a. ***Project Title***: **Speed Breeding Experiment for Producing Transgenic American Chestnut Pollen**

b. ***Summary*** *(not more than 100 words)*

This project deploys and compares three high-light methods for rapid pollen production from transgenic American chestnut seedlings. Based on discussions with SUNY-ESF staff and a literature review, our three high-light methods are: (1) growth chamber, (2) plant tent, and (3) greenhouse, exposed to seasonal light. Each has advantages and disadvantages we will assess. Two undergraduate interns will refine methods associated with growing seedlings, controlling pests, and rapidly producing and storing pollen. We will deep-freeze the resulting transgenic pollen while awaiting federal evaluation for public release. The PI and the students will produce a research poster and article for *Chestnut* journal.

c. ***Principal Investigator***: Professor Thomas Klak, PhD, Department of Environmental Studies, University of New England, Biddeford Maine

d. ***Duration of project***: 1 year, Dec. 1, 2019 to Dec. 1, 2020

e. ***Total amount requested***: $10,000.

Please list sources and amount of matching funding for the same project:

e.1. PI Uncompensated Time: The PI will devote an average of approximately six (6) hours per week to this project throughout its duration. A significant part of the PI time will be training and overseeing students in all of the greenhouse and growth chamber methods, data collection, and data analyses associated with growing chestnut seedlings that he has refined over the past four years.

e.2. Project Infrastructure provided by the University of New England: Most of the essential infrastructure for this project is provided at no cost by the host institution, the University of New England (UNE). More specifically, the UNE plant growth chamber has been recently inventoried to cost $46,000 if it were replaced, and it's been very little used until my recent speed breeding experiments with chestnut seedlings. Additionally, the PI has a greenhouse that UNE provides, and that includes electricity (and electrician support), heating and cooling, fans, lighting timers, a room-sized humidifier, a PAR meter, PH testing equipment, and various other inputs. UNE also provides freezer space at -80C. Sara Fitzsimmons suggested there is no need to attempt to monetize the above infrastructure as matching costs. But clearly their value is substantial and represents a large subsidy both to this speed breeding experiment and to the larger chestnut restoration project.

e.3. Undergraduate Student Chestnut Restoration Work Beyond the Grant-Funded Internship:
- Fall Semester 2019 Directed Research course: Two or three Environmental Science undergraduates will enroll in a Directed Research course with the PI during Fall semester 2019 entitled *Environmental Research: American Chestnut Restoration*. Students will be trained in and will deploy techniques associated with the speed breeding of chestnut seedlings, among other aspects of chestnut restoration work. Data collection will be central to the students' responsibilities.
- Spring Semester 2020 Ecological Restoration course: Each Spring semester the PI teaches an advanced environmental science course enrolling 24 undergraduates. A key component of this course is an overview of the American chestnut project. In the UNE greenhouse, these students grow hundreds of seedlings from wild-type mother trees from Maine and beyond. The best seedlings are then planted in

Germplasm Conservation Orchards. During Spring Semester 2020, these ecological restoration students will be able to engage, not only the genetic diversity component of chestnut restoration (seedlings from wild-type mother trees), but also in the blight-tolerant component (from transgenic chestnut seedlings). While these students will not directly conduct the speed breeding experiments, they will be regularly apprised of project developments by the project-funded student interns. I approach the best students in this Ecological Restoration course to join my lab and greenhouse team for the next stage of our chestnut restoration work.

- **Summer (May-August) 2020 Funding for Student Interns**: The two UNE student interns this grant would fund during Spring Semester 2020 will apply for Summer 2020 funding through UNE's SURE program (Summer Undergraduate Research Experience); although funding is not guaranteed *a priori*, this on-going speed breeding project will be highly competitive to be funded.

f.1. ***Short-term goals of the project:***

- Produce transgenic American chestnut pollen under high-light conditions.

- Compare and contrast three high-light methods to assess the pros and cons of each.

- Develop and document successful methods for combatting pests that effect chestnut seedlings in growth chambers and greenhouses (e.g., mealybugs, fungal gnats, mold, powdery mildew).

- Deep-freeze desiccated pollen at -80C and periodically test samples of it for viability.

- Provide two undergraduate Environmental Science majors at UNE with a hands-on science experiment that contributes to the American chestnut restoration project; specifically, students will learn to deploy speed breeding techniques to help restore a flagship species of the eastern US forest.

- Work with the two undergraduate students to disseminate the results of this speed breeding experiment through a symposium research poster (May 2020) and an article to be submitted to *Chestnut* journal (Dec. 2020).

f.2. ***Long-term goals of the project***:

- Following federal transgenic approval, we will work with TACF and ESF to deploy the stored transgenic pollen to pollinate wild-type trees and their GCO (Germplasm Conservation Orchard) progeny in Maine and in New England more broadly (working with colleagues in the VT/NH and Mass/RI chapters).

- Continue to refine speed breeding methods to produce greater quantities of pollen from the next generations of regionally-diversified transgenic seedings (T3, T4, T5).

g. ***Narrative*** (no more than five (5) pages)

1. **Introduction**

As we await federal evaluation of the transgenic American chestnut, there is much to do to prepare for its release and dissemination across its native range. Demand for transgenic pollen will be great, and so establishing pollen production and storage methods at locations besides SUNY-ESF is warranted.

Speed breeding is an emerging method in plant (including chestnut) research; it shortens the time from seed to sexual maturity. The rapid generational advancement of speed breeding is valuable to TACF's 3BUR project. It is especially valuable to the transgenic project because of its clonal beginnings and the need to breed transgenic chestnuts with a wide variety of wild-type chestnuts over generations.

The PI's prior chestnut propagation experience is considerable. During the last four years, he and his students have deployed an LED-lit greenhouse to grow thousands of wild-type American chestnuts from seed. The seedlings have been planted in germplasm conservation orchards that the PI manages with landowner partners in multiple locations across Maine. A few of these wild-type seedlings have produced pollen as early as only two years in the field, attesting to successful speed breeding from greenhouse to field trials. The proposed project aims to intensify the speed breeding effort through enhanced high-light indoor conditions.

Recent discussions with Jared Westbrook and Andy Newhouse on how UNE could contribute to the transgenic chestnut program led to the PI applying to USDA-APHIS in May 2019 for restricted permitting. APHIS granted the PI permission to move transgenic chestnut material from ESF to Maine, to work to produce transgenic pollen in indoor facilities only, and to either deep-freeze the resulting pollen or to return it for use at ESF. Transgenic pollen production at UNE will adhere to the stipulations of the authorization provided by APHIS on May 31, 2019 (APHIS Notification No. 19-148-102n; copy available from the PI upon request).

In July 2019, the PI and two other New England TACF representatives obtained training at ESF in speed breeding and controlled pollination techniques for making transgenic and wild-type chestnut crosses (T3). The work included collecting and using pollen that was fresh from growth chamber and greenhouse seedlings, as well as deploying pollen that had been frozen for one year at -80C.

The PI's current APHIS permit allows up to 50 (fifty) transgenic chestnut seeds and/or seedlings to be transported from ESF to UNE for indoor lab/greenhouse pollen production experiments. In July 2019, 12 (twelve) T2 seedlings were moved in this way to begin the test of experimental speed breeding techniques at UNE. By December 2020, when the project period ends, these T2 seedlings will be more than 18 months old. This should be more than enough time to produce pollen from some of them as they are distributed evenly among the three high-light treatments (explained below).

In continued cooperation with ESF, the proposed project would move an additional 38 transgenic seedlings (T2) or seeds (T3) to UNE by December 2019. After being sowed indoors in January 2020, these transgenic seedlings will be 11 months old by the conclusion of the project period in December 2020. This will provide sufficient time for some of the seedlings in the second allotment to produce pollen under the three high-light conditions described below. Those seedlings that have not produced pollen by age 11 months will continue to be exposed to the high-light treatments after the project period.

2. **Three Speed Breeding Methods**

At the University of New England, the lab and greenhouse in which the PI works occupy rooms 243 and 242 in the Alfond Center for the Health Sciences. There, the PI has organized and begun to equip the three speed breeding arrangements described below. Over recent months, the PI has begun experimenting with these speed breeding methods, first using wild-type American chestnut seedlings, and then more recently with the initial twelve transgenic seedlings. For seedlings subject to each of the three methods, the PI continues to measure and adjust the levels of PAR (photosynthetically available radiation). The aim is to incrementally ramp up the PAR light intensity and also the daylength to levels similar to the peak summer maximum. These are among the methods for which the PI will train the undergraduate students.

Students will be responsible for the monitoring, adjustments, measurements and data recording and analysis.

a. ***growth chamber***

Several recent studies have successfully deployed growth chambers for speed breeding of plants other than chestnuts (mainly annual crops; Watson et al. 2018; Ghosh et al. 2018). ESF has developed proven systems for rapidly producing pollen from transgenic chestnut seedlings, which this project will emulate. ESF staff have deployed high-light, and humidity and temperature-controlled systems in growth chambers and modified greenhouses. They have achieved a 6-18-month cycle from planted seed to pollen-bearing seedling (Baier 2012).

The UNE growth chamber is a Percival model PGC-105HID high-intensity discharge, and is comparable to those at ESF. The UNE growth chamber is programmable to control light intensity, daylength, temperature, and relative humidity, and has two sets of lamps. The six MH (Metal Halide) lamps provide blue and violet spectrum PAR wavelengths to stimulate vegetative growth. The six HPS (high pressure sodium) lamps provide red and orange spectrum PAR wavelengths to stimulate flowering and fruiting. Total light intensity is up to 1250 µmoles/m2/s of light irradiance measured at 24" from the lamps (i.e., similar to full summer peak PAR sunlight, as deployed at ESF; Baier 2012). Total shelving floor area at UNE is 1.5 square meters.

The growth chambers at ESF and UNE are valuable speed breeding assets. However, they have limitations related to space (and therefore the number of seedlings) relative to a greenhouse. Additionally, a growth chamber like that at UNE requires an investment in the tens of thousands of dollars. Operating costs and repairs are also expensive. Growth chambers are therefore cost prohibitive for most chestnut restoration enthusiasts. Thus, it is valuable to compare growth chamber speed breeding with other methods that are lower-cost and therefore more accessible.

b. ***plant tent***

Some researchers have extended their growth chamber projects by asking whether they can achieve similar results at much lower costs. Both the up-front and on-going infrastructural requirements of a plant tent with LED grow lights are vastly less expensive than those of a growth chamber. Researchers have successfully sped up annual plant cycles using plant tents and have provided instructions for establishing such a system including measuring growth parameters, even at home (Katagiri et al. 2015; Ghosh et al. 2018). A plant tent system, if successful for speed breeding chestnuts, would make speed breeding accessible to a much wider community involved in the collective chestnut restoration project.

The plant tent at UNE is a Vivosun 48"x48"x80" Mylar Hydroponic Grow Tent (1.5 square meters of floor space, same as the growth chamber). It is equipped with several 1000w Full Spectrum LED Grow Lights on timers. It is located in a corner of the UNE greenhouse, and it has temperature and relative humidity controls. The experiment will therefore compare and contrast speed breeding via growth chamber and plant tent, while attempting to hold constant parameters such as PAR lighting levels, daylength, soil moisture, fertilizer, temperature and relative humidity as much as possible. Students will record these data.

c***. greenhouse setting, exposed to seasonality***

Speed breeding under high-light conditions usually eliminates winter dormancy; plants are subjected to extended periods of intense PAR light without seasonal changes. Speed breeding often deploys near-maximum PAR lighting for 16 to 22 hours per day depending on the plant, throughout the year (Baier

2012; Watson et al. 2018). What does this method exclude that is valuable to the sexual maturation of a deciduous, perennial plant? As researchers have explained with reference to plants other than chestnuts: "Flowering, tuberization and bud set are just a few of the many different responses in plants that are under photoperiodic control" (Jackson 2009 p.517).

To our knowledge, the direct role (if any) that seasonality plays in chestnut pollen production is not known. In order to test whether winter dormancy plays a role in advancing seedlings toward pollination, in this treatment, seedlings will receive LED lighting in our UNE greenhouse, but the PAR level and duration will change seasonally. Lighting will be decreased during the winter to a photoperiod of only six hours; PAR levels will be similarly lowered for the winter, and temperatures will also be decreased. Temperatures, light intensity, and the photoperiod will then be increased again to reach peak summer PAR by June 2020.

3. **Conclusion**

This research proposes to produce transgenic American chestnut pollen under high-light conditions, comparing and contrasting three high-light methods to ascertain the pros and cons of each. Two student interns will refine methods associated with growing seedlings, and rapidly producing and storing pollen. Throughout the experiment's duration, the students will also test and document methods for combatting pests that effect chestnut seedlings in growth chambers and greenhouses (e.g., mealybugs, fungal gnats, mold, powdery mildew). Pollen produced by our speed breeding methods will be desiccated and frozen at -80C to preserve it for a year or more. Samples of the frozen pollen will be periodically tested for viability.

The project will provide two undergraduate Environmental Science majors at UNE with an extended, hands-on science experience that contributes to the American chestnut restoration project. Students will learn to deploy speed breeding techniques to help restore a flagship species of the eastern US forest. The PI will work with the students to disseminate the results of this speed breeding experiment through a symposium research poster (May 2020) and an article to be submitted to *Chestnut* journal (Dec. 2020).

Following federal transgenic approval, the PI and future students will continue to work with TACF and ESF to deploy the stored transgenic pollen to pollinate wild-type trees and their GCO (Germplasm Conservation Orchard) progeny in Maine and in New England more broadly (working with colleagues in the VT/NH and Mass/RI chapters). Beyond the grant period, the PI and students will continue to refine the speed breeding methods to produce greater quantities of pollen from the next generations of regionally-diversified transgenic seedings (T3, T4, T5).

4. **Literature Cited**

Baier, K. et al. 2012 "Chestnuts and light: Early flowering in chestnut species induced under high-intensity, high-dose light in growth chambers" *Chestnut* May/June, pp.8-10.

Ghosh, S. et al. 2018 "Speed breeding in growth chambers and glasshouses for crop breeding and model plant research" *Nature Protocols* volume 13, pp.2944–2963.

Jackson, S. D. 2009 "Plant responses to photoperiod" *New Phytologist* Volume 181, pp.517–531.

Katagiri, F. et al. 2015 "Design and construction of an inexpensive homemade plant growth chamber" *PLoS ONE* volume 10, pp.e0126826.

Watson, A. et al. 2018 "Speed breeding is a powerful tool to accelerate crop research and breeding"

Nature Plants volume 4, pp.23–29.

h. ***Timeline, showing start and completion dates for each goal***:

1. Dec. 2019: introduce student interns to the experiment; purchase equipment and supplies; obtain additional transgenic seeds/seedlings from ESF.

2. Jan.-Feb. 2020: student interns sow of transgenic seeds, and establish of the three techniques and on-going measurement logs.

3. March-April 2020: student interns continue to advance the experiment and document trends.

4. May 2020: student interns compile and present initial results in a symposium research poster.

5. June-August 2020: students continue monitoring and collecting data on seedlings in the three speed breeding methods.

6. Sept.-Dec. 2020: students consolidate findings since the project began in the Summer 2019 and report them in an article submitted to *Chestnut*.

i. ***How results will be measured and reported:***

Measurement:

- We will record over time the seedling propagation techniques and results (e.g., watering sequence, fertilization rates, PAR levels, challenges such as pest outbreaks and control methods, seedling conditions).

- We will measure the comparative seedling growth rates associated with the three speed breeding methods.

- We will measure pollen production and volume.

- We will test frozen pollen viability over time.

Reporting:

- The two student interns will jointly present their speed breeding findings to date in a poster at the annual undergraduate research symposium at the University of New England in May 2020. Once the project term is completed in December, 2020, the students will work with the PI to report the full project findings in an article to be submitted to *Chestnut* journal.

j. ***Breakdown of how and when funds will be spent***:

- Two student interns, Spring semester (Jan.-May) 2020; these two interns will earn 3 credits toward their undergraduate degrees in Environmental Science:
Pay is for 12 hours per week x 16 weeks x $12/hr = $2,304 x 2: $4,608

- 50 RootMaker® pots, 2-Gallon Round, with shipping (Dec 2019): $250

- Additional & Replacement LED solar lights (Dec 2019-Sept 2020): $1,300

- Soil (Jolly Gardener (mainly peat; includes aged pine bark; Dec 2019): $400

- Fertilizers (Miracid, Osmocote, Micronutrients; Dec 2019): $342

- Watering system: nutrient distributor, PH regulator, & watering (Dec 2019): $1,000
(note: tap water in UNE lab building is ~7.3PH; chestnuts prefer 5.5PH; too high of water PH and seedlings cannot absorb Miracid)

- Plant pest control products (note: greenhouse & growth chamber conditions are challenged by a variety of pests, which require on-going diagnosis & experimental treatments; e.g., nematodes can be useful for root-eating insects; Dec 2019-Sept 2020). $700

- Miscellaneous lab & greenhouse supplies (Dec 2019-Nov 2020): $500

- PI travel to ESF for 2 work days (4 days including travel) for additional speed breeding training, to assist with the transgenic project, and to move T3 transgenic chestnut seeds and/or seedlings, as APHIS authorization permits (Winter 2019-2020): $900

k. ***Brief Curriculum Vitae*** *(CV) for the Principal Investigator, including recent publications and grants received. Please restrict the CV to two (2) pages.*

THOMAS KLAK – Email: tklak@une.edu; Phone: 207-391-9496

1. Professional Positions

A. *Education*
Ph.D., 1987, Geography, University of Wisconsin-Madison
M.S., 1982, Geography, University of Wisconsin-Madison
B.A., 1979, Augustana College, Rock Island, IL

B. *Recent Professional Positions*
2011 - Professor, Department of Environmental Studies, University of New England
2009 - 11. Director of the Latin American, Latino/a, and Caribbean Studies Program, Miami University
2003 – 12. Professor, Geography Department, Miami University (now Professor Emeritus)

C. *Topical Specializations*
- sustainability
- local ecological restoration and native landscaping
- the theories, discourses, practices, and ecological consequences of development

2. Publications and Other Scholarly Achievements

a. Selected Recent Publications

2018a Klak, Thomas and Mathew Sutherland, "Planting Pure American Chestnuts on Wild and Remote Long Island, Maine" *Chestnut: The Journal of the American Chestnut Foundation* Spring, 32(1)12-14.
2018b Klak, Thomas, "Preserving and Making Accessible American Chestnut Biodiversity in Maine" *Chestnut: The Journal of the American Chestnut Foundation* Winter, 32(2)10-12.
2018c Klak, Thomas, "TACF-Landowner Partnerships: An Unfolding Story from Maine" *Chestnut: The Journal of the American Chestnut Foundation* Fall, 32(3)10-12.
2017. Collins Tracey, Chris Indorf, and Thomas Klak, "Creating regional consensus for starting school later: A physician-driven approach in Southern Maine", *Sleep Health: Journal of the National Sleep Foundation,* Vol. 3, pp.479–482 https://doi.org/10.1016/j.sleh.2017.10.002
2017. Noah Perlut, Thomas Klak, and Eldar Rakhimberdiev, "Geolocator data reveal the migration route and wintering location of a Caribbean Martin (*Progne dominicensis*)", *The Wilson Journal of Ornithology* Vol 129, No. 3, pp. 605–610.
2016. Thomas Klak and Joseph Simonowicz, "Relationships between World Heritage Sites and their Contiguous Communities: Cross-Scale Analysis and Dominica Case Study," chapter in: *Global Change and the Caribbean: Adaptation and Resilience*. Barker, D., D. McGregor, T. Edwards and K. Rhiney (editors). Kingston, Jamaica: University of the West Indies Press.
2016. Edward L. Jackiewicz and Thomas Klak "Mass and Alternative Tourisms in Latin American and the Caribbean" in *Placing Latin America: Contemporary Themes in Geography*. (Third Edition) Ed Jackiewicz and Fernando Bosco (editors). Lanham, Maryland: Rowman & Littlefield.
2016. Thomas Klak, "Economic and Geopolitical Vulnerabilities and Opportunities" in *Placing Latin America*: *Contemporary Themes in Geography*. (Third Edition) Ed Jackiewicz and Fernando Bosco (editors). Lanham, Maryland: Rowman & Littlefield.

2016. Thomas Klak. "The Importance of Intercultural Engagement in Study Abroad: Experiences in Dominica" *International Agenda* http://www.schoolcraft.edu/department-areas/international-institute/international-agenda Vol 13, No. 2, pp. 14-17 & 23.

b. Editorial and other Professional Experience

A. *Experience as an External Reviewer*
(1) Manuscripts Reviewer for 40+ Scholarly Journals
(2) Book Proposal or Manuscript Reviewer for 13 Publishers
(3) Research Proposal Reviewer for 3 Organizations
(4) Applications for Promotion and/or Tenure Reviewed for 12 Universities

B. *Recent Elected or Appointed Offices in Professional Organizations*
2019- Member, Science & Technology Committee, **The American Chestnut Foundation**.
2016- Member of the Board of Directors & Chair of the Gene Conservation Committee, Maine Chapter, **The American Chestnut Foundation**.
2011-13. Advisory Board Member, Bowman Expeditions, **American Geographical Society.**

C. Selected Academic Awards
2007. **Carl O. Sauer Distinguished Scholarship Award** from the Conference of Latin Americanist Geographers.
1988. **Nystrom Award**, Association of American Geographers (an annual competition based on oral and written presentations of recent PhD dissertations in geography).

c. Recent Grants

2019-20. Principal Investigator, for project "Preserving Wild American Chestnut Trees' Genetic Diversity: A Comparative Analysis of Greenhouse & Field Methods" UNE Mini-Grant & Research Infrastructure Programs (includes matching funds from Maine Chapter of The American Chestnut Foundation & Plantra Inc.), $16,581.
2017-8. Principal Investigator, for project "Can Early Exposure of American Chestnut Seedlings to Fungal Blight Enhance the Selection of Resistant Trees? UNE Mini-Grant Program (includes matching funds from Maine Chapter, The American Chestnut Foundation.) $7,000.
2015-6. Principal Investigator, for project "UNE Healing Garden" Grant from Davis Foundation/Campus Compact, $4,000.
2012-3. Co-Principal Investigator (with Noah Perlut) for project "Identification of the migration and wintering grounds of the Caribbean Martin (*Progne dominicensis*)" Grant from the Blake-Nuttall Fund, $5,000.
2008-10. Principal Investigator, for project "The Impact of Free Trade and Neoliberal Economic Policies on Rural Land Uses, Employment, And Migration Patterns in The Windward Lesser Antilles" Grant from the American Geographical Society and US Army, $26,000.

d. Recent Advanced Undergraduate Course Offerings

- Sustainability and Ecological Restoration
- Environmental Research: American Chestnut Restoration
- Permaculture: Landscape Design with Nature
- Biodiversity and Sustainable Agriculture

l. *A **Conflict of Interest** or Commitment (COI or COC) statement:* There are no known potential Conflicts of Interest or Commitment.