Stratification and light promote germination of ratroot (*Acorus americanus* (Raf.) Raf. [Acoraceae]) seeds harvested in northeastern Alberta

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ABSTRACT

Seeds of *Acorus americanus* (Raf.) Raf. (Acoraceae), or ratroot as it is commonly known, were harvested from 3 locations in each of 2 y and subjected to combinations of light and dark treatments, 30 d of stratification, 3 germination temperature regimes, and 4 storage durations (zero to 24 mo). When germinated under ambient conditions, we determined that light is required for germination and that stored seeds germinate better after stratification (moist conditions at 2–4 °C). Observations on ideal storage time and germination temperatures, however, were inconclusive. This species is an important plant for Aboriginal peoples of northern Canada, and it is included in oil sands mining reclamation efforts.

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KEY WORDS
aquatic plant, boreal forest, oil sands, Aboriginal peoples, Acoraceae

NOMENCLATURE
ITIS (2014)

CONVERSIONS
km * 0.62 = mile
(°C x 1.8) + 32 = °F

Acorus americanus (Raf.) Raf. (Acoraceae), or ratroot as it is commonly known, is a species significant to Aboriginal peoples of northern Canada. Found in wetlands of the parkland and boreal regions of Alberta, this species has come to the attention of oil sands mining operators as one to include in wetland reconstruction efforts. Many studies have focused on the medicinal attributes of the closely related species, *A. calamus* L. (Keddy and Ellis 1985; Motley 1994; Kim and others 2009; Sigma-Aldrich 2010; Divya and others 2011), and we examined vegetative propagation (Smreciu and others 2014); however, germination of either ratroot species has not been closely examined. In an effort to provide operators with pertinent cultural information, we initiated an analysis of ratroot germination.

Although limited germination occurs without treatment, we expect that ratroot seeds would benefit from stratification (moist and cold conditions), like many other boreal wetland and upland species (Baskin and Baskin 2001), including *A. calamus* L. (Keddy and Ellis 1985). Other common requirements for wetland species are light and heat (Baskin and Baskin 2001). These conditions replicate the effects of a natural drawdown where retreating water exposes seed and dark soils. Because seeds are not always planted immediately, the effect of storage time was also evaluated.

Figure 2. Immature ratroot spadix. Photo by Wild Rose Consulting Inc

MATERIALS AND METHODS

Ratroot seeds were harvested from 3 naturally occurring wetlands within 200 km of Fort McKay (lat 57°11′12.3″N, long 111°38′12.3″W) in northeastern Alberta (Figure 1). The northernmost wetland was an outlet of Kearl Lake, where plants grew in soil adjacent to a stream in which water levels fluctuate from season to season and year to year. The southernmost wetland was Willow Lake, with plants growing along the shoreline. Plants were floating as part of a cattail (*Typha latifolia* L. [Typhaceae]) mat, connected to the shore by roots of a large willow tree. Roughly halfway between these two, Shipyard Lake lies on the Suncor mine lease. Although close to mining operations, the lake itself is relatively undisturbed as it falls in an environmental buffer maintained adjacent to the Athabasca River. Plants are found throughout the lake, floating on the surface as a constituent in cattail mats. These mats are not anchored and their configurations change frequently.

Figure 1. Map of Alberta showing the location of Fort McKay relative to Fort McMurray and Edmonton. Map by Jim Marin Graphics

Seeds of ratroot were harvested in 2 consecutive years to incorporate variations in seed production and quality (Figure 2). Spadices were allowed to dry for a minimum of 1 wk (up to 1 mo) before stripping seed capsules by hand. Capsules were broken up by scrubbing them on a No. 8 sieve (2.36 mm opening), catching seeds in a No. 18 sieve (1 mm opening), and finally winnowing.

Cleaned seeds were transferred to paper envelopes and stored at ambient conditions (18–22 °C and 30–50% relative humidity) for 0, 6, 12, and 24 mo. Fresh seeds and those in each storage interval were germinated in darkness (wrapped in foil) or in fluctuating light and one of 3 temperature variables (5/15 °C, 10/20 °C, and 15/25 °C) controlled by a germination chamber with a photoperiod of 8 h light/16 h dark. For each storage duration, light, and temperature treatment combination, 2 dishes of 100 seeds were placed on germination paper and moistened. Tests ran for 56 d (8 w). By the end of the second storage interval (1 test of fresh and 1 test of 6 m old seed), it was clear (germination <5%) that light is required for germination of ratroot seeds. In an effort to conserve seeds and costs, dark treatments were excluded from the 12 and 24 mo storage tests and the subsequent fresh and 6 mo storage tests.

All data presented are for seeds receiving a cycle of 8 h light and 16 h dark. A multivariable analysis of variance was conducted to assess the effect of site, stratification, storage time, and germination temperature at a significance level of $P < 0.05$.

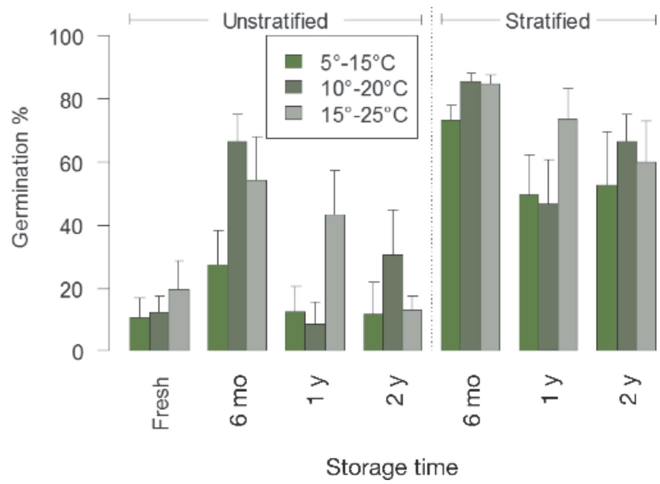

Figure 3. Ratroot germination with and without stratification across 3 temperature regimes and 4 storage durations.

RESULTS AND CONCLUSIONS

Germination was observed in all treatments (except dark conditions) on all harvested seedlots (Figure 3). Storage time, stratification, and germination temperatures all significantly affected germination percentage (Table 1). No three-way interactions were significant, but the site x storage and stratification × storage interactions were. We detected no discernable pattern to the site x storage interaction, but non-stratified seeds show a greater decrease in germination with lengthening storage time than do seeds that are stratified. The warmest germination temperature range (15/25 °C) resulted in significantly greater germination percentages than the lowest range (5/15 °C) ($P = 0.0346$); neither was significantly different from the middle range of 10/20 °C. Seeds stored for 6 mo germinated more than seeds stored for any other tested period ($P < 0.05$).

TABLE 1

Analysis of variance table for germination data.

Variable	Df	Sum sq	Mean sq	*F* value	*P* value
Site	2	3383	1691	2.1745	2.1745
Stratification (Strat)	**1**	49109	49109	63.883	< 0.0001
Storage	3	19659	6553	9.6125	< 0.0001
Temperature (Temp)	2	5605	2803	4.1110	0.0210
Site × Strat	2	399	199	0.2926	0.7473
Site × Storage	6	10807	1801	2.642	0.0238
Site × Temp	4	1494	374	0.5480	0.7012
Strat × Storage	2	402	201	0.2947	0.0238
Strat × Temp	2	188	94	0.1377	0.8717
Storage × Temp	6	7818	1303	1.9113	0.0927
Site × Strat × Storage	4	1160	290	0.4253	0.7898
Site × Strat × Temp	4	1139	285	0.4175	0.7954
Site × Storage × Temp	12	3093	258	0.3781	0.9667
Strat × Storage × Temp	4	935	234	0.3430	0.8479
Site × Strat × Storage × Temp	8	714	89	0.1309	0.9976
Residuals	63	42948	682	—	—

Overall, fresh seeds germinated less than any other tested period ($P < 0.05$). In general, 6 mo of storage and warmer temperatures benefit germination. Stratification improves germination percentages over the range of germination temperatures and storage times. Seed storage conditions were not tested in this study but it would be worth examining seeds stored at low temperatures under low relative humidity to determine if germination of older seeds can be improved compared with that observed in this study. This approach would inform whether the seed storage behavior is orthodox.

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