National Leadership Grants for Museums

Sample Application MG-245983-OMS-20
Project Category: Collections Care and Public Access

Center for Plant Conservation

Amount awarded by IMLS:	$476,040
Amount of cost share:	$0

The project description can be viewed in the IMLS Awarded Grants Search:
https://www.imls.gov/grants/awarded/mg-245983-oms-20

Attached are the following components excerpted from the original application.

- Narrative
- Schedule of Completion

Please note that the instructions for preparing applications for the FY2021 National Leadership Grants - Museums grant program differ from those that guided the preparation of FY2020 applications. Be sure to use the instructions in the Notice of Funding Opportunity for the grant program and project category to which you are applying.

NARRATIVE: RNA integrity as a powerful metric of aging in preserved seed collections of wild rare plant species

Project Justification

1.1 What we propose to do.

Botanical gardens are instrumental in conserving the nation's botanical heritage. The Center of Plant Conservation (CPC) coordinates a National Collection of 1600 rare and endangered plant species held by its network of botanical institutions across North America. High rates of plant extinction have led botanical gardens to use seed banking as a cost effective conservation strategy. Thus the National Collection is maintained primarily as seeds that are stored in "orthodox" storage at -18 C in the freezers of botanical gardens. CPC's seed banks aim to keep seeds alive for decades or centuries as safeguards against the loss of wild populations. Because most research has been conducted on crop species, no one actually knows how long many rare plant seeds can survive in seed banks. We know that seeds vary widely in their longevity; some species are damaged in orthodox storage, some survive less than ten years, while others persist for several hundred years. Consequently, seed bank curators typically monitor viability through time using germination tests to detect mortality. This "gold standard" curation method is labor intensive, consumes valuable seeds, and does not detect change before death occurs. In other words, good curation potentially undermines the purpose of rare seed collections: to safeguard diversity.

To ensure better stewardship of rare plants in seed collections, CPC's national office, its network of botanic gardens, and the National Laboratory for Genetic Resource Preservation (NLGRP) propose to test aging and longevity in wild rare plant species seeds in a new way. Recent research with crop species shows that biochemical indicators can reliably measure seed aging and predict seed longevity. RNA integrity number (RIN) has shown particular promise as a metric of seed aging. Applying this new technology to wild species will be especially useful for curators, given the enormous variation wild seeds display in germination requirements and storage behavior.

To assess the usefulness of this cutting-edge research in conservation collection curation, we propose the first known application of RIN to assess aging of seeds from wild rare plant species. CPC's network of botanical institutions will recollect fresh seeds from plant populations corresponding to seed accessions currently held in freezer storage for than 15 years or more at a CPC seed bank. For 20 of 100 study species, we will simulate seed aging by exposing fresh seeds to dry heat, allowing us to examine seed deterioration in biochemical markers and germination until all seeds have died. From this aging experiment, we can establish a standard equation that relates RIN declines directly to seed storage half-life, the point at which half of the seeds remain viable. For all 100 species, we will measure "real time" seed deterioration in orthodox storage by performing germination assays and a panel of newly-available measurements (including RIN) on the fresh and long-held frozen accessions. To test existing hypotheses about seed longevity, we will build a predictive model evaluating how aging rates of seeds covary with phylogeny, habitat, climate, and plant traits. We connect this model to an interactive web application that enables user to predict seed longevity from these traits, which would inform species conservation plans for the thousands of rare plant species not included in this study. Results of the study and their implications for curation will be discussed and refined into new best practice guidelines through a terminal webinar, which will allow broad participation of research partners and other conservation professionals. We will communicate our results and data publicly through peer-reviewed publications, through CPC's online multi-media *Best Practice Guidelines* for rare plant curation methods, and directly with the country's top conservation seed banking professionals at the CPC National Meeting.

To achieve these outcomes, we request ~$476,040 from the Institute of Museum and Library Services National Leadership Grant for Museums to be dispersed over three-years. The funding would support all field and lab work, coordination, web development, and data analyses.

See Appendix III for bibliography of works cited

Specific field-wide needs this project will address.

Problem: The longevity of orthodox seeds in storage is not known for most wild rare plant species

Nearly 600 plant species have gone extinct in the past 250 years[2], a rate 500 times that of natural background extinction, posing existential threat to global biodiversity. Orthodox seed banking is a cost effective defense against plant extinction[3]: involving just two initial steps 1) desiccating seeds and 2) placing them in the freezer at -18ºC. The majority of rare plant seeds survive this treatment, at least initially. However, like all living things, seeds age and degrade over time. Uncertainty about the length of time a seed collection can survive obscures the need to replace collections and thus presents an obstacle to plant collections planning.

Though there are rigorous standards set forth for the collection of seed from wild plant populations and reintroducing rare plants into the wild[1,4], there is an absence of widely adopted recommendations about optimal time that seed collections should remain in frozen storage. One misconception is that all seeds will live for decades, if not centuries. With no guidelines on seed lifespan for most plant species in the US, seed bank curators could improperly store a valuable collection or fail to notice the death of seed from an irreplaceable plant population. In fact, few seed bank collection plans have clear "exit strategies" for seeds in frozen storage, working instead under the tenuous assumption that the seeds will be ready when needed. A better understanding of seed longevity could guide botanical institutions in their curation and collection strategies – helping to prioritize seed collections to be regenerated, returned to the wild, or recollected before they die in the seed bank.

The patterns found in agronomic species give hope that a better understanding of seed longevity is achievable, even though there are a few comparative studies and most show stark differences among plant species[5–10]. For instance, a study of 276 species at NLGRP demonstrated familial patterns of seed longevity, with Brassicaceae and Apiaceae being especially short-lived, and Fabaceae, Solanaceae and Chenopodiaceae being particularly long-lived [6]. Some evidence suggests that species originating from dry environments tend to produce longer-living seeds[6,7], a hypothesis we intend to test further in this proposal.

Problem*: *Germination assays used to monitor viability do not predict seed longevity

The fact that seed viability is only determined through germination tests presents a major hurdle to assessing longevity and decline in rare plant accessions. First, germination is binary: seeds are either alive or dead. Therefore, germination tests can only detect seed deterioration after the onset of seed death, when it is almost too late to regenerate or reintroduce irreplaceable plant populations. Further, 50-100 seeds are required in a germination assay to generate the statistical power necessary to detect a minor loss of viability before the inevitable abrupt decline. Consuming this amount of seeds in multiple monitoring tests over a 30-year period could completely deplete many rare plant accessions, which may have 500 seeds or less. Finally, germination results are confounded by seed dormancy, which masks viability of live seeds and is characteristic in most wild-collected seed accessions. For many seeds in CPC's collections, there is no *a priori* information on germination requirements, and research shows that the requirements change with storage time, making it difficult to interpret seed aging from germination tests. Thus, though germination tests are helpful in assessing current viability and learning about propagation requirements, they do not predict seed longevity and have limited utility in informing priority actions for curation.

Solution: Apply newly developed biochemical indicators of seed aging in storage, such as calculating RNA integrity numbers, to wild rare plant species

To understand how long seeds live in storage, we must first understanding how seeds die. Our strategy in this proposal is to use methods used to study food or drug stability[6,9] to increase aging rates

without compromising the physical structure of dried seed cytoplasm. Biochemical indicators such as RNA integrity number (RIN) detect degradation of seeds before seeds lose viability. RNA is easily damaged from reactions that cause aging. Over time fragmented molecules accumulate in cells and are easily detected by changes in the size distribution of fragments[9,11]. As an example of the procedure, note that high quality RNA has large amounts of 18s and 25s ribosomal subunits (peaks 3 and 4, respectively, in Fig. 1), while these proportions are reduced in degraded RNA (region 2 in Fig. 1). RIN is determined by a propriety algorithm [12] that reproducibly indexes RNA quality from 1-10 based on the detected proportions of ribosomal subunits. Fortunately, RIN can be easily quantified from the RNA extracted from the dry material of a single seed (2-5mg), meaning relative few seeds are needed to characterize average RIN for an accession.

NLGRP has pioneered the use of RIN for seed longevity, with very promising initial results. RIN has been shown to decline over time at a range of storage temperatures and relative humidity < 50% (Fig. 2). Because decline is linear, changes in RIN can be observed before differences in germination viability can be detected. Therefore, RIN appears to track seed aging more effectively and directly compared to germination assays. Furthermore, the rate of RIN decline (RIN_{slope}) is significantly correlated with germination half-life (P_{50}) in many tested agronomic species [9]. In addition to its linearity and predictive power, RIN assays require only 4 to 5 replicates per time point, or only 4 to 5 seeds, which is an order of magnitude less than the 50-100 seeds required for a germination assay. Consequently, adoption of RIN testing into curation practice could generate cleaner, more predictive data while wasting fewer precious rare plant seeds.

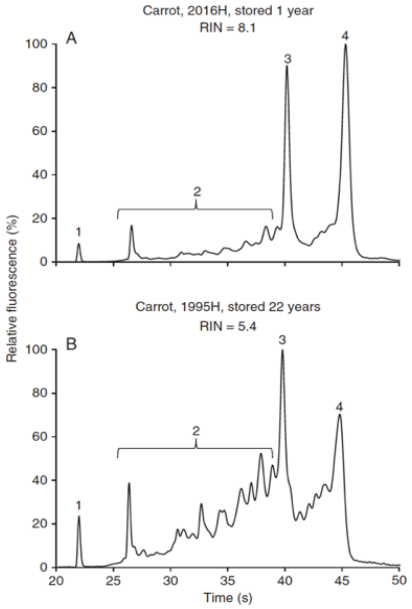

Figure 1. RNA electropherogram from carrot seed accession with differing RIN values (Fleming et al. 2018)

To assess the suitability of RIN testing and other bioindicators for seed longevity, we propose a series of experiments on wild collected seed to inform an analytical model of longevity patterns among various taxonomic, habitat, and growth conditions. Our experiments will test the following hypothesis:

If RNA integrity is a real-time indicator of seed health in wild rare plant species, then:

1) *RNA integrity numbers of seeds in storage should decline linearly over time regardless of storage temperature*
2) *Declines in seed RNA integrity number should be detectable earlier during the course of seed storage than loss of germination-measured viability,*
3) *The rate of decline in seed RNA integrity number (RIN_{slope}) should correlate with seed half-life (P_{50})*

Experiment A: We propose to be the first to use RIN to measure aging of seeds from diverse wild plant species. Our plan is to compare RIN and germination rates in approximately 100 seed accessions that have been stored for at least 15 years at -18ºC (the frozen seed treatment). We will also measure RIN and germination rates of seeds newly collected from the same population by the CPC network (fresh seed treatment). This will provide two time points from which we can calculate RIN_{slope} for 100 species.

In addition to RIN, we will measure three biophysical markers also implicated in the dynamics of seed aging: lipid (triacylglycerol) enthalpy, volatile compounds, and seed coloration. These metrics may provide mechanistic explanations for RIN declines: lipid crystallization[13] and the reactive oxygen species found in volatile compounds are known causes of seed (and RNA) damage. On the other hand, if lipid phase changes occur at a temperature threshold, this variable may account for observed non-linearity in

RIN with time in wild species. In this case, it may be necessary to use RIN and lipid enthalpy in tandem to predict longevity. Seed coloration is a very worthwhile metric to study because if changes in seed color correlate closely with RIN or germination based measures of aging; it would provide the botanical community economical and non-invasive metric of seed longevity that could be implemented in any seed bank with a digital microscope.

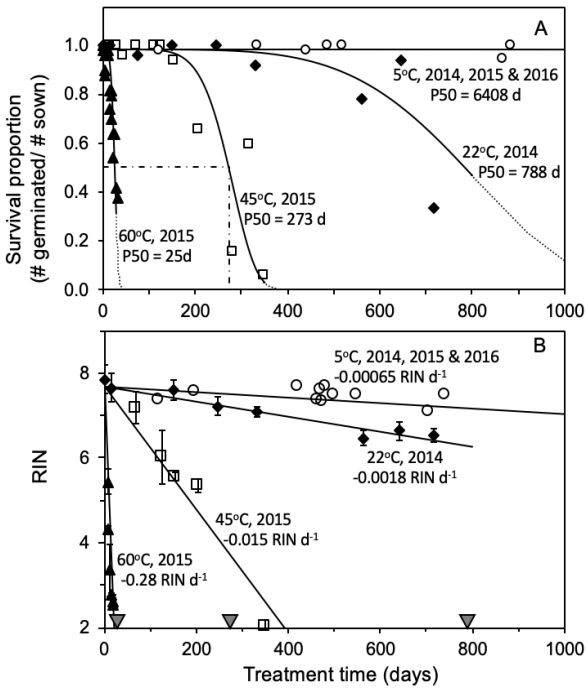

Figure 2. Declines in germination percent and RIN in soybean accessions monitored at a variety of storage temperatures. Walters et al. (in press).

Experiment B: Because none of target species have been in storage more than 35 years (Appendix I), it is possible that we will not see sufficient loss in germination to reliably calculate P_{50} long-lived species. Therefore, we will provide the needed link between RIN_{slope} and P_{50} by simulating aging in a subset of 20 freshly collected accessions by subjecting them to 55ºC under dry conditions. This treatment causes seed deterioration within 1 year (Fig 2A), and allows us to collect RIN and germination measurements at regular intervals.

Model Development: Whereas statistical differences in germination rates may be difficult to discern over a short timescale and are strongly influenced by non-aging phenomena such as dormancy, RIN_{slope} could provide a cleaner statistical target that could lead to more reliable inferences about the covariates of seed longevity. Given the potential of RIN as a metric of seed aging, assessing RIN in a diversity of plant species will improve our ability to predict seed longevity from phylogenetic, ecological, geographic, and climatic factors. We will utilize publicly available trait, distribution, and climate data to select the species for this study to maximize contrast in the factors predicted to influence seed longevity and test the following predictions about ex-situ seed longevity:

	Hypothesized Longevity in Orthodox Storage	
Species Trait	*Short-lived*	*Long-lived*
Habitat	Riparian, wetland, alpine	Grassland, Desert
Life form	Tree, shrub	Herbaceous, annual
Seed Mass	Large	Small
Taxonomic Group	Poaceae, Brassicaceae	Chenopodiaceae, Fabaceae
Bloom Period	Spring	Fall
Successional Status	Late Successional	Pioneer

From this analysis we will be able to improve general guidelines about longevity for orthodox seed storage, and generate specific predictions of seed longevity for all 4400 species ranked as globally rare in North America based on known species traits, distribution, and habitat preferences. By connecting our predictive model to an interactive web app, we will enable seed collectors to explore our existing longevity predictions and predict longevity values for additional species by inputting known traits. We envision that plant curators can use these recommendations to inform species conservation plans, including which species to prioritize, which storage solution is best suited for a species, and how best to curate seeds in banks.

1.2 Benefits for plant collections practitioners

Improving methodology for monitoring seeds in long-term orthodox storage is critical to the advancement of seed banking, a practice that is a cornerstone biodiversity conservation strategy. Most

directly, this study will generate RNA-derived and germination-derived seed longevity estimates for 100 wild rare plants species currently stored in long-term collections. This information produces a timeline for curation, recollection, or reintroduction of invaluable plant material that is currently not available. Without a clear plan, many of these species are poised to stay in storage indefinitely, even beyond their possible utility in providing living material to conserve our botanical heritage. More broadly, we believe that measuring RNA degradation rates in a wide diversity of species will provide guidance for future collections and subsequent storage recommendations based on observed patterns in the tested plant families, species, ecology and plant traits, which can be applied to plants the world over, including wild crop relatives, rare and endangered species, and culturally significant plant varieties. Through our collaboration with over a dozen seed banks, this project will educate the plant collections community about emerging technologies for estimating seed longevity, with the possibility of introducing new practices more broadly.

Collecting and processing seed is by far the most expensive aspect of seed banking. Yet letting the seeds die in storage means that the investment is wasted. By discussing results at CPC National Meetings and through a terminal webinar, the collaborators on this project will create a framework for testing seed longevity via RNA and other biochemical methods in wild rare plants species that will endure long after the term of this study. Collaborators can learn how to implement the technology in their own institution or through partners via lay and peer-reviewed publications, and CPC's web resources at saveplants.org.

1.3 Planned project collaborations

Conserving plants requires an integrated approach that involves active participation of collaborators from many private and government sectors. This project requires translating basic science about the mechanisms of seed aging into implementable curation activities for seed banks to promote a conservation goal. As such, this project relies on the expertise of NLGRP whose unparalleled facilities and expert staff provide the perfect setting to test the products of the recent 'genomics revolution' on valuable wild rare species. CPC has acquired pledges from 16 of the country's foremost rare plant conservation experts to re-collect and/or provide stored seeds for this project. This level of buy-in from botanical garden professionals, who also work to conserve these species in the wild, is key to acquiring fresh seed through recollections, and to ensuring that the curation recommendations arising from this study will be embraced by the botanical community. These botanical institutions will also provide accessions not held at NLGRP and will be critical participants in the decisions about species selection and discussions of results and curation implications. Finally, CPC will integrate collection and research findings of world-class botanical gardens and the USDA-NLGRP to address best management practices for valuable, small, heterogeneous and under-studied wild rare species. Through its 35 year history, the CPC network has generated numerous peer reviewed publications [4,14–16] that form the basis of our IMLS-funded online platform for Plant Conservation methods[1]. CPC not only has the experience and reputation for conservation synthesis, but also the mouthpiece to publicize these findings to the target audience.

1.4 Alignment with the goals of National Leadership Grant for Museums: Collections Care and Public Access Program

This study is squarely in line with the overarching IMLS National Leadership Grant Goal of *building capacity* in museums. Our overall goals are to engage more botanic garden professionals in genomic tools that enhance curation activities and improving technologies for accessions that are hard to curate. Through our evaluation of RIN as a metric of seed aging in a collaborative multi-institution framework, we will be re-evaluating existing seed curation paradigms and *sharing best practices and innovations* to the plant conservation community. By assessing the factors that predict RNA degradation

in storage, we will *identify trends* in seed curation that will allow extrapolation of results to extend beyond the study species and *will help organizations make informed decisions* about when to recollect, regenerate, or out-plant seed collections. By including a large number of collaborators and a terminal webinar, we will be enriching and *developing the plant conservation workforce*.

With its focus on curation practice, this project appeals directly to the objectives of the Collections Care and Public Access Program of the National Leadership Grant. Understanding how long seeds survive in frozen storage is critical *to caring for, conserving, and managing the collections that represent the natural foundations of our shared heritage*. The RIN method proposed for evaluating seed aging is a new application of *state-of-the-art collections care* that has the potential to prevent against the loss of invaluable plant material. We will be engaging in *cross-sector collaboration*, integrating members of the non-profit conservation community and a government-sector National Laboratory in a project that will *strengthen existing networks to enhance and sustain collections care*.

Project Work Plan

2.1 Specific project activities as numbered on Schedule of Completion

A1-A2. CPC National Office will begin coordinating species selection immediately in September 2020. CPC has compiled a preliminary list of 202 candidate species that have been stored for at least 15 years at either NLGRP or other participating seed bank (Appendix I), which we will amplify and refine once funded. Species selection will be informed by a CPC-generated database of attributes including habitat, life form, bloom period, seed mass, and feasibility of recollection communicated by partners. We will use these data to ensure we have the statistical power to test hypotheses about how each of these parameters influence seed longevity. We will select 100 additional species for inclusion in the comparison of RNA- and germination-based viability estimates of orthodox stored and freshly collected seed. A subset of 20 of these species will be selected for the simulated aging study (Exp B), which will use an additional 800 fresh seeds from the newly collected seed accession. After species selection is finalized, we will expand this database to include geographic distribution parameters, climate parameters, species functional traits, and a phylogenetic tree of study species for both the study species and all 4400 species to be included in our predictive model of seed longevity.

A3. After species selection, CPC will finalize initial subawards with Participating Institutions by January 2021. CPC will renew subawards each year (January 202, January 2022). Granting new contract each year will allow us to incentivize collections early in the funding cycle and redirect resources to participants with high collections capacity if needed to reach our collections goal.

A4. Seed from selected study species stored according to orthodox seed standards at a Participating Institution other than NLGRP will be shipped to NLGRP by April 2021. Participants will send 100-200 seeds from each accession. NLGRP will store these subsamples according to orthodox seed standards until they are tested.

A5. Participating Institutions (PIs) will collect proposed species seasons from the same wild population as the seed accessions held in orthodox storage between January 2021 and May 2023. PIs will strive to recollect rare species according to the CPC Best Practice Guidelines[1]. PIs will send to NLGRP a minimum of 800 processed fresh seeds for the simulated ageing experiment and a minimum of 100 fresh seeds for the fresh vs. frozen comparison (Experiment A) . Species selected for the simulated ageing experiment (Experiment B) will be sent to NLGRP by February 2022 to allow enough time to complete the experiment in the term of the grant. The remaining collections will be sent to NLGRP by January 2023. PIs will submit field collections data for each accession using the CPC PI web portal (ww.saveplants.org/login).

A6-A9. NLGRP will begin testing viability of frozen seeds in January 2021 using germination methods established through years working with these species. We plan a suite of tests beyond the

conventional germination assays and newer RIN assessments, to include plant trait assessments (triacylglycerol crystallization tendencies, emission of volatile compounds, seed mass, and seed (dis)coloration). For germination assays, 50-100 seeds will be pretreated to break dormancy and monitored weekly until seeds germinate or become mushy when lightly squeezed. RNA will be extracted from 5 replicates (typically 5 seeds). NLGRP will conduct parallel germination and RIN assessments upon receipt of fresh seed samples. For the ~20 species selected for the dry ageing experiments, NLGRP will dry seeds at 5% relative humidity, package seeds in foil laminate containers, and incubate packages at 55ºC. Germination and RIN will be assessed in three month intervals for 18 months. We will end germination assays when there is no observable germination. NLGRP's data records will be continuously available through a shared web platform set up by the CPC National Office.

A10-A11. CPC and NLGRP will give project progress updates to the Participating Institutions and the rest of the CPC network annually at the CPC National Meeting (2021, 2022, 2023).

A12-A13. Upon completion of laboratory testing in 2023, the CPC National Office and NLGRP will work jointly to analyze for submission of a synthesis paper summarizing seed shelf-life for peer review. NLGRP will analyze the relationship between RIN_{slope} and P_{50} for different storage temperatures. CPC will create a statistical model of evaluating the factors associated with RIN_{slope} in our 100 study species and will use database of species traits compiled in **A2** to correlate longevity (through RIN_{slope}) to other factors and predict seed longevity for 4400 rare plants in North America. These predictions will be shared publicly via an interactive web application hosted on the CPC website.

A14. In July 2023, we will hold a half-day terminal webinar for participants and other stake holders in the plant conservation community where CPC and NLGRP staff will discuss implications of seed longevity measurements for seed curation and conservation planning. CPC National Office will work with NLGRP and PIs to synthesize the recommendations from the terminal webinar into their web-based best practice guidelines, and publicize our findings through conference presentations and web outreach.

A15. In August 2023, CPC will circulate a project summary with a final performance evaluation survey to all 50 long-term seed banks in North America.

2.2 Project maturity level

Pilot studies of agronomic species have demonstrated that RIN may be a useful metric of seed longevity in a broader suite of species. Therefore, we contend that this project is in the "scaling" phase because we would like to expand the use of this technology to a new domain (wild rare plants) and to a new group of users (plant conservationists, botanic gardens). Using RIN-based estimates of seed longevity in conservation seed banking greatly expands beyond preventing loss of agricultural seed lots to saving irreplaceable plant biodiversity.

2.3 Project planning and management. *Activity numbers included parenthetically*

Project planning and management will be divided between the CPC and NLGRP. CPC Data Scientist, Dr. Katie Heineman, will coordinate species selection (**A1**) and contracting of fresh seed collection with CPC PIs (**A3-A5**). Dr. Heineman will also compile species covariates for species selection and statistical models (**A2**), and create web-based data forms for sharing field collection information and laboratory generated results (**A6**). Dr. Christina Walters, NLGRP, will oversee the laboratory seed aging/longevity testing and quality control in data collection (**A7-A9**). CPC will plan the logistics of CPC National Meeting and terminal webinar (**A10,A15**). Dr. Walters will lead the analysis of laboratory results and writing of scientific manuscript(s) related to seed performance in a genebank (**A13**), and CPC National Office will lead the analysis of seed longevity traits with phylogenetic and ecological factors and the development of the web app displaying predictive model results (**A12**). All manuscripts will be developed in collaboration with the principals of this proposal and targeted for peer-review. CPC will

ensure the synthesis of recommendations are shared in public databases and CPC Best Practice Guidelines, and will write and circulate the final performance evaluation survey (**A13-15**).

2.4 Ensuring Diverse Perspectives and End User Engagement

A broad variety of professionals are potential end users of the insights and recommendations generated from this project: long-term seed banks, land managers, agricultural companies, academic researchers, and government agencies. By involving the seed banks that store wild rare plants in recollection, we ensure that the seed longevity insights from this project direct their future curation practice. We will encourage the perspective of land managers and policy makers by inviting the members of government agencies to the terminal webinar, with goal of more explicitly including timelines of seed recollection or out planting in species recovery and conservation planning and developing a new set of best practice guidelines. We will also use our professional network to publicize the recollection of these seeds as a resource for academic studies through our peer-reviewed manuscripts, upload to public databases, and communication through social media and web resources.

2.5 Project risks

1) Seed collection is inherently unpredictable. Not all plant populations fruit every year, especially in small rare plant populations with few adult individuals. We realize that, despite our best efforts to select feasible species for recollection, we will not be able to collect all of the species selected by our intial process. To offset this risk, our current candidate species list contains two times more species (~200) than we are proposing to analyze (Appendix I), allowing us some wiggle room to shift species without sacrificing sample size of our hypothesized trait groups.

2) Our project hinges on the 15-year-old seed having observable RNA deterioration. Fifteen years is likely an insufficient duration to observe changes in viability, and possibly RIN, in many seeds (see Fig 2A for timescales at 5C). Because RNA degradation rates follow classic temperature dependencies[9] the risk of no change can be mitigated by including additional species to our simulated ageing experiments (Experiment B).

3) Estimates of survival *times* for seeds stored in the freezer (Experiment A) hinge on a strong correlation between RNA degradation rates (RIN_{slope}) and seed half life ($P50^{-1}$) for the 20 species subjected to 55C treatments (Experiment B). A poor correlation in our "standard curve" would indicate a disconnect between our biochemical assay (RIN) and our functional assay (germination). A major reason for this disconnect could be the intrinsic heterogeneity of wild-collected seeds with large within-population variability in the rapidity of aging. We would address this contingency by examining the effect of more RIN replicates on the average RIN value. We would also test correlations with other biochemical assays we are developing such as volatile emissions and lipid crystallization, to determine if the inclusion of these variables improves prediction of $P50^{-1}$ from RIN_{slope}.

Time, financial, and personnel resources needed

The proposed period of this project is September 2020 to August 2023. This project requires $476,040 in support to achieve proposed outcomes. The majority of this request will fund botanical institutions to recollect seed accessions from wild populations, which CPC will reimburse at $2800 per collection ($280,000). We request $105,000 to fund laboratory work of frozen and fresh seed accessions at NLGRP including the cost of labor, laboratory supplies, and overhead. We request $4500 for travel to NLGRP staff to travel to the CPC National Meeting in each year of the grant. We request $60,400 for coordination, research, web development and administrative duties of the CPC National Office.

Key personnel for this project include CPC National Office staff, NLGRP, and Participating Institutions in the CPC network. **Joyce Maschinski, CPC President and CEO,** is a world-renowned expert in the reintroduction of rare plants from *ex-situ* collections. She orchestrated the last CPC Island

Press publication, co-authored CPC guidelines for rare plant reintroduction, and co-wrote the newest set of CPC guidelines for seed collection, curation and banking. **Dr. Christina Walters, Supervisory Plant Physiologist at NLGRP,** is a leading researcher in the field of seed banking whose lab pioneered the use of RIN as a metric of seed longevity in agronomic plant species. She has published five publications on this topic in the past three years. **Lisa Hill, Biological Lab Science Technician at NLGRP,** has 25 years of experience working with seeds and helped develop the biochemical assays required for this analysis. **Dr. Katherine Heineman, CPC Data Scientist**, has technical experience in data management, statistical analysis, and web building. Dr. Heineman currently coordinates data sharing and synthetic analysis with four of the PIs in the study for the California Plant Rescue seed collecting initiatives. **CPC PIs**, as represented by the16 leading botanical institutions who have expressed their support, are committed to recollecting seed for this project (See Letters of Commitment in Appendix II). Collectively, this group has published more than 200 papers on the conservation and curation of rare plant species, and hold over 1200 rare plant species in orthodox seed collections.

2.6 Tracking progress & Sharing results

CPC will track progress on seed collections by requiring seed collectors to submit data through the NLGRP form on the CPC website prior to reimbursement. We will track progress on lab work by creating a web form for NLGRP to upload data as laboratory assays are completed. Each year we will present interim progress and results at the CPC National Meeting. We will make laboratory seed longevity data for this study available through the CPC website (saveplants.org), peer reviewed scientific literature and publicly accessible trait and seed databases. We will also post standard operation procedures for seed biochemical assays as part of web CPC Best Practice Guidelines. We will share the general findings and conservation recommendations through our terminal webinar, CPC National Meeting, and our web-based publicly accessible CPC Best Practice Guidelines. We will leverage the CPC social media accounts, list serves, professional conferences to amplify the message posted in the specified channels.

3.1 Intended results

Overall results are expected to 1) increase the applicability of a new curation tool to measure seed deterioration of seeds before mortality is detected; 2) provide the first estimates ever of how long seeds of rare plant species from North America survive in a seed bank; 3) guide seed storage practices for rare species to ensure target longevities are met through orthodox storage freezers; and 4) develop ecological correlates to predict factors that correspond to short and long-lived seeds. These results will have major impact to the seed banking world for agriculture and conservation, in a way that increases the efficiency and effectiveness of curation of seed collections. Moreover, these results will provide critical missing information about ecotypic variation in seed longevity that addresses profound questions in diverse fields, such as persistence of seeds in soils; molecular genetic traits that confer seed longevity; and assays that portend lost functionality in diverse solid-state materials such as dried foods, drugs, and plastics.

3.2 Anticipated change in knowledge, skills, and behavior of intended audience

The work will demonstrate to the seed banking community that one must care for seeds after they are collected and stored, in contrast to a pervasive mindset that seeds in a seed bank will last forever. The 'new reality' will ensure that seed collections are replenished in a timely fashion to ensure high quality collections stay valuable. Decision-makers will also be confronted with hard data to show that the cost of seed banking extends beyond collection in the wild, and maintaining a collection requires a business model that ensures the initial investment of getting seeds pays off. Finally, the work will

introduce an alternative for viability monitoring that provides quantitative data using a fraction of seeds and is more predictive to seed longevity.

3.3 Potential barriers for adoption of new practices

Although some gardens may lack personnel and financial resources to the wide adopt RIN as a seed longevity metric, collaborations are possible. Understanding the broad patterns of seed longevity we plan to model will help with species-specific recollection, regeneration, or outplanting plans. We hope to make resources for both RNA extraction and seed recollection more available to seed banks by actively publicizing the results of this experiment to agencies that fund plant collections work. In addition, providing actionable, species-specific insights, this project will help seed banks allocate limited resources to the species with the particularly short life spans, while reducing intensive curation attention (germination testing) on especially long-lived seeds.

3.4 Measurement of project success

The establishment of RIN as a metric of seed ageing would be an important scientific and curatorial advance that impacts seed banks from all sectors, including agriculture. Moreover, providing new knowledge about the potential longevity of seeds from North American species will provide important information on the botany, reproductive biology and evolutionary potential that currently doesn't exist. This project will generate recommendations for seed banking, and how these are embraced by the plant collections community will be an important metric of success. To measure the attitudes of this community, we will circulate a performance evaluation survey to 50 participating and non-participating seed banks identified through Botanical Gardens Conservation International garden search, including a summary of the patterns of seed longevity and recommendations identified through this project. Survey questions will focus on three topics: 1) characterization of the institution's size, staffing, and collections priorities 2) the likelihood that the institution will change existing practice based on new seed longevity recommendations and 3) the likelihood that the institution will consider RIN determination for seed longevity testing in rare or high value seed collections.

3.5 Tangible products from project

This study will generate a collection of rare plant seeds consisting of paired accessions sampled from the same population decades apart. This collection is valuable for both the present study and future research addressing climate change, habitat fragmentation, and other threats facing endangered plants. We will produce a publicly available dataset and peer-reviewed publications that describe methods used to test seed viability using and RIN, as well as other seed traits and provide estimates of seed longevity using both rates of germination and change in RIN for 100 rare plants species. Educational/professional development products will include revised *CPC Best Practice Guidelines*, laboratory protocols for RIN, lipid, volatile compound, and seed coloration, and a web-tool predicting species seed storage longevity from available trait and geographic data.

3.6 Sustaining Benefits

CPC will sustain the benefits of this project by codifying the seed longevity recommendations into its industry standard Best Practice Guidelines, which are widely adopted throughout the plant conservation community. To put these recommendations into action, CPC will continue its long track record of securing funding for seed collection and re-collection to enable longevity testing in additional species and to replace valuable accessions nearing the end of their shelf life. NLGRP will collaborate in sustaining this benefit by providing RIN analysis for to seed banks in a fee-for-service model, while working to reduce the cost of this analysis through automation. CPC will support maintenance and hosting of longevity data and seed longevity web app as part of its suite of web resources.

Schedule of Completion: RNA integrity as a powerful metric of aging in preserved seed collections of wild rare plant species

Activity	Description	2020	2021				2022				2023		
		Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Project Coordination & Planning													
A1	Select candidate species for recollection and Simulated Aging												
A2	Compile database of rare plant species traits (model covariates)												
A3	Coordinate subawards with seed collectors												
Seed Collection and Material Transfer													
A4	Transfer seed in long term frozen storage lots to NLGRP												
A5	CPC Participating institutions recollect seed from wild populations of selected species												
Laboratory Seed Longevity Testing													
A6	Create web forms for sharing laboratory results												
A7	Seed biochemical testing of frozen seed accessions (100 acc - Exp. A)*												
A8	Seed biochemical testing of recollected accessions (100 acc - Exp. A)												
A9	"Simulated Aging" on recollected seeds (20 acc* 6 times= 120 tests- Exp. B)**												
Collaboration and Training Activities													
A10	Progress reports to collaborators at CPC National Meeting												
A11	Terminal Webinar for Seed Bank Community												
Analysis and Synthesis													
A12	Create predictive model of seed longevity with asociated web app												
A13	Share data & analysis publicly through submission to peer-reviewed manuscripts and public trait databases												
A14	Synthesize recommendations into CPC Best Practice Guidelines in conjunction with terminal webinar												
Final Performance Evaluation													
A15	Circulation of community surveys to evaluate performance												

*In Experiment A ("Real Time" aging) the following characteristics will be tested at two time points (immediately after collection and after many years of storage): RIN, germination %, triacylglycerol levels, volatile compounds, and seed coloration

**In the "Simulated Aging Experiments" the following seed attributes will be tested on freshly collected seed accession at six time points at 55 C: RNA, germination %, triacylglycerol profile, volatile compounds, and seed coloration