Nova Scotia Habitat Conservation Fund Project: Development of a Seed Bank for Nova Scotia Flora (2013-14)

Project Manager: Dr. Robin Browne (K.C. Irving Environmental Science Centre and Harriet Irving Botanical Gardens, Acadia University)

Award: $9500

Final Report for Research Activity

This research project was intended to provide key foundational information and accelerate technical advancement toward the establishment of a seed bank program for native species in Nova Scotia. Implementation of enhanced cryogenic seed storage capabilities at the K.C. Irving Environmental Science Centre and Harriet Irving Botanical Gardens (Irving Centre) is expected to make significant contributions to future initiatives aimed at the protection of native plant species essential to habitat conservation programs.

The specific research objectives for this project were:

1. Collection of viable seed from at least 10 select native plant species in Nova Scotia
2. Development of collection, processing, drying and freezing protocols for cryogenic storage
3. Viability and germination testing prior to cryogenic storage
4. Viability and germination testing post cryogenic storage

Outcomes achieved during the research period:

1. A total of 11 species were selected for study based on consultation with the Herbarium Curator (Ruth Newell) and Conservation Horticulturist (Melanie Priesnitz) at the Irving Centre. Selection of the initial test species was based on expected availability of seed, access to collection sites, abundance of populations and taxonomic diversity. The selected species included: *Carex crinita, Glyceria striata, Solidago canadensis, Spiraea latifolia, Rosa virginiana, Scirpus cyperinus, Carex lurida, Calamagrostis canadensis, Oenothera biennis, Juncus canadensis, Geum macrophyllum*

2. Seed collections were completed in the summer and fall months of 2013. Single populations of each species were sampled, with sufficient estimated quantities of seed to complete the research objectives. Samples were brought to the Irving Centre to be prepared for testing under controlled conditions. A period of after-ripening was completed using aluminum trays laid on shelving in a designated processing area (Fig. 1). Seeds were extracted and cleaned manually using rubber tubing, sieves, and aspiration, following by examination and dissection of subsamples to verify viability. Mass determinations were also completed for future reference and estimation of seed number for sampling. A total of 120 seed were removed prior to further treatment to determine germination capacity. Seeds were imbibed for 1 hour in running water using mesh infusers (Fig. 2), and placed on Petri plates with agar solidified media (Fig. 3). Each of six plates contained 20 seed. Three plates were placed under 16 hour photoperiod at 21-25 C,

while three were placed in a fridge at 4 C for 6-8 weeks to test for possible stratification requirement prior to placing under the same germination conditions as the non-stratified test group. Germination was recorded weekly for at least one month. Final observations were usually completed within 4 months.

The remaining seeds were desiccated under controlled conditions using a two chamber system to reduce seed moisture levels. A series of preliminary trials were completed to determine appropriate desiccation conditions. Seeds were placed on open Petri plates with a filter base, inside a bell chamber, which was also inside a large plexiglass chamber (Fig. 4). Both chambers contained fresh silica wells to reduce internal humidity levels. These levels were monitored with a combination of relative humidity (RH) sensors and a HOBO data logger. The RH levels inside the bell chambers were held at < 20% for two weeks. Seeds were then removed and portioned into sample vials while working inside the plexiglass chamber through arm ports, to reduce the impact of possible RH spikes while completing the transfer. Vials were placed inside a storage container and moved to a - 20 C freezer (Fig. 5).

3. The species were separated into three groups based on initial viability and germination tests, as follows:

    Viable, germination > 80%

    Glyceria striata
    Spiraea latifolia
    Oenothera biennis
    Geum macrophyllum

    Viable, germination 35 – 60 %

    Carex crinita
    Carex lurida
    Juncus canadensis

    Low viability and or germination < 15 %

    Solidago canadensis
    Rosa virginiana
    Scirpus cyperinus
    Calamagrostis canadensis

The four species with germination frequencies of > 80 % were selected as the primary test material. These seed samples were advanced to desiccation treatments and long term cryogenic storage at – 20 C. Samples from the other species have been kept at RT and 4 C for ongoing germination trials and possible future comparative trials with subsequent collections.

4. Two months following freezing, the first set of seeds from the four primary test species were removed from cryogenic storage. Germination tests were repeated as done prior to freezing. Among all species tested, no reduction in germination rates were observed following exposure to - 20 C for two months.

Summary, Implications of the Project, Recommendations for Future Research

This project demonstrated the feasibility of developing a seed bank program for native plants in Nova Scotia. Methods developed for seed collection, processing, desiccation and freezing proved effective with four selected test species. The occurrence of other species with low viability and or low germination rates in this study underscores the importance of expanding the sampling regime, as well as more extensive research to examine aspects of seed maturation, dormancy and germination requirements.

It is expected that the seed bank program will eventually become an integral component to future conservation activity at the Irving Centre. Ongoing research is expected to develop a long term seed bank program that includes a wider range of native species, particularly those from wetland habitats.

The potential importance of having a functional seed bank in this region has been evidenced already by recent requests for seeds of native species.

Other research horizons may eventually explore the potential of ultra low cryo storage (-196 C in Liquid Nitrogen) as a preservation strategy, particularly for “recalcitrant” species (ie those which do not produce “orthodox” seeds that respond well to desiccation treatments).

Subsequent to the completion of trials during the project research period, additional germination tests have verified the effectiveness of – 20 C following controlled desiccation. In addition, out-planting trials following germination of seed after cryogenic storage have demonstrated the capacity to maintain and grow native plants under ex situ conditions (Fig. 6). This information may prove useful in future research projects, particularly involving site restoration trials.

Future collections are expected to also include at risk species in the Acadian Forest Region, as a strategy to mitigate against possible reduction in biodiversity and continued habitat loss.

Acknowledgement: The author thanks the Nova Scotia Habitat Conservation Fund (contributions from hunters and trappers) for supporting this work. Ruth Newell and Melanie Priesnitz were involved in the selection of species and seed collections. Mike Muldoon, Rebecca Ryan and Nicole White provided technical support for seed collection, processing and germination trials. Rodger Evans is the group leader of the seed bank project.

Report prepared by: Robin Browne
August, 2015

Figures

Fig. 1 Sample being after-ripened following collection, prior to seed extraction and cleaning

Fig. 2 Seed samples prepared for imbibing prior to germination testing

Fig. 3 Germination test with one of the seed samples (*Geum macrophyllum*)

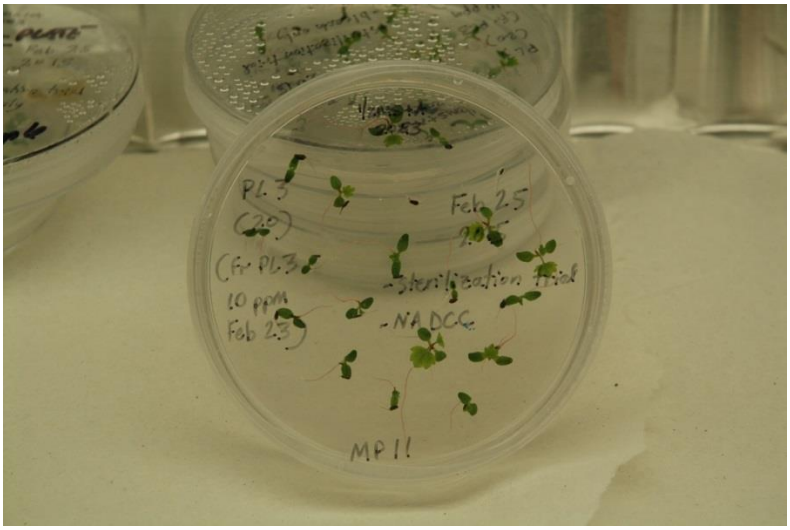

Fig. 4 Experimental desiccation system involving two chambers to reduce humidity levels

Fig. 5 Seed samples being placed in -20 C freezer

Fig. 6 Seed bank out-planting trial for *Glyceria striata*