11-3-2004

Collaborative Research: Developing Methods to Study Age-Related Changes in the Physiology of Forest Trees

Michael S. Greenwood
Principal Investigator; University of Maine, Orono, Greenwd@UMENFA.Maine.Edu

Final Report for Period: 09/2001 - 08/2004 **Submitted on:** 11/03/2004

Principal Investigator: Greenwood, Michael S. **Award ID:** 0110021

Organization: University of Maine

Title:

Collaborative Research: Developing Methods to Study Age-Related Changes in the Physiology of Forest Trees

Project Participants

Senior Personnel

Name: Greenwood, Michael

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Day, Michael

Worked for more than 160 Hours: Yes

Contribution to Project:

Post-doc

Graduate Student

Name: Ward, Margaret

Worked for more than 160 Hours: Yes

Contribution to Project:

Ms. Ward is an MS student supported by this grant with supplemental support from a departmental teaching assistantship. She joined the project in the spring of 2002 and is completing her degree this semester. In addition to her input as a research assistant for this project, her thesis study (Age-related changes in morphology and anatomy in red spruce foliage) is directly related to the goals of this research effort. Her portion of this study has provided valuable insights into anatomical trends that shed light on the mechanisms underlying age-related decreases in photosynthetic efficiency reported for this species and mechanisms associated with hydraulic compensation in older (larger) trees. She has presented her results with an oral presentation at the Ecological Society of America Annual Meeting (2004), is currently in the final stages of writing her thesis, and is preparing a jouirnal manuscript on her findings.

Name: Adams, Stephanie

Worked for more than 160 Hours: Yes

Contribution to Project:

Ms. Adams is a MS student supported by this grant and by supplemental funding from a departmental teaching assistantship and Maine Agricultural and Forest Experiment Station funding to PI's Greenwood and Day. She joined the project in the summer of 2003, and should finish her thesis by the end of summer 2005. Ms. Adams thesis research will examine age-related differences for diurnal trends in photosynthetic performance in red spruce, modeling whole tree water relations, and evaluation of pathways controlling age-related change relative to photosynthetic attributes. She presented initial results of the diurnal trend analysis at the Eastern Canada/USA Forest Science Conference (Oct 2004), is currently working on her thesis, and we expect her study to result in two journal publications. Her future plans including pursuing a Ph.D. in physiological plant ecology.

Undergraduate Student

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Oregon State University

Greenwood and Day at the University of Maine and Barbara Bond at Oregon State University have developed this project as a collaborative effort. Parallel reciprocal grafting studies are underway in red spruce in the Northeast and Douglas-fir in the Northwest, both being economically and ecologically important species in their respective regions. Although these conifers have many physiological attributes in common, such as declining productivity with tree age, our two-species approach is important due to known differences in key aspects of age-related change between these species. Such different trends may be due to the large differences in maximum height between these two species, factors related to climatic differences, or interactions of these factors.

Other Collaborators or Contacts

Dr. Barbara Bond, Oregon State University, has been pursuing a parallel study in Douglas-fir, further described under Partner Organizations. Dr Bond will provide detailed reports of her part of this collaborative study separately.

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)

Findings: (See PDF version submitted by PI at the end of the report)

Training and Development:

Education and training

At the University of Maine, we have used this grant to support, with research assistantships, two Master of Science students, Margaret H. Ward and Stephanie L. Adams . In both instances, NSF funding was supplemented by departmental teaching assistantships and Maine Agricultural and Forest Research Station funding (to Greenwood and Day).

While participating in this project, both Ms. Ward and Ms. Adams have received broad experience in ecophysiological experimentation and in both laboratory and field research techniques. Specific skills learned have included: operating state of the art gas exchange and chlorophyll fluorescence measurement systems in conjunction with experimental manipulation of photosynthetic conditions, quantitative carbohydrate analysis and spectrographic pigment quantification, preparation and appraisal of histological samples, photomicroscopy, and use of computer image analysis systems, all followed by statistical analysis and interpretation of results. In addition they have been trained in diverse horticultural skills by managing experimental greenhouse populations and establishing our field-based grafting studies, have experienced the design and implementation of field research programs and their associated logistics, and become competent in the safe operation of hydraulic lifts used for canopy access.

As teaching assistants in the undergraduate course Introduction to Forest Biology, Ms. Adams and Ms. Ward received extensive hands-on experience with teaching outdoor and indoor lab exercises, grading tests and student reports, and the opportunity to interact with a diverse group of undergraduate students. Ms. Adams, whose goal is an academic research/teaching career, opted to further her teaching skills by taking advantage of an opportunity to serve as a teaching assistant for two semesters.

Our training approach includes a strong emphasis on outreach and communication of results. Students are required to give two seminars on their thesis research, one close to the beginning of their program that outlines their planned study and research approaches, and a second near completion of their thesis, oriented towards results and interpretation. In addition, Ms. Ward has delivered two oral presentations of her work, one at the Northeastern Biological Graduate Student Conference (2004) and the other at the Ecological Society of America 89th Annual Meeting (2004). Ms. Adams presented a poster of her early results at the Eastern Canada/USA Forest Science Conference (2004) and plans to present an oral paper at the ESA 90th Annual Meeting in 2005.

Outreach Activities:

Greenwood is presenting a general-audience seminar on tree maturation as part of the University of Maine's Forestry Seminar Series in November 2004, and will present an invited lecture on maturation in trees to a nationwide conference of arborists and horticulturists in Washington, DC, this fall. Day presented an overview of our research efforts to a diverse group of forest policy and management professionals at the recent Eastern Canada/USA Conference in Fredericton, NB, Canada (October 2004). As a Project Learning Tree participant, Day visits both elementary and secondary school classes with slides depicting the challenges and opportunities of research in forest ecology and physiology, with many from this project. These presentations and related workshops for science teachers are intended to interest students in science in general and forest research in particular, and make them aware that scientists are seeking the answers to many interesting and important questions in their local forests.

Journal Publications

Ward, MH, MS Greenwood, and ME Day, "Linking structure and function during age-related trends in the foliage of red spruce", Tree Physiology, p. , vol. , ( ). in preparation

Books or Other One-time Publications

Ward, MH, ME Day, MS Greenwood, SL Adams., "Age-related trends in red spruce needle anatomy and their relationship to declining productivity", (2004). abstract collection, Published
Editor(s): Ecological Society of America
Collection: Ecological Society of America 89th Annual Meeting (August 1-6, 2004, Portland, Oregon) Abstracts
Bibliography: The Ecological Society of America, Washington, DC, USA

Day, ME, MS Greenwood, SL Adams, and MH Ward, "How trees age: research on mechanisms controlling age-related trends in productivity of red spruce", (2004). conference proceedings, Published
Editor(s): MacLean, DA
Collection: Proceedings of the Eastern CANUSA Forest Science Conference
Bibliography: University of New Brunswick, Frederickton, NB, Canada

Ward, MA, "Age-related change in the morphology and anatomy of red spruce needles and their relationship to declining productivity with tree age", (2004). Thesis, Submitted
Bibliography: MS Thesis, The Graduate School, University of Maine, Orono, Maine, USA

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

Although numerous age-related trends have been described in forest tree species, the physiological mechanisms controlling age-related change are poorly understood (Day et al. 2002). In analogous fashion, the contribution of age-related changes in morphology and physiology to the widely documented phenomenon of declining productivity with tree age is currently the subject of much debate among physiological ecologists (cf. Bond 2000, Thomas and Winner 2002). Much of the equivocal evidence brought to this debate results from confounding correlations between tree age, size and change in environment that occur over a tree's lifespan (Day et al. 2000). Further disparities in published findings result from research on species that may have evolved different strategies to confront similar challenges or, perhaps, different challenges faced by trees growing in dissimilar environments (Thomas and Winner 2002). In this project, we have developed an experimental approach, reciprocal grafting, that promises to overcome the first, and certainly the most significant, impediment to advancing our understanding of these ecophysiological processes at the tree-level. In addition we have initiated an inter-institutional program that uses parallel studies on two conifers that differ in climatic range, growth form, and life-strategies as a model test of species-specific approaches and factors related to dissimilar climates.

Literature cited

Bond BJ (2000) Age-related changes in photosynthesis of woody plants. Trends in Plant Science 5(8): 349-353
Day ME, Greenwood MS, Diaz-Sala C (2002) Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control. Tree Physiology 22:507-513
Thomas SC, Winner WE (2002) Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. Tree Physiology 22:117-127

Contributions to Other Disciplines:

This research has ramifications that extend beyond the study's tree-level ecophysiological approach. By identifying key attributes that exhibit age-related trends and, more importantly, identifying the pathways regulating change in those attributes we establish the foundation for exploring the genetic underpinnings of ageing in trees. For specific traits, we are developing evidence for both the existence of transduction pathways that induce age-related differences in gene expression and for age-related change in gene expression that ultimately determines the direction, magnitude, and reversibility of such changes. In the other direction of scale, quantifying age-related change and the underlying mechanisms will permit these factors to be addressed in process models that seek to understand, quantify, and predict many phenomena associated with forest growth and yield, from timber production in uneven-aged silviculture to the role of forests in carbon sequestration.

Contributions to Human Resource Development:

The diverse approaches of this project include use of state-of-the-art instrumentation for field research, laboratory analytical procedures, and histological techniques, provided an exceptional environment for training future researchers. Two female researchers, a group underrepresented in forest science, have been supported and trained, both of whom plan to pursue careers in research and teaching. We have also found, through presentations to diverse public audiences, that our 'treetop' research with complex instruments in the upper forest canopy easily captures the attention and imagination of students and the non-scientific public.

Contributions to Resources for Research and Education:

In this phase of our research, we have established a viable population of reciprocal grafts in the crowns of juvenile, mid-aged, and old red spruce trees that will, if funding permits, continue to yield important insights into questions on the physiology and regulation of age-related change for many years to come. In fact, the most valuable data will be obtained as we follow the development of these grafts and planned additional grafts. Ultimately, we will be able to perform return-grafting experiments where scions from these grafts are grafted to their original donors to further test current hypotheses relative to age-related change and new suppositions generated from this research.

Contributions Beyond Science and Engineering:

This research on a commercially important timber species has also yielded impacts of a practical nature by enhancing qualitative understanding of the implications of distributions of stand leaf area among age classes in uneven-aged silviculture, an increasingly important management approach in temperate conifer forests. In addition, silviculturists have enthusiastically received collateral findings from this study related to age-class specific access to resources such as water and nutrients in multi-cohort stands.

Categories for which nothing is reported:

Any Web/Internet Site

Any Product

Activities related to the project

In April of 2002, we established a population of reciprocal grafts between juvenile (9 y, 1 m mean height), mid-aged (mean of 60 y, 10 m) and old (120+ y, 25 m) cohorts of red spruce, using a rented self-propelled hydraulic lift. In the mature trees (5 mid-aged and 5 old), 18 scions, 6 of each age-class, were grafted to each understock with an equal number of single grafts in the crowns of juveniles. We recorded graft survival, first year growth, and basic morphological and physiological data in August 2002. In the 2003 grafting season for red spruce (April-May), we made additional grafts to explore alternative grafting techniques, and inventoried over-winter survival of the 2002 experimental population (98% survival).

Figure 1. Grafting into the crown of an old-growth red spruce.

Later in the 2003 field season, we collected morphological and physiological data from the 2002 experimental graft population and from comparable understock foliage. We collected foliage samples for detailed morphological and anatomical study from our grafted populations, understock trees and an expanded sample of non-grafted trees. *In situ* gas exchange data were collected with a portable IRGA-based gas exchange system (Figure 2). During the following fall, winter and spring, Margaret Ward prepared foliar sections and made extensive observations and measurements of needle anatomy both on natural understock and grafted foliage. This was followed by data analysis, development of her thesis and a presentation at the Ecological Society of America Annual Meeting (M. Ward, Summer 2004). The ESA meeting also provided a setting for discussions of our findings and future research directions with our Oregon State collaborator (B. Bond) and other interested individuals.

In 2004 we were able to rent a self-propelled hydraulic lift (Figure 3) for two months, which permitted studies on an array of physiological attributes. Morphological, growth, and gas exchange data were collected from the experimental grafts and understock foliage. Age-related differences in water potential were evaluated, and data to enable modeling of whole-tree hydraulic conductance were collected. Ms. Adams and Ms. Ward followed diurnal patterns in gas exchange attributes across age classes, to test the hypothesis that age-class differences in photosynthetic rates result from differential responses to midday water stress (Yoder et al 1994). We have collected data from experimental *in situ* manipulation of CO_2 concentrations in the measurement chamber of our portable gas exchange system that will provide insight into fundamental differences in photosynthetic

Figure 2. Collecting *in situ* gas exchange data.

capacity between age-classes, as proposed by Day et al. (2001). Our ongoing analysis of the reciprocal grafting data is providing the first experimental evaluation of alternative mechanisms regulating age-related change proposed by Day et al. (2002).

Ms. Ward is currently in the final stages of preparing a thesis on age-related change in morphology and anatomy of red spruce foliage and a journal manuscript describing her findings. Ms. Adams is currently analyzing the physiological data collected in the summers of 2003 and 2004, and plans to have her thesis finished within a year. Her portion of our study should yield at least two additional journal publications.

Figure 3. Taking measurements in the grafted crowns of old-growth red spruce trees with a self-propelled hydraulic lift.

Literature cited

Day ME, Greenwood MS, White AS (2001) Age-related changes in foliar morphology and physiology in red spruce and their influence on declining photosynthetic rates and productivity with tree age. Tree Physiology 21:1195-1204.

Day ME, Greenwood MS, Diaz-Sala C (2002) Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control. Tree Physiology 22:507-513.

Yoder BJ, Ryan MG, Waring RH, Schoettle AW, Kaufmann MR (1994) Evidence of reduced photosynthetic rates in old trees. Forest Science 40:513-527.

Developing methodology to study age-related change in forest trees

The reviewers of this proposal, despite great enthusiasm for the concept and science behind our proposed study, were concerned that we would not be successful with a critical component of the proposed work, which involved grafting of scions from young to very old trees, and vice versa, in red spruce (*Picea rubens*) in the NE United States and Douglas-fir (*Pseudotsuga menziesii*) in the NW. We were funded to conduct a pilot project to determine whether viable grafts were possible, especially with very old trees as understock. The grafting was very successful in red spruce, with a 56% survival rate on mid-aged and old understock (Figure 1) and an 81% rate on juvenile understock. The reviewersí concerns were well founded for Douglas-fir. In the first year of the study (grafts performed in 2002), 100% of the grafts on to old rootstock failed. The grafts were repeated in 2003 year using different procedures, and weíre happy to report success ñ 30% of the grafts on to old rootstock and 48% of grafts on young rootstock survived. We are now confident that our reciprocal grafting approach is a viable and productive method for studying the ageing process in forest trees and can overcome the confounding of size, age and environmental factors that have hampered previous efforts to understand the regulation of age-related change.

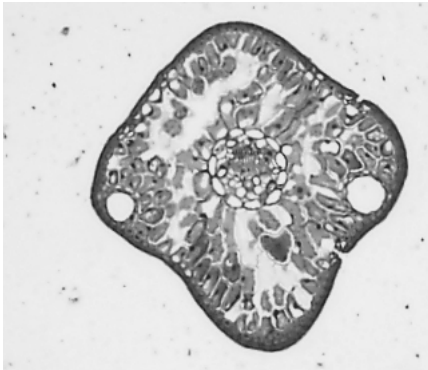
Figure 1. Juvenile scion growing in the crown of an old (151y) red spruce after two growing seasons. Arrow points to the graft union.

In addition to our primary goal of developing successful methods and techniques for studying questions associated with age-relate change in trees, this phase of our effort has substantially increased our understanding of the physiology of ageing in trees and its relationship to age-related declines in productivity. These phenomena have wide ranging implications, from managing multi-aged forests for multiple outputs to understanding the role of forests in global carbon dynamics.

Age-related change in foliar anatomy and physiology

Our previous research showed that photosynthetic rates decline with age in red spruce and provided evidence that this was not related to stomatal limitation of gas exchange, as had been described for several Pacific-Northwestern (PNW) conifers (Day et al. 2001). Margaret Wardís study established that needle anatomy plays a likely role in this phenomenon, with needles from older trees both becoming both more massive and having proportionally less internal air space. This combination of factors substantially increases internal resistance to CO_2 diffusion. In addition, age-related trends in internal needle structure may decrease the rate of CO_2 conductance between its gas-phase in internal air spaces and as a dissolved gas the cytoplasm of mesophyll cells where photosynthesis occurs.

The results of Stephanie Adamís research into age-related differences in diurnal cycles of photosynthesis provides evidence of inherently lower photosynthetic capacity in the foliage of old compared to young red spruce. This is consistent with the suppositions of Day et al. (2001), but, again is in contrast with studies of PNW conifers (e.g., Yoder et



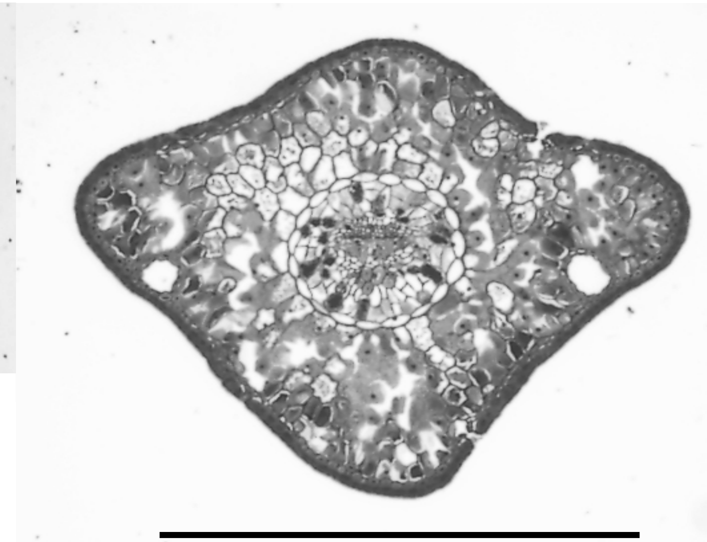


Figure 2. Cross sections of needles from 2 y (above) and 120 y (right) red spruce trees

al. 1994). In several PNW species, photosynthetic capacity, as evidenced by early morning photosynthetic rates, does not differ across age-classes. However, greater midday water stress (or other factors) results in lower midday photosynthetic rates in older trees, and, ultimately, decreases their productivity. In Ms. Adamís study, photosynthetic rates were consistently lower in older trees, irrespective of time of day, suggestive of a lower photosynthetic capacity. We will evaluate this hypothesis further by analyzing the results of a series of A/C_i response curves performed on foliage from our subject age-classes. These manipulative experiments use models of photosynthetic response to altered CO_2 concentrations to estimate the capacity of various components of the photosynthetic ëmachineryí.

Several studies have implicated roles for hydraulic limitations in age- (size-) related change including effects on photosynthetic rates (Yoder et al. 1994, Hubbard et al. 1999) and foliar morphology (Koch et al. 2004, Woodruff et al. 2004). Other studies have suggested that hydraulic changes with tree size are balanced to some extent by increased conductivity of water conducting elements (Becker et al. 2000, McDowell et al. 2002). This study provides evidence that structural hydraulic compensation occurs in red spruce needles (increased xylem and increased diameter of xylem lumena), and data collected will allow us to model and test age-related differences in whole-tree conductivity. Initial analysis and observations suggest that water stress may play roles in inducing mature-type developmental traits, but confirmation of this will require further experimentation.

Mechanisms controlling age-related change

Our ultimate goal is to understand the developmental regulation of age-related change. Day et al. (2002) proposed three hypothetical pathways by which genetic, size-complexity, and environmental factors might regulate age-related trends in the foliar meristem, the tissue producing new foliage. In the *extrinsic model*, the characteristics of developing foliage are altered by factors external to the meristem such as increased resistance in the tree hydraulic system or increases in ambient leaf-to-air vapor pressure deficits. Extrinsic control implies plastic, reversible change. In the *intrinsic model,* changes in ontogenetic behavior derive from genetically programmed developmental changes, analogous to aging processes in animals. The third model, *extrinsic-intrinsic,* combines attributes of both intrinsic and extrinsic pathways in that genetic ëprogramsí that alter meristem behavior are induced by external cues. In contrast with a strict extrinsic mechanism, these changes in gene expression have very limited reversibility. One of the principle reasons for developing our reciprocal grafting approach is to provide a method with the potential to distinguish between these pathways.

Our preliminary findings indicate that some ëage-markersí, such as increasing needle width with age (Figure 3), may be controlled by interactions of both extrinsic and intrinsic mechanisms. For example, juvenile scions grafted onto juvenile understock have similar needle widths to non-grafted juveniles, as does old scions on old understock compared with old understock. From this we might conclude that all scions on old understock have wider needles than those on juvenile understock, suggesting extrinsic control between understock groups. However, within understock groups, juvenile scions always have narrower needles than those on old scions, indicative of intrinsic or extrinsic-extrinsic control. In contrast, photosynthetic capacity (Figure 4) would appear to be most strongly influenced by external factors (extrinsic or, perhaps, extrinsic-intrinsic). Nevertheless, old scions grafted onto juvenile rootstock appear to have a ëmemoryí that may somehow restrict photosynthetic capacity. A similar retention of old tree attributes in red spruce scions on juvenile rootstock was described in Day et al. (2001). Now that the methodology has been established we hope to develop a clearer understanding of these mechanisms by following reciprocal grafts over longer time periods and by return-grafting studies where the shoots of grafted scions are returned to individuals of their original age classes.

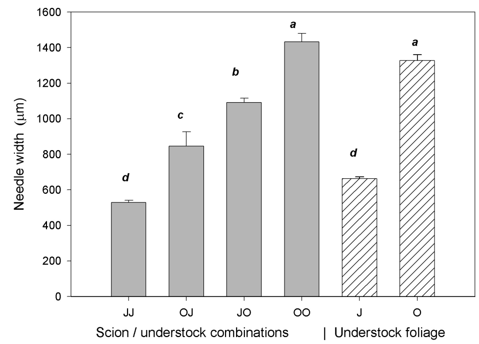


Figure 3. Needle width for red spruce reciprocal grafts and understock. The first letter indicates scion donor age-class, the second that of understock: J = juvenile, O = old. Data for mid-aged class grafts were not statistically different from old-growth and is omitted for clarity. Letters give Tukey HSD classes (α = 0.05), and bars indicate one standard error.

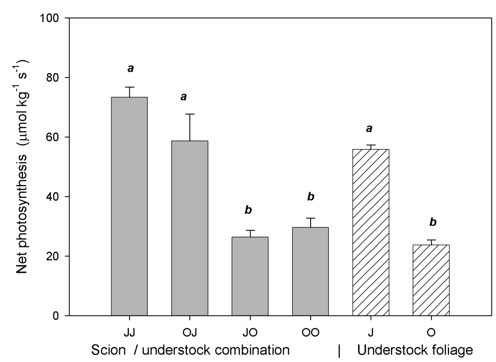


Figure 4. Light-saturated net photosynthesis for reciprocal grafts between juvenile and old red spruce, compared to juvenile and old understock

Literature cited

Becker P, Meinzer FC, Wullschleger SD (2000) Hydraulic limitation of tree height: a critique. Functional Ecology 14:4-11.

Day ME, Greenwood MS, White AS (2001) Age-related changes in foliar morphology and physiology in red spruce and their influence on declining photosynthetic rates and productivity with tree age. Tree Physiology 21:1195-1204.

Day ME, Greenwood MS, Diaz-Sala C (2002) Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control. Tree Physiology 22:507-513.

Hubbard RM, Bond BJ, Ryan MG (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiology 19: 165-172

Koch, G.W., S.C. Sillett, G.M. Jennings and S.D. Davis (2004) The limits to tree height. Nature 428:851-854

McDowell NG, Phillips N, Lunch C, Bond BJ, Ryan MG (2002) An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. Tree Physiol. 22:763-774

Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? Plant, Cell, Envir. 27:229-236

Yoder BJ, Ryan MG, Waring RH, Schoettle AW, Kaufmann MR (1994) Evidence of reduced photosynthetic rates in old trees. Forest Science 40:513-527.