Seed quality and comparison of planting techniques for Atlantic white-cedar, *Chamaecyparis thyoides* (L.) B.S.P., at Arlington Echo Outdoor Education Center, Anne Arundel County, Maryland.

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Abstract

Atlantic white-cedar is an extremely local tree on the Severn and Magothy Rivers in Maryland with a total of nine sites. Recruitment in Atlantic white-cedar stands is known to be effected by variation in germination rates due to poor seed quality, insect damage, and variation in embryo dormancy. Reforestation success may depend on the size of the seedling used and where it is planted within the wetland matrix. Our goal was to compare the survival of rooted cuttings, seedlings, and freshly harvested cuttings stuck in the ground and to determine the cause for our average germination rate of 9% with 1997 Atlantic white-cedar seed. Rooted cuttings, seedlings, and freshly collected stems were co-planted at thirty-nine plots across environmental gradients including Atlantic white-cedar forested wetland, sedge hummocks in scrub-shrub wetland, and seepage slopes at fresh/tidal interfaces. Our greatest survival in planting across an environmental gradient was obtained in Atlantic white-cedar forested wetland (62-68%) while the lowest survival occurred on seepage slopes at fresh/tidal interfaces (5-10%). None of the freshly harvested cuttings stuck in the ground survived. We found that 15% of seed produced was good quality while most seed was of poor quality

(85%). One of the reasons for the high rate of poor quality seed was insect destruction of the megagametophyte and embryo. A possible source of the low germination rate observed in 1997 Arlington Echo seed may thus be due to insect parasitization.

Introduction

Atlantic white-cedar, *Chamaecyparis thyoides* (L.) BSP, is a coastally restricted obligate wetland tree (Laderman 1987). Little (1950) reported the difficulty of regenerating stands of this tree due to variable seed germination rates, herbivore grazing, and competition. Recruitment in Atlantic white-cedar stands is also effected by variation in germination rates due to poor seed quality, insect damage, and variation in embryo dormancy (USDA 1974; Laderman 1987).

Atlantic white-cedar is extremely local on the Severn and Magothy Rivers, Maryland with a total of nine sites (Sheridan et al. 1999). Sheridan et al. (1999) suggested the restoration of these Atlantic white cedar habitats be based on a sound ecological foundation. An essential component of Atlantic white-cedar restoration in Maryland is an understanding of the factor(s) controlling fitness. An effort was therefore launched to determine seed quality and survival rate of Atlantic white-cedar planted across an environmental gradient at Arlington Echo Outdoor Education Center in Anne Arundel County, Maryland.

Materials and Methods

Seed Quality

Arlington Echo was visited on October 3, 1998 and an extension pole was used to collect branches bearing cones from a total of seven trees. Diameter and position of tree within the site were recorded. Branches bearing cones were then removed from trees and both tree and branch numbered for future reference. Cones were only collected from three large trees due to the difficulty of reaching cone bearing branches, even with a 15 foot extension pole. Atlantic white-cedar is self-pruning (Musselman pers. comm.) and branches are sparse to non-existent in the lower reaches of mature trees. Higher fitness of seeds from mature trees has been reported (Boyle and Kuser 1994) and we were particularly interested in obtaining seed from mature trees. Cone bearing branches were also collected from four saplings in the marsh edge at Arlington Echo.

Cones were then removed from branches and each cone individually packaged in a labeled #1 coin envelope for drying. Envelopes were placed in the drying oven on October 13, 1998 for ease of seed removal following the methods of Boyle and Kuser (1994). Envelopes were removed from the oven on October 15, 1998. Oven temperatures ranged between 35-44^{0}C. Seeds were then manually extracted from cones, counted, the envelope labeled with the number of seeds, and seeds returned to the envelope. Debris from seed extraction was then discarded.

Once all seeds were counted, one tenth of the seed containing envelopes were randomly selected for each tree and set aside for seed quality analysis. The remaining seeds were then used to determine average weight per seed for each tree. Seed for each tree was combined into one envelope, the seeds weighed, and average weight determined by dividing total weight by total number of seeds. Seed quality was assayed by cutting the seeds open with a razor blade and examining the cross section. Healthy white gametophytic tissue was scored as good quality while brown or shrunken tissue, or empty seeds, scored as poor quality. Healthy cross sections were placed in a 0.05% tetrazolium solution to determine viability.

Survival Study

Three different treatments (seedlings, rooted cuttings, and fresh non-rooted cuttings) were used to measure the survival rate of Atlantic white-cedar propagules across three environmental gradients at Arlington Echo (Atlantic white-cedar forest, sedge hummocks in scrub-shrub wetland, and seepage slopes at the fresh/tidal interface). Atlantic white-cedar naturally grows in all three habitats at the site. All propagules were collected at Arlington Echo.

Propagule Preparation

Seed was collected from 17 trees and saplings on 10/22/97 by clipping branches with an extension pole pruner. Seed was removed from cones, cleaned, weighed and stored in coin envelopes based on tree number. Seed was then sowed on the surface of a pre-moistened peat/sand mix in shallow trays measuring 50cm long, 28cm wide, and 5cm deep. Seed was labeled to identity of tree origin. Seed was then allowed to stratify outside under a shelter for one month at the Meadowview Biological Research Station (MBRS). Soil moisture was maintained through careful surface watering. Trays were then brought into a greenhouse on 3/25/98 and percent germination measured on 5/11/98. Germinated seedlings were repotted on 6/27/98 into 6cm pots and placed in bottom-watered beds in full sun. Seedlings were mulched in the fall with pine straw for winter protection and mulch removed the following spring.

Cuttings were made from the same trees from which seed was collected on 10/22/97. Cuttings ranged in size from 7-15cm. Cuttings were dipped in Rootone, labeled as to tree origin, and placed in moist peat/sand beds under benches in the Meadowview greenhouse on 10/24/97. Greenhouse temperatures were maintained above freezing through occasional use of a wood stove. Cuttings were potted in late June and early July 1998 and rooting percent recorded. Potted cuttings were then placed in bottom-watered tanks at MBRS and placed in full sun. Plants were then mulched in the fall with pine straw for winter protection. Mulch was removed in the spring of 1999.

Propagule Planting and Data Collection

Forty-five planting sites across the three environmental gradients were selected and flagged on 5/14/99. Seedlings and rooted cuttings were bare rooted on 5/14/99 and placed in a dilute Miracid solution. The average height of seedlings and rooted cuttings was 8 and 23cm, respectively. Seedlings and cuttings were transported to Arlington Echo on 5/15/99. Fresh cuttings averaging 28cm in length were then made from a recently toppled, living tree. Each planting site received a total of six implants consisting of pairs of seedlings, rooted cuttings, and fresh cut Atlantic white-cedar stems. Planting material was labeled as to treatment type. Survival and growth data was then collected the following year on 3/26/00.

Results

Seed Quality

Oven drying of seeds was not an effective way to open white-cedar cones. We still had to manually "crack" many cones to get all the seeds and what was even worse was the unpredictable rise in oven temperature to 44^{0}C which could have compromised future germination experiments with this seed.

Seeds were extracted from a total of 741 cones with a yield of 5608 seeds. The average number of seeds per cone ranged from 6.5 to 8.6 depending on the tree (Table 1). Tree diameter did not appear to effect the average number of seeds per cone. There was no significant difference in average seed weight between trees and overall average weight of seed was 0.0009g (Table 2). Work on white-cedar seed from Arlington Echo in 1997 resulted in the same average seed weight as well (8647 seeds/8.2g = 0.0009g/seed).

Good quality seed (ca. 3mm in length) ranged from 1-62%, depending on the tree, with an overall average of 15.4% (Table 3). There also seemed to be an association between smaller diameter trees and better quality seed. Most seed, however, was of poor quality with an overall average of 84.6% (Table 3). Some of the poor quality seeds were also very tiny (less than a millimeter). One of the reasons for the high rate of poor quality seed was destruction of the megagametophyte and embryo by some kind of larva. Many poor quality seed contained tiny frass particles and in many cases the translucent larva was still present. Boyle and Kuser (1994) reported three categories for their poor quality seed (brown or deformed embryos, insect damage, empty). We found it difficult to make the first two distinctions. We were also not satisfied with the accuracy of the tetrazolium test because of the difficulty in properly slicing the seed to expose the embryo. Boyle and Kuser (1994) reported a similar difficulty with tetrazolium test interpretations and instead relied on other visual aspects of seed quality, as we have done.

Survival Study

Germination of the 1997 seed used for producing seedlings averaged 9% (range 4-14%) and average seed weight was 0.0009g. Average rooting of cuttings in the greenhouse was 34% (range 0-68%).
When seedlings and rooted cuttings were planted back at Arlington Echo across the three environmental gradients the highest survival was obtained in the Atlantic white-cedar forest. Survival was lower in both the scrub-shrub and seepage slopes at the fresh/tidal interface (Table 4). None of the fresh cut Atlantic white-cedar stems survived in any environmental gradient

Discussion

Germination tests with 1997 seed from Arlington Echo involved 17 trees and resulted in an average germination rate of 9% (range 4-14%) and average seed weight of 0.0009g. This experiment with 1998 seed from 7 trees resulted in the same overall average seed weight and a possible explanation for the low observed germination rate. We originally suspected a high level of inbreeding depression in Arlington Echo Atlantic white-cedar because of the limited population size (88 trees). We thought that inbreeding depression was being expressed in seed quality (hence the low germination rate) and that examination of seed would disclose deformed embryos which would support this hypothesis. We did find some deformed seed which suggests a certain amount of inbreeding but do not think inbreeding is the major cause of low germination.

Megastigmus thyoides has been reported as a new pest of Atlantic white-cedar (DeBarr, pers. comm. to K.O. Summerville). *Megastigmus* lays its eggs in the seeds of Atlantic white-cedar and destruction of seed can be over 90% (Summerville pers. comm.). Potentially the larva observed in Arlington Echo Atlantic white-cedar seed is *Megastigmus*. An effort should be made to confirm this identification and determine this species ecological interaction in the Atlantic white-cedar ecosystem. No work has been done on the life cycle of *Megastigmus* (Summerville pers. comm.).

We think that the source of the low germination rate in 1997 Arlington Echo seed may be principally due to seed destruction by the parasite observed in this study. Although Atlantic white-cedar seed can take up to 3 years to germinate (Laderman 1989) the low germination rate with 1997 seed (9%) can largely be explained as the result of poor quality seed (85%) found in this study. Presumably delayed germination phenomena would explain the rest of the difference. Addressing both parasitization and germination enhancement of Atlantic white-cedar seed should therefore be a productive field for conservation biologists in the future.

The extreme localization of Atlantic white-cedar stands on the western shore of Maryland, and at Arlington Echo in particular, has been an active topic of conversation among Maryland conservation biologists, land managers, and decision makers. Atlantic white-cedar at Arlington Echo survive best in habitats (e.g. Atlantic white-cedar forest) where they are currently most abundant. In contrast, survival of Atlantic white-cedar at Arlington Echo is lower in habitats where they occur at a lower frequency (e.g. scrub-shrub and seepage slopes at the fresh/tidal interface). Many of the Atlantic white-cedar stands on the western shore of Maryland are in a similar landscape position and hydrogeoological setting as Arlington Echo. While pollution and sedimentation have reduced the extent of habitat available for Atlantic white-cedar on the western shore of Maryland this study demonstrates that their current localization may largely be due to limited appropriate habitat for survival.

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Table 1. Average number of seeds per cone for Atlantic white-cedar at Arlington Echo

	Tree number							
	1	2	3	4	5	6	7	Total
tree diameter	43.7	44.9	41.3	6.4	1.9	5.0	4.4	
total seeds	734	1050	1281	926	277	473	867	5608
# cones	85	129	198	125	39	60	105	741

Average	8.6	8.1	6.5	7.4	7.1	7.8	8.3
Stnd. dev.	3.1	3.4	3.4	2.8	2.3	3.1	3.3

Table 2. Average seed weight for Atlantic white-cedar at Arlington Echo

	Tree number							
	1	2	3	4	5	6	7	Total
# seeds weighed	659	935	1136	847	252	431	777	5037
weight (grams)	0.481	0.857	0.777	1.046	0.244	0.620	0.464	4.489
avg. weight*	7.2	9.2	6.8	12.3	9.7	14.4	6.0	9.0

X^2	0.19	0.001	0.055	0.112	0.002	0.143	0.075	0.407[t]

*average seed wt. x 10^{-4}

[t] Not significant, d.f. = 6, $P < 0.05$

Table 3. Seed quality of Atlantic white-cedar at Arlington Echo

	Tree number							
	1	2	3	4	5	6	7	Total
# seeds	75	115	145	79	25	42	90	571
Good quality								
#	1	14	8	27	11	26	1	88
(%)*	(1.3)	(12.2)	(5.5)	(34.2)	(44.0)	(62)	(1.1)	(15.4)
Poor								

quality								
#	74	101	137	52	14	16	89	483
(%)*	(98.7)	(87.8)	(94.5)	(65.8)	(56.0)	(38.0)	(98.9)	(84.6)

* % x 100

Table 4. Percent Survival of Atlantic white-cedar planted at Arlington Echo

	Treatment	
Gradient	Rooted Cuttings	Seedlings
Atlantic white-cedar forest (n=8)	62	68
Sedge hummocks In scrub-shrub	40	42

(n=21)		
Fresh/tidal interface (n=10)	10	5