THE RESEARCH FOUNDATION
The State University of New York

SUNY COLLEGE OF ENVIRONMENTAL SCIENCE AND FORESTRY

Office of Research Programs
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Syracuse, New York
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March 9, 2012

Dr. Fred Hebard
TACF Grants Coordinator
The American Chestnut Foundation
TACF Research Farms
29010 Hawthorne Drive
Meadowview, VA 24361

RE: 12093835/9936

Dear Dr. Hebard:

The enclosed proposal entitled, "Preservation and multiplication of elite backcross American chestnut hybrids by micropropagation" is being submitted for your consideration by The Research Foundation of SUNY with a place of business at the SUNY College of Environmental Science and Forestry.

Technical responsibility of the project rests with the SUNY College of Environmental Science and Forestry and has been assigned to the technical representative identified below. Questions about the substance of the application should be directed to the technical representative or to the Office of Research Programs. This office will also respond to questions on the budget. Please reference the number assigned to this application in your correspondence or inquiries.

If action on the application is favorable, the award should be made to the Research Foundation of State University of New York which will provide fiscal administration. Negotiations concerning the terms and conditions of the award should be conducted with the administrative representative identified below.

Sincerely,

for Dr. Neil H. Ringler
Vice Provost for Research

Administrative Representative
Ms. Justine Gordon
The Research Foundation of
State University of New York
PO Box 9
Albany, NY 12201
(518) 434-7105

Technical Representative
Dr. Charles A. Maynard
State University of New York
College of Environmental Science
and Forestry
Syracuse, New York 13210
(315) 470-6560/6986

cc: Dr. Charles A. Maynard
Dr. William A. Powell
Mr. William Nicholson

Preservation and multiplication of elite backcross American chestnut hybrids by micropropagation

Summary

One of the bottlenecks for the breeding program that is dealing with quantitative resistance is the ability to quickly multiply the B3F3 trees demonstrating the highest levels of blight resistance. This study will collect dormant stems the twenty most promising individuals from the TACF breeding program and clonally establish them in aseptic tissue culture. Therefore the germ line will be stored almost indefinitely in tissue culture. Each genotype will be clonally replicated *in vitro*, and the plantlets will be rooted and acclimatized according to our established protocol. Field-ready young trees will be available for planting throughout each State chapter's breeding programs in 2013.

Principal Investigator(s) and Institutional Affiliation(s)

Charles A. Maynard
Professor
State University of New York, College of Environmental Science and Forestry, Department of Forest and Natural Resources Management, One Forestry Dr, Syracuse NY 13210

William A. Powell
Professor
State University of New York, College of Environmental Science and Forestry, Department of Environmental Forest Biology, 1 Forestry Dr, Syracuse NY 13210

Allison Oakes
Ph.D. Graduate Student
State University of New York, College of Environmental Science and Forestry, Department of Environmental Forest Biology, 1 Forestry Dr, Syracuse NY 13210

Duration of project

Approximately six months from starting date in September 2012.

Total amount requested

$2,715

Short and long-term goals of the project

The short-term goal of the project is to provide clonally propagated trees from up to twenty elite lines of B3F3 trees to the TACF breeding and restoration programs. These trees would be available to the American Chestnut Foundation and affiliates' plantings and seed

orchards. We can maintain the most promising individual trees produced by the TACF backcross breeding program almost indefinitely by means of micropropagation. In this way the genotype will not be lost if anything happens to a particularly promising tree.

The long-term goal is to demonstrate the methods and utility of micropropagating elite lines of highly blight resistant B3F3 American chestnut trees. The success of this project may encourage other conservation and restoration projects to consider micropropagation as a tool for recovering threatened wild populations.

Narrative

"Why is shoot culture better than seedlings?"

One of the greatest strengths of a breeding program is also a weakness. The genetic recombination which occurs during fertilization creates novel genotypes and maintains genetic diversity within a population. The corollary is that no offspring will have the exact genotype of the parent. In the case of exceptional B3F3 trees, its offspring may not have the characteristics of the parent that make it so promising.

One tree will only remain one tree. In addition to the risks of disease or accident destroying a promising genotype, only having one individual presents other limitations. In order to cross a B3F3 tree with another from another orchard, pollen has to be collected and shipped. The amount of female flowers will be limited to what one tree can produce. If clones of the B3F3 trees were available, any orchard could have copies of the exact B3F3 tree they needed, which would also increase the number of flowers available for crossing.

An interesting example of tissue culture preserving a novel genotype is the case of the Bramley Apple. A single apple tree, planted by chance in an English garden in 1809, produced exceptional fruit. Cutting were taken and grafted, and the variety was named "Bramley" after the owner of the garden. Over 200 years later, Bramley apples are still grown and sold, the original tree has been weakened by age and honey fungus. Shoot tips and leaf buds were collected, and using as similar process as our shoot forcing technique they grew in aseptic culture. Today a clone of the original Bradley Apple is growing well next to its progenitor, and Bradley Apple clones are available for orchards across Britain to reintroduce into breeding programs (Univeristy of Nottingham 2009).

Vegetative propagation has been proposed as a method of restoring an 2.3 km double alleé of lime trees planted in the 1700s, now a World Heritage Site (Gerzson, Szilagyi, and Bede-Fazekas 2012). Seedling offspring were deemed too variable, whereas cloned offspring would have the same desired growth habit as the trees planted over 200 years ago.

Tissue culture has been used to successfully save and propagate many threatened species in Australia and New Zealand, such as epiphytic and terrestrial orchids, an endangered fern, and the famous Wollemi pine (Ashmore, Hamilton, and Offord 2010). Unlike these studies, culture conditions have already been optimized for the target species – American chestnuts have been micropropagated in our Tree Improvement Lab for over 15 years.

The current success rate of the shoot-forcing/*in vitro* method is 100%. We now have four

individual B3F3 trees in aseptic culture, currently being multiplied prior to rooting. The parent trees were planted at the Lafayette Rd. Experiment Station in 2010. About 30 6" pieces of stem were collected, doing less harm to the parent tree than regular pruning. The collection took place on January 21st 2012, and we had established aseptic cultures by February 26th. We could conceivably force shoots in late fall, multiply during the winter, and have 20-30 field-ready clones of each chosen tree by April 2013.

Reference list

Univeristy of Nottingham. 2009. "Turning Back the Clock to Save the Bramley Apple." *Alpha Galileo Foundation*. http://www.alphagalileo.org/ViewItem.aspx?ItemId=56383&CultureCode=en.

Ashmore, Sarah E., Kim N. Hamilton, and Catherine A. Offord. 2010. "Conservation Technologies for Safeguarding and Restoring Threatened Flora: Case Studies from Eastern Australia." *In Vitro Cellular & Developmental Biology - Plant* 47 (1) (November 3): 99–109. doi:10.1007/s11627-010-9320-9.

Gerzson, L., K. Szilagyi, and A. Bede-Fazekas. 2012. "The Long Term Preservation of an 18th Century Gene Bank Heritage." *Applied Ecology and Environmental Research* 10 (1): 47–64.

Timeline, showing start and completion dates for each goal.

September 2012
Identify which twenty trees will be chosen for the program.

October-November 2012.
Collect dormant stems from selected B3F3 trees from the TACF orchards.
Initiate the shoot forcing procedure developed during the fall tissue culture class at SUNY-ESF.

December 2012
After three weeks, most stems will flush out buds.
Stem and meristem tissue will be placed in aseptic culture.
Each culture will be transferred to fresh media and multiplied every three weeks.
In addition to multiplication, large and leafy plantlets will be selected to undergo the rooting process.

January 2013
Root 66-75% of cultures each transfer, for a stable 1:3 or 1:4 multiplication ratio (depending on clone line).

January 2013-April 2013

Rooted plantlets will be acclimatized in potting mix in growth chambers, then moved to the greenhouse to continue hardening off. 20-30 clones of each tree will be ready for transplanting to the field in April 2013.

April 2013 - ?
Maintain clone lines in culture for additional uses and prepare for planting.

How results will be measured and reported.
The percentage of successful shoot forcing, establishment *in vitro*, the multiplication rate of each tree culture (taken during each monthly transfer), and monthly totals of plantlets will be recorded. The rooting and acclimatization percent success of each culture will also be recorded, along with monthly production/loss total in the growth chamber and greenhouse.

Breakdown of how funds will be spent

$565	Labor, including shoot forcing procedure, micropropagation, rooting, greenhouse care, and glassware washing
$1200	Materials, including shoot forcing solutions, tissue culture media, pots, potting mix, and tags
$750	Travel, for trip to and from Syracuse, NY to Meadowview, VA for cutting collection
$200	Publication costs

$2715 Total

Timeline for when funds will be needed
August 2012 – Travel funds = $750
December 2012 – $1965.

BIBLIOGRAPHIC SKETCH

Charles A. Maynard

Department of Forest and Natural Resources Management
College of Environmental Science and Forestry, SUNY
1 Forestry Drive
Syracuse, NY 13210-2787

Education and training:

Ph.D.,	Forest Biology & Wood Science, Iowa State University, Ames, Iowa	1980
M.S.,	Forest Biology, Iowa State University, Ames, Iowa	1977
B.S.,	Forest Management, Iowa State University, Ames, Iowa	1974

Research and Professional Experience

Professor (1994-present), Associate Professor (1986-1994), Assistant Professor (1983-1986), and Research Associate (1980-1983) Faculty of Forestry, SUNY College of Environmental Science and Forestry	1980-present
Research Assistant, forest genetics. Iowa State University, Forestry Department.	1977-80
Forestry Extension Assistant. Iowa State University, Forestry Department.	1975-77

Societies and Professional Memberships

American Association for the Advancement of Science
Society of American Foresters

Teaching experience

Forest Genetics and Tree Improvement, a senior-level three-credit elective course dealing with the application of plant breeding methods to long-lived perennials: phenotypic selection, provenance and progeny testing, breeding, seed orchard management, seed and pollen collection, storage and testing, as well as tissue culture propagation, isozyme analysis and DNA isolation, and germplasm conservation.

Tissue Culture Methods, a senior-level/graduate-level three-credit elective course covering tissue culture as applied to woody plants. The course covers basic cell biology and plant physiology, media optimization for a new species, explanting, multiplication, rooting, and acclimatization of tree species. Laboratory research is emphasized.

Introduction to Arboriculture, a senior-level three-credit elective course covering the practice of arboriculture. The course covers site evaluation, species selection, planting, pruning, fertilization and the identification and removal of hazard trees in an urban environment.

Collaborators and Affiliations **(see attached document),**

RESEARCH **(See attached Current & Pending list for my Recent Grants)**

Major Research accomplishments

I've been at this game for a long time! In the early 1980s, I worked closely with the New York State Department of Environmental Conservation, Division of Lands and Forests on their applied forest genetics program. One of my major accomplishments for DEC was to write and begin implementation of a 10-year plan, outlining goals and strategies for tree improvement by the State of New York (Maynard 1983b). Priorities were set among the different species. Field tests were re-measured (Easley and Maynard 1986, 1987; Maynard 1983a). Pesticides were tested for controlling seed orchard insects (Valenti, Abrahamson, and Maynard 1990). Seed was collected from three Norway spruce (*Picea abies*) seed orchards; I even had a crew doing plus-tree selection for Norway spruce "plus trees." From all of this material, slightly over 150 Norway spruce seedlots were established in field tests.

Concurrent with my traditional forest genetics activities, I was working with a series of graduate students on developing tissue culture propagation methods for black cherry (*Prunus serotina*). This project led to a number of important

accomplishments. We were the first to establish, multiply, and root black cherry *in vitro* (Tricoli, Maynard, and Drew 1985), the first to use gibberellin to break dormancy in a forest tree species - it had been used several times on woody horticultural crops (Kavanagh, Maynard, and Drew 1987; Drew, Kavanagh, and Maynard 1988), and the first to identify an enhancing effect of dimethylsulfoxide (DMSO) on bud break (Kavanagh, Lee, Drew, and Maynard 1993). We were also one of the first research groups to identify an inhibiting effect of blue light on root formation (Fuernkranz, Nowak, and Maynard 1990). Our half-acre planting on Heiberg Forest is the oldest field planting of tissue culture propagated black cherry in the country (Maynard 1994). The project, through initial field results, was summarized in a book chapter (Maynard, Kavanagh, Fuernkranz, and Drew 1991).

In 1988, I began working with American chestnut (*Castanea dentata*), first on pollen collection and storage techniques (Maynard 1991d), then in collaboration with Dr. William Powell, on engineering blight resistance into American chestnut. Early in the project, I transformed a selectable-marker gene into chestnut callus tissue and confirmed transformation using the polymerase chain reaction (PCR) technique (Maynard 1991a). My graduate students and I also worked on rooting techniques (Maynard, Satchwell, and Rieckermann 1993), and more recently on embryogenesis and acclimatization (Xing, Satchwell, and Maynard. 1996). In June of 2006 we finally succeeded in transforming, regenerating, acclimatizing and outplanting two American chestnut plantlets in the field. In June of 2007 we planted about a dozen transgenic chestnut plantlets, and in 2008 we planned it out more than 20. As of late December 1011, we have more than 300 transgenic American chestnut trees planted in six locations around the state. We don't yet have blight resistance, BUT we are very close!

PUBLICATIONS

Cameron, K.D., I. S. Phillips, R. F. Kopp, T. A. Volk, C. A. Maynard, L. P. Abrahamson, and L. B. Smart. (2008). Quantitative Genetics of Traits Indicative of Biomass Production and Heterosis in 34 Full-sib F1 *Salix eriocephala* Families. Bioenergy Research (Bioenerg. Res. 1:80–90.

Maynard, C.A., Powell, W.A., Polin-McGuigan, L.D., Viéitez, A.M., Ballester, A., Corredoira, E., Merkle, S.A. and Andrade, G.M. (2008) Chestnut In: Kole, C. and Hall, T.C. (eds.) *A Compendium of Transgenic Crop Plants*. Volume 9. Wiley-Blackwell Pp. 169-192.

Newhouse, A.E., N.S. Kaczmar, C.A. Maynard, and W.A. Powell (2007) American Elm. In: Kole, C. and Hall, T.C. (eds.) A Compendium of Transgenic Crop Plants. Volume 9. Wiley-Blackwell (In Press).

Rothrock, R.E., Polin-Mcguigan, L.D., Newhouse, A.E., Powell, W.A. and Maynard, C.A. (2007) Plate flooding as an alternative *Agrobacterium*-mediated transformation method for American chestnut somatic embryos. *Plant Cell, Tissue and Organ Culture* 88: 93-99

Welch, A.J., A.J. Stipanovic, C.A. Maynard, and W.A. Powell. (2007) The effects of oxalic acid on transgenic *Castanea dentata* callus tissue expressing oxalate oxidase. Plant Science 172:488-496.

Polin L.D., H. Liang, R. Rothrock, M. Nishii, D. Diehl, A. Newhouse, C.J. Nairn, W. A. Powell, and C.A. Maynard. (2006). Transformation of American chestnut (*Castanea dentata* (Marsh.) Borkh.) somatic embryos. Plant Cell Tissue and Organ Culture. 84:69-78.

Merkle, S.A., Andrade, G.M., Nairn, C.J., Powell, W.A. and Maynard, C.A. (2007) Restoration of threatened species: A noble cause for transgenic trees. *Tree Genetics & Genomes* 3: 111-118

Powell, W. A., P. Morley, M. King and C. A. Maynard. 2007. Small stem chestnut blight resistance assay. Journal of The American Chestnut Foundation 21(2): 34-38

BIOGRAPHICAL SKETCH

William Allen Powell
COLLEGE OF ENVIRONMENTAL SCIENCE & FORESTRY
State University of New York
1 Forestry Drive
Syracuse, NY 13210-2788
Phone: (315) 470-6744

Education:

Ph.D., Biology, Graduated: 1986
Utah State University, Logan, UT
Dissertation: Differential accumulation of poly(A)+ RNA and polypeptides between virulent and dsRNA-induced hypovirulent strains of *Cryphonectria* (*Endothia*) *parasitica*

B.S. with honors, Biology, Graduated: 1982
Salisbury State University, Salisbury, MD

Professional Experience:

Director of the Council on Biotechnology in Forestry 2001-present
Professor 2003-present
Area: Biotechnology in Forest Pathology
College of Environmental Science and Forestry
State University of New York
Syracuse, NY 13210

Associate Professor 1995-2003
SUNY-ESF

Assistant Professor 1989-1995
SUNY-ESF

Postdoctoral Associate 1986-1989
Area: Molecular Plant Pathology
University of Florida, Department of Plant Pathology
Gainesville, FL 32611

Teaching within the past 5 years:

EFB307 Principles of Genetics (3 credits)
EFB308 Principles of Genetics Lab (1 credit)
BTC497 Research Design & Professional Development (1 credit)
BTC425/EFB625 Plant Biotechnology (3 credits)

Publications within the past 5 years:

Book chapters:

H. Liang, P.A. Kumar, V. Nain, W.A. Powell, J.E. Carlson (2010) Selection and Screening Strategies. In: C. Kole, C.H. Michler, A.G. Abbott and T.C. Hall (eds) Development and Deployment of Transgenic Plants. Springer-Verlag – Berlin, Heidelberg, New York, Tokyo, Vol. 1, pp. 85-143

Andrew E. Newhouse, Nicholas S. Kaczmar, Charles A. Maynard. American Elm, in Kole, C. and Hall, T. C. (eds.), "Compendium of Transgenic Crop Plants: Transgenic Forest Tree Species", Blackwell Publishing, Oxford, UK, 2008, pp 241-262.

Maynard, C.A., W.A. Powell, L.D. Polin-McGuigan, A.M. Viéitez, A. Ballester, E. Corredoira, S.A. Merkle and G.M. Andrade (2008) Chestnut. In: Kole, C., Hall, T.C. (eds.) "A Compendium of Transgenic Crop Plants: Forest Tree Species", Blackwell Publishing, Oxford, UK, 2008. Pp169-192

Peer reviewed journals:

Barakat, A., M. Staton, C. Cheng, J. Park, N. B. M. Yassin, S. Ficklin, C. Yeh, F. Hebard, K. Baier, W. Powell, S. Schuster, N. Wheeler, A. Abbott, J. E. Carlson and R. Sederoff. 2012.Chestnut resistance to the blight disease: insights from transcriptome analysis. BMC Plant Biology (in press)

Sisco, P.H., Sederoff, R.R., Tomkins, J.P., Carlson, J.E., Kubisiak, T.L., Staton, M.E., Hebard, F.V., Anagnostakis, S.L., Powell, W.A. and Smith, C.P. 2009. The United States National Science Foundation project on developing tools for the study of the Fagaceae: *Castanea*, *Quercus*, and *Fagus*. Acta Hort. (ISHS) 844:267-274

Barakat, A., D. S. DiLoreto, Y. Zhang, C. Smith, K. Baier, W. A. Powell, N. Wheeler, R. Sederoff, J. E. Carlson. 2009. Comparison of the transcriptomes of American chestnut (*Castanea dentata*) and Chinese chestnut (*Castanea mollissima*) in response to the chestnut blight infection. BMC Plant Biology. 9:51 (pp. 1-11)

Newhouse, A.E., F. Schrodt, H. Liang, C.A. Maynard, W.A. Powell. 2007. Transgenic American Elm Shows Reduced Dutch Elm Disease Symptoms and Normal Mycorrhizal Colonization. Plant Cell Reports 26:977-987

Welch, A.J., C.A. Maynard, A.J. Stipanovic, and W.A. Powell. 2007. The effects of oxalic acid on transgenic *Castanea dentata* callus tissue expressing oxalate oxidase. Plant Science 172:488-496

Powell, W. A., P. Morley, M. King and C. A. Maynard. 2007. Small stem chestnut blight resistance assay. Journal of The American Chestnut Foundation 21(2): 34-38

Rothrock, R., L. McGuigan, A. Newhouse, W.A. Powell and C.A. Maynard. 2007. Plate Flooding as an Alternative Agrobacterium-Mediated Transformation Method for American Chestnut Somatic Embryos. Plant Cell Tissue and Organ Culture 88:93-99

Merkle, S.A., G.M. Andrade, C.J. Nairn, W.A. Powell and C.A. Maynard. 2007. Restoration of threatened species: a noble cause for transgenic trees. Tree Genetics and Genomes 3:111-118

Allison D. Oakes

250 Thurber St, Syracuse NY 13210 - (315) 730-8024 - adoakes@syr.edu

Summary

- Six years lab experience in the Tree Improvement Lab at SUNY College of Environmental Science and Forestry performing undergraduate and graduate level research
- Preparation of clear, concise oral and Powerpoint presentations for the annual NYS American Chestnut Foundation meetings as well as lab meetings
- Teaching and communication experience through salsa dance teaching, undergraduate and graduate teaching experience

Career History & Accomplishments

Research Assistant, *SUNY Research Foundation.* *2009-present*

- Investigating the use of endosymbiotic bacteria and mycorrhizae to increase acclimatization success of tissue-cultured American chestnut
- Media and lab preparation for the Fall 2010 BTC 426-626 Plant Tissue Culture class, culture maintenance and regeneration
- Promoted from half time to full time in July 2010

Program Director, *La Familia de la Salsa* *2008-2012*

- Organize dance events, classes, and workshops for a local Latin Dance school, promote events through weekly email newsletter, website, and online social networks
- Assistant instructor for introductory, intermediate and advanced salsa classes

Research Aide, *SUNY Research Foundation* *2006-2009*

- Washed glassware, made media, cleaned lab.
- Learned proper aseptic technique and began performing monthly stock transfers.
- Set up and performed multiple experiments in both lab and greenhouse.

Education

PhD Candidate in Plant Science and Biotechnology *2009-2011*
SUNY College of Environmental Science and Forestry*, Syracuse NY.*
Completion Date: August 2014

B.S. Degree in Biotechnology *2009*
SUNY College of Environmental Science and Forestry*, Syracuse NY.*
Graduated cum laude with 3.4 G.P.A.

Teaching Experience

Spring 2008 – Undergraduate TA for EFB 120 Zoology

- Assisted teaching lab lectures and exercises, led study groups before each exam, held make-up labs.

Fall 2008 – Undergraduate TA for BTC 401/601 Molecular Biology Techniques

- Prepared reagents and materials for lab exercises, gave pre-lab lecture and directed lab.

Spring 2010 – Graduate TA for BTC 401/601 Molecular Biology Techniques

- Prepared reagents and materials for lab exercises, gave pre-lab lecture and directed lab, monitored lab notebooks and proctored exams.

Spring 2008 to present – Assistant Instructor for La Familia de la Salsa

- Assist during large group classes, assist with private lessons, organize payment plans.