime during the summer growing season. Aquarium heaters were also placed in the tank water to maintain water temperature above 20°C. Salinity was maintained at 7-8 by mixing ambient estuarine water with fresh water. Propagule viability and plant growth were determined as described above.

Comparison of planting methods.

Mesocosm experiment. To investigate alternative planting methods for *P. perfoliatus and S. pectinata* propagules, over-wintering buds and tubers were planted into pond sediments (see above) in outdoor mesocosms at the HPL facility in early May 2006. All propagules were placed in water from the collection site (HPL ponds) and kept in cold storage (4°C) for 8 weeks until the experimental planting.

Three planting methods were used to compare effectiveness of alternative deployment approaches for below-ground propagules of *P. perfoliatus* and *S. pectinata*. For both species, five

replicate planting units were generated by using two propagules in each of three planting methods: (1) inserting into balls of potting clay with propagule growing tip exposed (Figure 3, left), (2) inserting into burlap bags (10 cm^2) stapled closed and containing small pebbles to make the bags negatively buoyant (Figure 3, right), and (3) inserting directly into the sediment by hand. For growth comparisons, 10 individual *P. perfoliatus* and *S. pectinata* plants (grown out from foliar cuttings) rooted in sediment turfs were also planted in each treatment. Transplanted plant-sediment turfs were used as "reference treatments" because previous studies (Melton 2002; Hengst 2006) have demonstrated that this method yields a high survival rate (>90 percent) in field and mesocosm plantings. Relative success of each planting method was measured as the number of shoots produced (after 6 weeks) per below-ground propagule or per initial rooted plant turf deployed.

Figure 3. *P. perfoliatus* buds inserted in clay balls (left) and burlap bags (right).

Field Testing. A field planting experiment was also conducted in Broad Creek estuary (a tributary of the Choptank River estuary in Chesapeake Bay) during the summer of 2006 (May-August). This experiment was designed to assess the effectiveness of hand-planting individually inserted propagules compared to rooted plant-sediment turfs as restoration protocols. Triplicate plots (9 m^2) were established at three sites (Chapel Creek, Irish Creek, Hambleton Island) with mean water depths of ~1 m. Each plot, which contained 12 propagule units (each consisting of 5 propagules) and 12 units with rooted plant-sediment turfs (10-12 plants per unit) of *P. perfoliatus* and *S. pectinata*, was marked using numbered and color-coded wooden stakes and wire flags.

Twice-monthly site visits were conducted to assess water quality conditions, shoot emergence from propagules, and plant survival. Salinity, dissolved oxygen, and water temperature were measured using a hand-held YSI 85 unit (Yellow Springs Instruments, Ohio), and water clarity was estimated from Secchi disk depth. Transplanting success was measured by the percentage of planting units (bud/tuber propagule and mature plants) that contained surviving shoots of *P. perfoliatus* or *S. pectinata*.

RESULTS

Natural propagule production and viability. Seasonal patterns of plant biomass and winter-bud/tuber production differed between the two SAV species. For *P. perfoliatus* (Figure 4, left panels), shoot and root biomass levels exhibited peaks in late summer and early winter, respectively. Seasonal variations in abundance of winter buds generally followed trends for total below-ground biomass. Peak shoot biomass for *S. pectinata* (Figure 4, right panels) also occurred in August; however, relatively high biomass levels were found in early spring as well. In contrast to *P. perfoliatus*, root biomass of *S. pectinata* peaked in spring and declined from late summer through winter. Propagule production (Figure 4, bottom right panel) associated with below-ground tissue of *S. pectinata* varied over the year, with a bimodal trend where peaks occurred in both June and October.

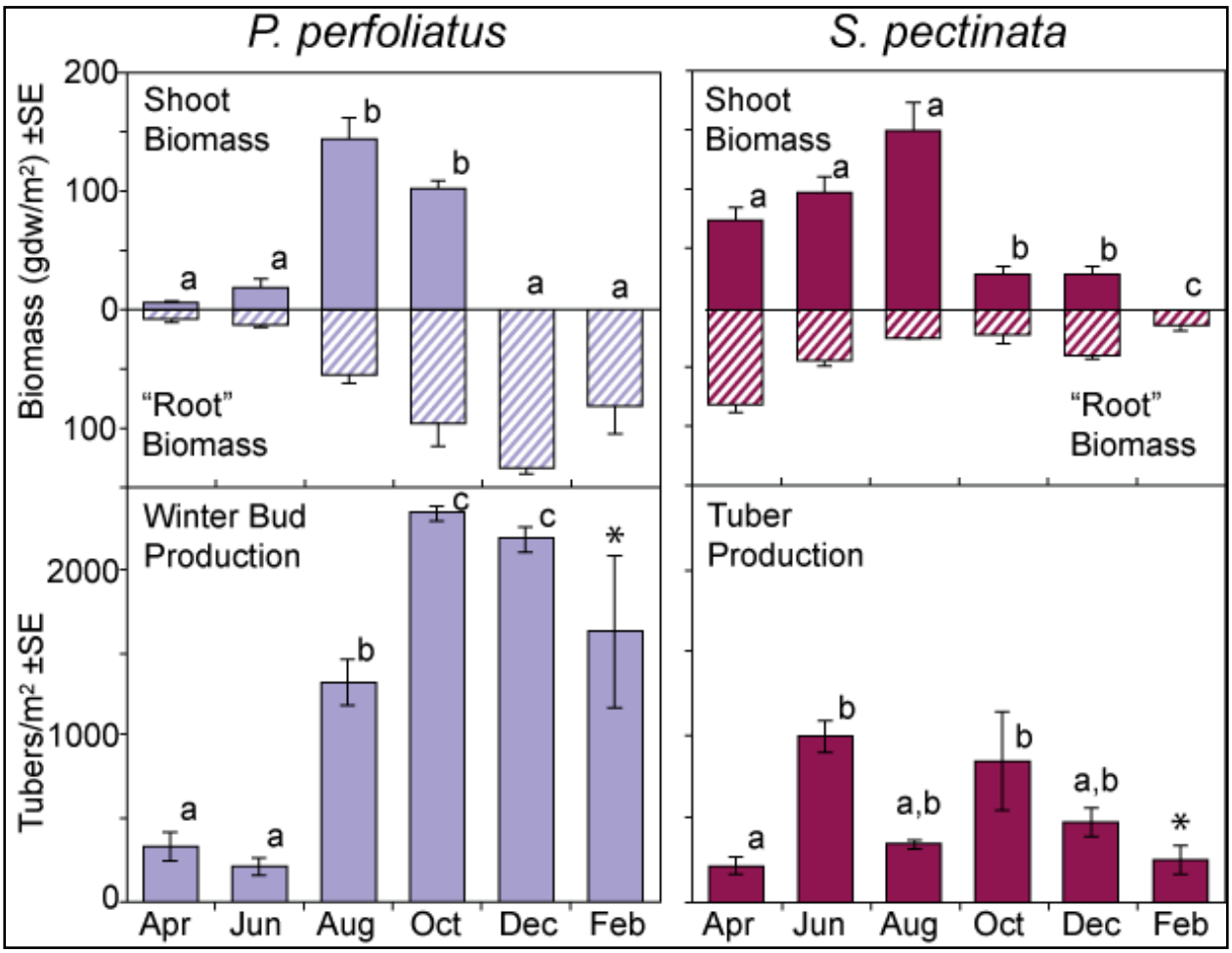

Figure 4. Seasonal cycle of shoot and root biomass accumulation (upper) and production of winter buds and tubers (lower) for *P. perfoliatus* and *S. pectinata* populations. Bar values indicate the mean ± 1 SE; (Values with the same letter are not significantly different.) * = data unavailable for statistical analysis.

Bud viability (data not shown) for *P. perfoliatus* peaked in winter (December–February), with an average of 80–100 percent of planted tubers producing shoots one week after planting. Over-wintering bud viability decreased in early spring (April) and fall (October) to 0–30 percent of the

buds producing shoots. Buds harvested in summer (June, August) were not viable (0 percent shoot emergence). To test effects of simulated natural cold exposure on propagule viability, buds harvested in each month were also subjected to a 6-week cold treatment. This increased *P. perfoliatus* bud viability in fall (October) by 40 percent, but had no effect on buds collected in spring-summer (April, June, August) or late winter (February).

Although *S. pectinata* tubers harvested during colder months (December–February) exhibited relatively high viability (50–90 percent), those collected in warm months (June–October) were not viable unless they were subjected to the 6-week cold storage treatment, which increased viability from zero to 80–90 percent. However, cold treatment only slightly increased viability (10–15 percent) of propagules harvested in colder months (December–April). The overall average viability of cold treated tubers harvested throughout the year approached 100 percent.

Subsequent plant growth (number of shoots produced per propagule planted after 6 weeks) was low for *P. perfoliatus* buds planted during warm months (<2 shoots per propagule planted) and was approximately the same with or without cold treatment. However, cold treatment in early winter (December) stimulated plant growth (12 shoots per propagule planted), and similar growth rates were observed for both direct planting and cold treatment in February (8-12 shoots per propagule planted). *S. pectinata* propagules planted directly (no cold treatment) during cold months (April, December, February) had relatively low subsequent growth rates (1-4 shoots per propagule planted). In contrast to *P. perfoliatus*, cold treatment of *S. pectinata* propagules resulted in elevated plant growth rates from propagules during all months (5-10 shoots per propagule planted).

Effect of salinity and cold storage duration. Salinity did not have an effect on *P. perfoliatus* or *S. pectinata* propagule viability with no significant difference between salinity treatments (top panel, Figure 5). Salinity had negative effects on plant growth in both plant species. Growth of *P. perfoliatus* was significantly higher in treatments of zero salinity compared to treatments of salinity = 12 (bottom panel, Figure 5). *S. pectinata* growth rates were lower in the higher salinity treatments, but differences were not significant (Figure 5).

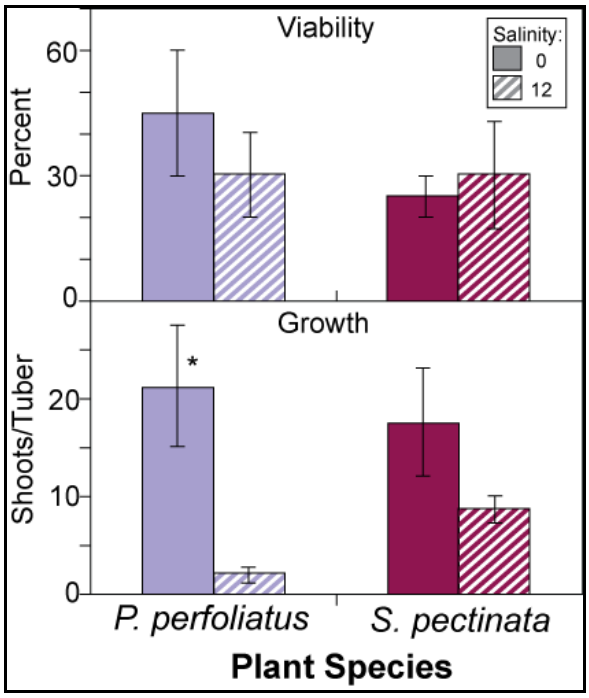

Figure 5. Effects of salinity on propagule viability (top) and growth (bottom) for *P. perfoliatus* and *S. pectinata* propagules planted (after 4 and 8 weeks of cold storage) in sediments with overlying water of 0 or 12 salinity. Asterisks (*) indicate significant salinity effects ($p<0.05$). Bars indicate the mean ± 1 SE; n = 4.

Plant growth declined with increased duration of cold storage treatment (beyond 8 weeks), with both *P. perfoliatus* and *S. pectinata* following similar patterns. Effects of cold storage on growth were most evident in the 0 salinity treatments (Figure 6), with highest rates occurring after 4- and 8-week storage periods (plant growth = 25-30 shoots per propagule planted).

For both SAV species, plant growth declined significantly with cold treatments at 12 weeks duration (10 shoots per bud) and 16 weeks duration (no shoots per bud).

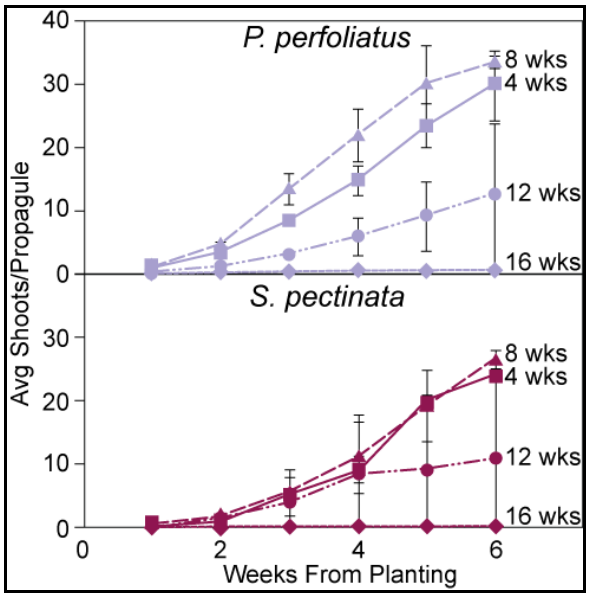

Figure 6. Weekly growth of *P. perfoliatus* (upper) and *S. pectinata* (lower) over 6 wks at 0 salinity in response to cold storage durations of 4, 8, 12 and 16 wks. Points indicate the mean + 1 SD; n = 2.

Artificially induced propagule production. Six weeks of cold treatment of *P. perfoliatus* plants had a positive effect on propagule production, with roughly twice as many propagules produced (2200 propagules m^{-2}) compared to both the controls and the 3-week treatment in both light regimes (~1150 propagules m^{-2}). For *S. pectinata*, tuber production rates after a 6-week cold treatment (average of 3700 m^{-2}) were slightly lower than rates in controls (5400 m^{-2}), but there were no differences between propagule production from plants receiving 3-week and 6-week cold treatments.

Cold treatment had a mixed effect on propagule viability. There were no differences in the number of *P. perfoliatus* shoots emerging from propagules generated from three weeks of cold treatment compared with controls (both ~ 40 percent). However, viability was reduced for propagules receiving the 6-week cold treatment (20 percent compared to 70 percent for controls). Cold storage of *P. perfoliatus* propagules did not increase propagule viability or growth for plants receiving either 3 weeks or 6 weeks of cold treatments. In addition, there were no significant differences in light treatments in either the 3- or 6-week cold treatment forcing experiments.

Three weeks of artificially induced cold treatment had a mixed effect on the viability of *S. pectinata* propagules, with highest rates for plants receiving treatments of cold and reduced light (100 percent) to lows of 25 percent in controls. Cold storage of tubers increased plant growth by 50-70 percent in both treatments and controls. There was no difference in viability (average of 70 percent) between light treatments in the 6-week cold experiment but controls had slightly reduced emergence rates (50 percent). Cold storage did not increase emergence rates in either experimental light treatment, but did increase it to 75 percent in controls.

Comparison of planting methods.

Mesocosm Experiments. All methods tested resulted in significant growth after 6 weeks for both plant species. For both species, tubers inserted in sediments by hand produced the highest rates of shoot production, while tubers placed in burlap bags exhibited the lowest shoot growth (Figure 7). For both plants, but particularly for *S. pectinata*, hand-planted tubers generated more surviving shoots after 6 weeks than did the other planting methods (including hand-planted turfs). Although shoot production rates from remotely deployed tubers (clay balls and burlap bags) were generally lower than rates from hand-planted rooted turfs, growth was still substantial

(1-2 shoots per tuber). It is encouraging to find that planting success from tubers was comparable to (or higher than) that seen previously for rooted plant turfs. The ultimate success of this approach for SAV restoration will depend on development of cost-effective methods for placing tubers in sediments under field conditions.

Field testing. Transplanting success (percent of planting units with healthy shoots) for plots grown from tuber deployment exhibited increased growth during the first half of the experimental period for both species (Week 2 (25 May) to Week 5 (13 June) (Figure 8). The percent survival of mature plants was statistically unchanged for *P. perfoliatus* and declined by 60 percent for *S. pectinata*. Although survival levels were generally 30–40 percent higher for plots planted with intact rooted plant turfs, trends for both tuber and turf plantings were parallel between weeks 5 and 10 (Figure 8). Survival declined for both types of plantings by week 10 (July 10) and disappeared by week 13 (August 9).

Water quality and physical conditions at the Broad Creek transplanting site changed radically between the beginning (May 10) and end (August 9) of the experimental period. Salinity dropped from 12 to 10, temperature increased from 19 to 27°C, morning levels of dissolved oxygen dropped from 6.3 to 5.2 mg/l, and Secchi disk depth decreased radically from 1.1 m to 0.5 m. At the time of initial transplanting deployments, Secchi depths (1.1 m) and associated light levels reaching the sediment surface (23 percent of light at water surface) were adequate to support SAV survival and growth (e.g., Kemp et al. 2004). These water clarity conditions, however, deteriorated (Secchi = 0.5–0.8 m) to levels well below required SAV criteria from late May through August in response to relatively

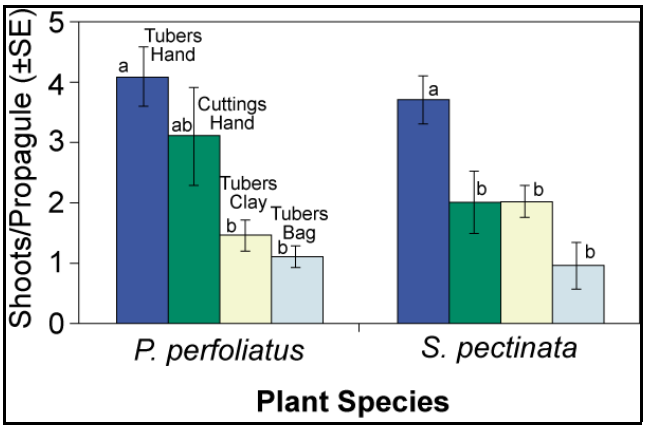

Figure 7. Bars indicate the mean ±1 SE (n = 4) for growth of *P. perfoliatus* (left panel) and *S. pectinata* (right panel) in mesocosms from tubers planted: directly by hand (dark blue), in clay balls (tan), and in burlap bags (light blue) as well as rooted shoots from cuttings (green). Growth is expressed as number of shoots produced per tuber planted after 6 weeks. (Values with the same letter are not significantly different at $p \leq 0.05$.)

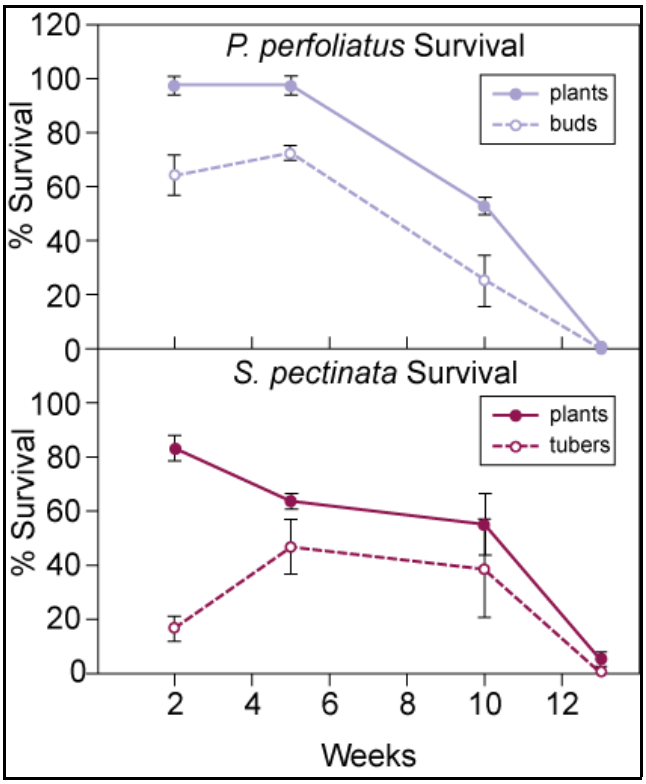

Figure 8. Comparison of survival of field planted buds (*P. perfoliatus*, top panel) and tubers (*S. pectinata*, bottom panel) with mature, rooted plants over the 13-week experimental period. Points indicate the mean ±1 SE, n = 3).

heavy rains. Thus, decreases in plant growth over the experimental period were attributable to declines in water quality.

Implications for restoration. The results of this study indicate that, under simulated field in situ conditions (estuarine ponds), *P. perfoliatus* over-wintering buds require an extended cold period (4–8 weeks) to be viable and to produce healthy shoots with strong subsequent growth. For this species, natural bud production, tuber viability, and plant growth were highest from buds harvested during cold months (October–February), when plants were dormant. While the number of propagules produced was highest in fall (October–December), plant growth from these propagules increased following an extended period of cold exposure either through an artificially induced cold storage treatment (6 weeks at 4°C) or a natural cold treatment (e.g., February). Experiments conducted to artificially induce *P. perfoliatus* production of viable buds in summer and early fall were generally unsuccessful, with generated buds having relatively low viability and growth.

P. perfoliatus buds can be stored in the cold (4°C) and dark for up to 8 weeks with no decrease in propagule viability or subsequent shoot growth. Transplanting experiments showed that under simulated and natural field conditions, these buds have the potential to survive and grow into mature plants as long as water quality conditions remain favorable. Although plant growth from *P. perfoliatus* buds was highest for hand-planted buds and turfs (requiring snorkeling or SCUBA), these studies demonstrated that buds can also be successfully planted using more cost-effective "remote" methods (clay balls, burlap bags) that are less labor-intensive. Propagule viability and subsequent growth were strongly affected by salinity, with significantly higher values at low salinities.

In contrast, *S. pectinata* tubers are produced throughout the year under natural conditions, with peak numbers in June and October. While tuber viability is especially low during warm months, they can be "induced" to sprout after a 6-week period of cold storage. Experiments with lowering temperatures and light during warm months did not induce mature *S. pectinata* plants to increase the number of tubers produced or their viability. However, 6 weeks of cold storage did increase viability of these tubers and subsequent shoot growth. In addition, because *S. pectinata* produces tubers year-round, there is little advantage in developing methods for "forcing" these plants into propagule production as a restoration protocol.

Tubers of *S. pectinata* can be stored for at least 8 weeks in cold/dark conditions with little decrease in viability. When stored for 12 weeks, shoot emergence decreased by 50 percent, and longer storage resulted in little or no shoot production. Both tuber viability and shoot growth increased significantly in freshwater compared to brackish conditions (salinity = 12). Field studies indicate that shoots grown from propagules generally survived equally well as those grown from mature rooted plant turfs. Similarly, healthy plant growth was observed in each of the investigated deployment methods. Therefore, "remote" deployment of cold-treated *S. pectinata* propagules harvested year-round can provide a cost-effective mechanism for restoring this SAV species.

CONTACT INFORMATION. For more information, contact Dr. Laura Murray, University of Maryland Center for Environmental Science, *murray@hpl.umces.edu*, or Dr. Deborah Shafer (*Deborah.J.Shafer@usace.army.mil*), Program Manager, Submerged Aquatic Vegetation Restoration Research Program, U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS, 39180. This technical note should be cited as follows:

> Murray, L., W. M. Kemp, D. Hinkle, and D. Shafer. 2009. *Production and field planting of vegetative propagules for restoration of redhead grass and sago pondweed in Chesapeake Bay*. EMRRP Technical Notes Collection (ERDC/TN SAV-09-1). Vicksburg, MS: U.S. Army Engineer Research and Development Center.

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