SEED DORMANCY AND GERMINATION OF NORTHERN RED OAK

by

George Martin Hopper

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
Forestry

APPROVED:

D.J.Parrish, co-chairman

D.Wm.Smith, co-chairman

L.H.Audy

H.E.Burkhart

J.D.Johnson

September, 1982
Blacksburg, Virginia

ACKNOWLEDGEMENTS

First, I want to thank the members of my graduate committee. Drs. David Wm. Smith and David J. Parrish, co-chairmen, were very cooperative and willing to help. They each contributed significantly to my education and research training. Dr. Smith's advice, silviculture teaching, and taking care of many administrative details were especially appreciated. Dr. Parrish was a valuable asset to my program in direction and advise of the research, teaching me seed physiology and editing the dissertation. I also acknowledge Drs. H.E.Burkhart, for his statistical and personal advise, L.H.Aung, for his philosophical perspective and encouragement, and Jon D. Johnson, for his laboratory help and serving as a replacement committee member. I give credit to

who offered me the chance to work on dormancy of northern red oak and was instrumental during my first year at VPI.

I am also grateful to the many students who helped directly in the research. and were helpful in seed collection, handling, and germination counts. and were valuable

in coding and inputing data. was especially helpful in laboratory organization and the seedling growth experiments.

Many graduate students listened to my philosophical-physiological rampages on acorns, dormancy, and growth and for this counciling I am thankful to all the forest biology graduate students, especially , , , and . I am humbled by their friendship.

And to my wife, , I owe the deepest gratitude for her support, understanding and encourgement during all my graduate education. Her perserverance and resilience gave me strength when it was most needed. And for .

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The most recent statistics on timber production in the United States indicate that hardwood growth exceeds hardwood removals; however, the majority of the growth occurs on stems of low quality and small diameter (USDA, 1980). Particularly in the southeastern United States, a prime hardwood-producing area, quality hardwood production falls short of demands. Annual demand for hardwood timber products from U.S. forests was 3.0 billion cubic feet in 1979 and is projected to be 9.6 billion cubic feet by the year 2030 (USDA, 1980). This increase reflects a projected demand for hardwood lumber for pallets, hardwood plywood and veneer for furniture, and roundwood for pulp and fuel. As early as 1969 the Southern Forest Resource Analysis Committee estimated that a two to three fold increase in growth and yield of commercially valuable hardwood timber must be realized to meet projected needs by 2010. The projected demand can be met by improving utilization, increasing forest land area and/or plant productivity. Commercial forest acreage, however, is decreasing, due to competitive pressure from agriculture, urban development, and conservation/preservation

interests (USDA, 1980). Thus, increases in productivity must rely on improved utilization, more intensive silvicultural practices and/or increased growth rates.

Commercial hardwood forests make up more than 60% of the forests of the eastern United States, and oaks (Quercus spp.) comprise more than 36% of the commercially valuable hardwood growing stock (USDA,1974a). In the hardwood forests of Virginia and West Virginia, 38% of the growing stock is oak; however, only 8.7% of this volume is comprised of large diameter oaks with high quality and form, i.e. select trees greater than 19 inch diameter at breast height (USDA,1978a; USDA,1978b). The scarcity of large diameter, high quality oaks is the result of past silvicultural practices such as high grading, livestock grazing, and wildfires (Smith and Linnartz, 1980). If the potential productivity of these low to medium quality sites in Appalachian forests is to be achieved, more intensive silvicultural practices must be employed. Unless quality oaks are propagated and used to regenerate suitable sites, the supply of good oak timber will inevitably diminish. Management practices such as site preparation, competition control, artificial regeneration, fertilization, and implementation of intermediate silvicultural operations must also be considered. Johnson (1975) noted that more intensive silvicultural practices must be

employed in order to maintain oak as a major component in bottomland hardwoods.

High costs and the qualified or limited successes of intensive silvicultural practices necessitates minimizing or eliminating factors that delay growth. A main thrust of tree physiology research has been the improvement of growth and yield of commercially important tree species. Tree growth is a relatively slow process and because of the differences in juvenile and mature growth rates and delays or repression of development, physiologists often must wait years before they realize the impact of their work. Methods in accelerating the growth process and maintaining or improving product quality and tree survival would be of great benefit.

The delays in germination of many tree seed has been of particular interest to forest scientists. In the red oak group (subgenus Erythrobalanus), a delay in germination is prominent (USDA,1974b). This delay, which varies qualitatively and quantitatively among species and genotypes, is called seed dormancy. A seed that fails to germinate when given gases, water, and temperature normally conducive to growth is dormant (Villiers,1972; Khan,1977). Acorn[1] dormancy within the red oak group has been investigated

[1]Although acorns are technically fruits, they will be refered to as seeds throughout this paper.

extensively, but a physiological cause common to all species cause has never been determined (Korstian, 1927; Bonner, 1970). Indeed, a single cause for this ecologically-diverse group seems unlikely.

Seed dormancy can create costly problems for nurserymen and seedsmen. The delayed and erratic germination associated with dormant seeds often results in nonuniform seedlings, reduced yield of high quality seedlings, and prolonged exposure to rodents and adverse weather conditions. The ability to control dormancy and germination would greatly reduce or possibly alleviate these problems. Positive germination control might permit rapid, early establishment of red oaks in the nursery. Germination control may shorten the time required to produce a plantable seedling such that 1-0 stock can be planted rather than 2-0 stock. Plantation establishment has proven its worth by more than doubling growth in cottonwood, ash, yellow poplar and sycamore (Smith and Linnartz, 1980); but an increase in oak growth in culture has not yet been realized.

Direct seeding in the field might be feasible if sowing times and seed treatments were optimized. Furthermore, the repression of early seedling growth often observed in northern red oaks may be eliminated with specific pregermination treatments. Thus a more competent seed in terms of germina-

tion would produce a more competent seedling in terms of growth.

While being able to promote acorn germination has definite advantages, methods for maintaining dormancy for several years or more would also be beneficial. Long term storage would decrease present dependency on annual seed production. Since individual oaks only bear seed in large quantities on three to five year cycles (USDA,1974b), maintaining viability and quality of the acorns in storage would insure supplies adequate to meet annual seed demand. Long term storage will become desirable when "gene banks" of superior red oak varieties are developed.

The underlying cause of red oak dormancy is unknown. Further, the effect of various storage and pregermination treatments, such as low temperature stratification and pericarp removal, on subsequent seedling growth have not been reported. It was therefore the purpose of this research to study the control of the dormancy and germination in Quercus rubra L. (northern red oak), a major hardwood timber producing species in the United States. The following objectives were established:

1. To determine the effects of stratification at a high and a low seed moisture level on germination of Quercus rubra L..

2. To examine the effect of pericarp removal on C. rubra's responses to stratification.

3. To study the adenylate energy metabolism of C. rubra seed during stratification and germination.

4. To examine the early growth of C. rubra seedlings in relation to pregermination treatments, stratification and pericarp removal.

LITERATURE REVIEW

Dormancy

Dormancy applies to more than just seeds. Villiers (1972) defines dormancy as a state of arrested development in which an organ or organism, by virtue of its structure or chemical composition possesses one or more mechanisms that prevents growth. Dormancy is internally imposed as a consequence of environmental conditions and of a developmental pattern that slows biochemical processes. Dormancy often can be reversed by changes in the environment, exogenous applications of growth substances, or other mechanical and physical manipulations (Logan and Pollard, 1976; Lewak and Rudnicki, 1977). Dormancy usually occurs in plant structures that are somewhat undifferentiated; however, these structures are always biochemically complex (Koller,1972). Dormancy, by definition, is endogenously regulated and, in nature, often synchronized with adverse climatic factors (Koller,1972). Plant structures may remain dormant for variable lengths of time; but, under natural conditions, the dormant period is relatively fixed. The length of time required to break dormancy may be shortened by chemically or

physically "forcing" the plant to grow; any man-made stimulus can, however, result in abnormal growth presumably due to the premature development at cytological and biochemical levels (Flemion,1933; Weever and Hough,1959; Garrard and Biggs,1963; Lewak and Rudnicki,1977).

In a broader sense, seed dormancy is a natural, adaptive phenomenon that has evolved through eons to provide for sporophyte survival during harsh environmental conditions (Koller, 1972; Villiers,1972). The result of such dormancy is often a suspension of life processes so that the plant can withstand adverse environments. The interest in this complex and intriguing phase of plant life is substantial, and many excellent reviews on seed dormancy have been written (Amen,1968; Villiers,1972; Khan,1977).

Dormancy in red oak seeds is broken by natural overwintering or by a longterm (90-120 days), low temperature (3-5 C) storage called stratification, to simulate overwintering (USDA, 1974b). Such a pregermination treatment is required to break dormancy of many tree seeds (Lewak and Rudnicki, 1977). During stratification, changes occur in morphology (Nikolaeva, 1969), anatomy (Vozzo,1975; Janerette,1978), and hormones (Nikolaeva, 1968; Dury,1977; Khan,1977; Hopper,1979). The increased germinability that occurs during stratification of seeds of various species is positively

correlated with such vital processes as respiration (Brown,1939; Vozzo,1973), nucleic acid synthesis (Osborn,1977), protein synthesis (Ching,1973a; Mayer,1977), enzymatic activity (Lewak and Rudnicki,1977), and adenylate energy metabolism (Ching and Ching,1972; Simmonds and Dumbroff,1974).

Influence of ripeness and storage on seed dormancy

The physiological behavior of forest species' seeds under various storage conditions has been studied extensively, but how seeds remain viable for only a few days, as does Salix nigra L. (black willow), or 150 years, as does Cassia multijuga Rich., continues to be a mystery (Stone,1959). According to Barton (1953), age of seeds is not as critical as conditions during storage. Generally, storage at low temperatures improves the germination of dormant seed of many species (Stokes,1953; Stone,1957; Flemion and Beardow,1965; Barton and Bray,1967; McLemore and Barnett,1967; Wilcox,1968; Kao and Rowan,1970; Thapiyal and Guta,1980). During stratification seeds are stored at low temperature under moist conditions, thereby simulating natural environs of the normal overwintering period. Changes occur during stratification that some authors contend are a continuation of ripening. Thus, some dormant seeds are said

to "afterripen" during stratification. Afterripening in other species may occur under very different conditions, yet increasing their germinability.

Embryo excision allows for germination of non-afterripened seeds in of some species. However, seedlings produced from these excised embryos are often "physiological dwarfs" and lack the ability to produce vigorous plants (Flemion,1933; Garrard and Biggs, 1963; Nikolaeva,1968). "Physiological dwarfs" exhibit stunted growth and malformed leaves and they are often spindly in form. For this reason, afterripening often appears necessary for production of vigorous seedlings from dormant seed. Woods and Blake (1981) reported no advantage of stratification for breaking dormancy in Pinus ponderosa (ponderosa pine). These researchers, however, conducted their tests on a seedlot that had been stored for four months, perhaps under conditions that promoted afterripening. In contrast, Scljanik (1968) showed that stratification of some cold requiring European forest trees may not be necessary and that dormancy may be initiated and progressively deepened during seed development. He reported production of normal seedlings from green (unripe) seeds of Rhus cotinus (European smoke tree), Cornus mas (cornelian cherry), Crataegus monogyna (hawthorn), and Fraxinus ornus (flowering ash). There was a two to three fold

increase in germination of seeds collected 4 to 8 weeks before natural shedding/abscission. Working with unripe seeds of Prunus persica (peach), Weever and Hough (1959) found that germination and seedling growth vigor varied with maturity of the embryo. Normally dormant Liriodendron tulipifera L. (yellow poplar) seeds will germinate and produce normal seedlings if harvested a month before natural seed dispersal (Bonner, 1977). Conversely, Pinus virginiana (Virginia pine) seed (dormant at maturity) germinates 8 weeks before cones are completely ripened, but they do not produce normal seedlings (Fenton and Sucoff, 1965). Similarly, Quercus nigra L. (water oak) acorns will germinate but will not produce normal seedlings when picked in advance of full maturity (Bonner, 1979).

Effects of stratification on germination and seedling growth usually have been reported to be positive. Stone (1957), working with dormant Pinus lambertiana L. (sugar pine), showed that stratification improved germination percent, speed of germination and seedling vigor. Farmer (1974) found that stratification had a slightly positive effect on the first flush of Q. rubra L. (northern red oak) seedlings. However, Allen (1962), studying the viability and germination of conifer seeds, suggested that fall sowing (a natural stratification process) yielded better seedlings than late spring sowing of artificially stratified seeds.

In 1912, Haack used the term "plant percent" to define the number of seedlings that become established per 100 seeds planted in a seedbed. Thus, plant percent gives an index of seed survival by omitting those seeds that germinate but fail to develop to a transplantable stage. Plant percent in sugar pine has been shown to decrease with seed age and storage time even though germination remains high (Stone, 1957). Barton (1953) published information that plant percent was an excellent survival index for P. taeda (loblolly pine), P. echinata (shortleaf pine), Ulmus americana (American elm), and Abies balsamea (balsam fir) after 16 years of storage. Dyachenko (1940) reported that plant percent and germination percent increased with stratification for P. strobus (eastern white pine), P. rigida (pitch pine), P. contorta (lodgepole pine), P. peuci (Balkan pine), and P. ponderosa (ponderosa pine); but he looked only at relatively short storage times.

Acorn storage, stratification, and dormancy.

Bonner (1971) reported that the seed storage of several bottomland red oak species was optimal at moisture contents of 70% (dry weight basis[2]) and at temperatures just above freezing. Although viability was maintained for 18 months,

---

[2]All moisture contents in this paper are expressed on a dry weight basis (100 C, 24 hr.)

these conditions resulted in sprouting during storage. Roe (1941) also reported that high moisture content was neccessary for northern red oak acorn storage and that viability severely diminished when seed moisture levels dropped below 52%. Holmes and Buszwicz (1955) showed Q. robor (English oak) seed remained partially viable (52% germination) after 42 months of storage at 1 C and 70% moisture content. Higher moisture content, up to 95%, gave adequate germination after 18 months storage but thereafter, seed deterioration and extensive pregermination was observed. Holmes and Buszwicz (1955) concluded that the best storage medium was dry sand or peat in a closed but not sealed container. Containers were left unsealed to allow for gas exchange.

The moisture content of acorns during natural stratification is critical to regeneration of some red oak species in the Mississippi River Valley of Illinois and Mississippi. Krajicek (1968) reported that drying out of Q. falcata var. pagodafolia (cherrybark oak) acorns caused a complete loss of germination in the field. He suggested dry autumn conditions may reduce cherrybark oak seedling establishment. Conversely, Q. nutallii (Nuttall oak), another bottomland red oak species, has been shown to germinate naturally only following afterripening under water (Johnson, 1979).

Farmer (1974) stored Q. rubra (northern red oak) seeds at 5 C. They were stored at their harvested moisture content, 64%, or first imbibied to 82%. He reported no difference in acorn germinability after 4, 10,or 16 weeks irrespective of moisture condition. Farmer also noted that cracking the acorn did not increase germination significantly; but, when gibberellic acid, GA,(a strong growth promoting compound) was added to the cracked seed, germination was increased 150% after only 4 weeks of chilling. He concluded that: 1) unimbibed cold "storage" and imbibed "stratification" were not different, 2) GA broke the dormancy of dormant acorns, 3) cracking the acorns did not improve germination greatly, and 4) northern red oak exhibits "embryo dormancy" at seed fall, and 5) this dormant condition may be alleviated by a period of 4 to 10 weeks at low temperature. "Embryo dormancy" implies that germination blockages are internally imposed and not the result of seedcoat or pericarp factors. This embryo dormancy hypothesis for red oaks has been supported and suggested by other researchers (Korstian,1927; McDermott,1941; Vogt,1970).

In opposition to the embryo dormancy model, Jones and Brown (1966) concluded that the pericarp structure causes dormancy in cherrybark and northern red oaks. By excising the embryos from the pericarps or clipping the pericarp from

the apical end, they were able to break dormancy. Jones and Brown suggested that stratification may overcome dormancy when cell expansion during stratification ruptures the pericarp. Other researchers have found the pericarp imposes some degree of dormancy in red oaks (Bonner, 1973; Johnson, 1979) and other dormant seed also (Crocker, 1948; Crocker and Barton, 1953). In sugar pine, seeds remain dormant following seedcoat removal (Stone and Duffield, 1950).

Germination

The metabolic changes that occur during germination have been reviewed (Ching, 1972; Khan, 1977), and the overall sequence is well characterized. An important, early event for dormant seed is rehydration or imbibition (Roberts, 1969; Lewak and Rudnicki, 1977). Following imbibition, hydrolytic enzymes, such as phosphatases, hydrolases, and cytochrome oxidase, are fully activated and biological oxidations may accelerate (Ching, 1973a). During germination, mobilization of food reserves from the storage tissues (nucellus, endosperm, perisperm, or cotyledons) provides metabolites for the growth and differentiation of the embryonic axis. This process continues until the seedling becomes fully autotrophic, at which point germination is considered completed.

Phytic acid is a major component of seed that provides phosporus for production of phosporylated energy-rich nucleotides. Phytic acid, myo-inositol hexaphosphate, makes up about 80% of the phosporus in seed and is stored as calcium, magnesium or manganese salts (Copeland, 1976). Phytases act on the phytate to release inorganic phosphorus for the production of high energy nucleotides (Atkinson and Morton, 1959). Williams (1970) reported that the rate of phytic acid synthesis was closely related to levels of adenosine triphosphate (ATP) in the cell. Energy availability is apparently a critical factor in dormancy and germination. Metabolic energy is needed to drive endergonic reactions. In Q. rubra, where a large component of the stored food reserves are lipids (Vozzo, 1973), a conversion to insoluble sugars must be accomplished for germination to take place. Biochemical processes for these conversions require energy, oxygen, and time.

Respiration rate is critical benchmark of changes in metabolic processes. As the acorn desiccates prior to abscission and during the onset of dormancy, respiration rate declines to low levels, and overall metabolism is slowed (Vozzo, 1975; Bonner, 1976). This retardation of growth processes is evident in many physical and biochemical parameters. Declines in dry matter accumulation, oxygen uptake,

cytochrome enzyme activities, and adenylate energy metabolism are each associated with maturation and deepening of dormancy. There is a resurgence of respiration when dormancy is being broken and germination begins (Roberts, 1969).

Vozzo (1973) reported that water oak acorns (50% moisture) stored in carbon dioxide atmospheres of 1 to 10% had elevated respiratory quotients (RQ)[3] as the seed consumed less oxygen and produced more carbon dioxide. Further, Vozzo reported that embryo excision increased aerobic respiration, coinciding with results of Jones and Brown (1966). Brown (1939) measured changes in RQ during stratification of northern red oak and found that the RQ decreased early during stratification and then remained constant. Brown suggested the decline in respiratory quotients reflected the conversion of lipids (the major storage component of red oak seed) to carbohydrates. He further pointed out that a period of carbohydrate accumulation is necessary before northern red oak acorns commence germination.

---

[3]Respiratory quotient is defined as the amount of carbon dioxide evolved divided by the amount of oxygen consumed.

Adenylate energy metabolism and energy charge

The pioneering work on cellular adenylate energy economy was done by Atkinson (1968). Atkinson developed the concept of "energy charge" (EC) to measure the relative availability of high free energy bonds in the adenylates, ATP, ADP and AMP. The equation for EC is,

$$EC = \frac{[ATP] + 0.5[ADP]}{[ATP]+[ADP]+[AMP]}$$

and the values range from zero to one.

Coupled with allosteric control, i.e. feedback regulation by concentration gradients, EC may be the most important metabolic control mechanism in living cells (Atkinson,1968; Shen et al.,1968). Adenine nucleotides balance (EC) can regulate partitioning of energy storing, energy yielding, and energy demanding processes when end product concentrations are low (Shen et al.,1968; Atkinson,1968). However, when EC is high, the metabolic pathways are controlled by allosteric inhibition. In short, competition among various reactions for adenylate energy depends on the momentary concentrations of any one product or the needs of the cell. When the feedback product is available from other sources, the EC ratio becomes maximally sensitive to regulation of the pathway. Furthermore, EC modulates relative per-

meabilites and membrane transport; a critical factor in deterioration of some seeds (Atkinson,1969; Ching,1972; Parrish and Leopold,1978).

Working with growing or starved E. coli, Chapman et al. (1971) found that active (marked by increased cell size/number) growth occurred only at EC values above 0.8. Cells remained viable between 0.8 and 0.5 EC, but cells died at EC values below 0.5. However,the crucial EC value for plant tissues may generally be somewhat lower. The biochemical significance of this is unclear, but it may be associated with the high vacuolation of leaf cells and simply a dilution effect (Santarius and Heber, 1965). Reported EC values of mature, dry seeds of several species have been extremely low, ranging from 0.1 to 0.3; however, the EC increases rapidly to a range of 0.6 to 0.9 during imbibition, stratification, and active germination (Olney and Pollock,1960; Brown,1962; Ching and Ching,1972; Simmonds and Dumbroff,1974; Haferkamp et al.,1977). Adenylate values for mature, wet seeds, such as oak, have not been reported. Imbibition of only four hours, gave rapid increases in ATP levels in soybeans (Ching and Ching, 1972), and shows the need for ATP in supplying energy for endergonic reactions, regulation of biosynthesis and protein synthesis during seed germination. ATP increases rapidly in lettuce seed upon water absorption at 20 C (Bomsel and Pradet,1968).

Seed vigor, defined by Ching (1973) as the potential for rapid and uniform germination and seedling growth under field conditions, has been reported to be postively correlated with adenylate energy metabolism. McDaniel (1969) found that seed size was positively correlated with seed and seedling vigor in several pure lines of Hordeum vulgare L. (barley). He further reported that the biochemical competence of the mitochondria, as measured by number of mitochondria and amount of mitochondria protein, was responsible for this seed vigor. McDaniel found that there was no genetic variation for mitochondrial metabolism. Thus, greater energy efficiency due to more mitochondria protein appeared to play an important role in rapid growth and development of seedlings. Ching and Danielson (1972) supported this hypothesis by publishing strong correlations between ATP levels and seed weight, seedling weight, and seedling height in Lactuca sativa L. (lettuce). They suggested that ATP concentration alone may be a useful biochemical parameter as an index of seedling vigor. Ching and Danielson also found that accelerated aging reduced ATP content, germination percent and seedling size. Crompton et al. (1978) found strong correlation between seed vigor of Virginia and Spanish peanuts and total adenylates and ATP.

Ching and Ching (1972) found a seven-fold increase in total adenosine phosphates in the embryo of ponderosa pine seed during two weeks of low temperature stratificaton. During that time, EC rose from 0.75 to 0.85. They concluded that adenosine phosphate levels of germinating pine seed reflect growth, organogenesis and morphogenesis of the developing plant. They further reported that a change occurred during the stratification period that was essential for vigorous growth. Ching and Kronstad (1972) reported that differences between some high-yielding and low-yielding varieties of wheat were related to adenylate energy levels. Similar varietal differences in adenylate energy pools with highly correlated growth rates have been reported in peanuts (Crompton et al.,1978) and in alfalfa (McDaniel,1976).

Anderson (1977) reported that depressed adenylate metabolism indicated deterioration of stored Glycine max (soybeans) and proposed that a decrease in the ability of the seed to maintain ATP synthesis resulted in the loss of germinabilty and vigor. Working with germinating soybeans, Anderson (1977a) found that, by adding exogenous adenine and adenosine, the levels of metabolically active adenine nucleotides could be increased. This ability to increase adenylate levels may be valuable in the stimulation of germination of dormant red oak seed.

In an effort to establish a causal relationship between energy charge and growth, Simmonds and Dumbroff (1974) experimentally altered the EC of dormant seed of Acer platinoides (Norway maple). After stratifying the seeds for 20 days, the seeds were moved to anaerobic conditions for 7 days. They found the ATP levels unchanged, but the ADP and AMP levels were significantly increased, thus lowering the EC. During anaerobiosis, growth of the excised axes responded very slowly to growth regulators. Simmonds and Dumbroff (1974) concluded that a lowering of the EC, by anaerobic conditions toward the end of stratification, decreases the sensitivity of excised embryos to growth regulators. Since this process was reversible, they concluded that a high EC was essential for hormonally induced elongation.

Analyzing ATP levels and EC ratios has promise in forest tree seeds, where long stratification periods are recommended (USDA, 1974b). Such energy analysis may reveal the range of adequate energy levels for prompt germination, thus allowing for preseasonal seedling growth in a greenhouse. In addition, seed lots that are potenially more vigorous may be detected by their elevated ATP levels and EC ratios, and thereby be screened in genetic selection and tree improvement programs.

METHODS AND MATERIALS

Seed collection

Acorns were collected from single sites in mid-October 1980 and 1981. The 1980 seed were taken from Butt Mountain in Giles County, Virginia (elevation 1000m). Approximately 18,000 acorns were collected within a 10 day period (October 15-25). In 1981, approximately 10,000 acorns were collected between October 22nd and 27th on the Virginia Tech campus, Montgomery County, Virginia (elevation 700m). In each year, the seedlot consisted of pooled seeds from all the trees over the collection period. There were 16 and 9 seed-producing trees for 1980 and 1981, respectively.

Seed handling

In 1980, the seeds were taken to the lab, and floated briefly to remove trash, debris, and bad seeds (any that floated). Average moisture content was determined gravimetrically from three samples of five acorns each. The samples were weighed fresh and then oven dried at 105 C for 24 hours (Bonner, 1977). Moisture content at collection was 64% (dry weight basis).

To dry acorns for low moisture (Lm) stratification, a fan was placed above a bilayer of seed and air circulated until a moisture percent of 50% was obtained. Acorns for high moisture (Hm) stratification were imbibed for 52 hours and reached a moisture content of 70%. The acorns for Lm and Hm stratification had moisture contents of 50% and 70%, respectively. Lm stratification seeds were placed in polyethylene plastic jugs (4 mil) and covered with loosen screw caps. For Hm stratification, seeds were put on moisten Promix BX* in trays and then covered with wet blotter paper. (Pericarp treatments, as outlined below, were imposed on some of the Hm seeds prior to placement in the trays). The entire tray was then fitted with a polyethylene cover to maintain high humidity within the tray. The plastic storage jugs and stratification trays were then transfered to a walk-in cold room where the temperature was maintained at 5 C (+/- 2 C) and approximately 70% relative humidity.

The acorns for the 1981 lot were treated similarly, except that Lm stratification was not performed. Seeds were floated for cleaning, imbibed to a mean moisture content of 83%, placed on moist Promix BX, and placed in the cold room. In addition, one half of the tray of seed was misted with 5%

*Promix BX is a trade name of a standard potting mix composed of peat, vermiculite and perlite. Use of trade name products does not constitute endorsement of said product by Viginia Polytechnic Institute and State University.

Phaltan (Ortho) fungicide to prevent fungal growth, which had been noted in 1980.

To facilitate testing procedures, each Lm stratification jug and each Hm stratification tray contained only seeds that were needed each date for the germination, seedling dry weights, and adenylate tests. For Lm stratification analysis (1980 only), the containers were filled with 225 acorns each. For Hm stratification, 135 seeds were placed on Promix BX trays in a grid of 7 x 15 seeds, so that three replicates of 33 and one rep of 34 seeds constituted each tray. The 1980 tests included three pericarp removal treatments;

1. pericarp removed prior tc stratification (-/-),
2. pericarp intact during stratification and removed prior to germination (+/-), or
3. pericarp intact during stratification and germination (+/+).

For Lm stratification (1980 only), only pericarp treatments +/- and +/+ were tested. During the 1980 Hm stratification, acorns were tested for germination and seedling growth (see below) following weeks 0, 2, 3, 4, 6, 8, 10, and 12. Seeds from Lm stratification were also tested for germination and

early growth but at longer intervals, following weeks 0, 4, 8, 12, 16, 20, 24, 28, and 32.

In 1981, testing emphasis was on Hm stratification and subsequent germination and EC. Hm stratification trays consisted of 100 acorns with four reps of 25 seeds each, and only two pericarp treatments were imposed,

1. pericarp removed prior to stratification (-/-), and

2. pericarp intact during stratification and germination (+/+).

Germination tests and adenylate analysis were conducted following 0, 1, 2, 3, 4, 6, 8, and 10 weeks of stratification for each of the pericarp treatments.

Pericarp removal

From the chalazal end of the acorn, approximately 1/3 of the acorn was cut with hand clippers. In this way the pericarp could then be removed easily from the remaining embryonic tissue. The embryonic axes remained intact and those seed that appeared to have damaged axes were discarded. The seedcoat or testa remained in place, attached tightly to the cotyledon and embryonic axis.

Germination procedures and conditions

Acorns from Hm stratification were germinated in place on moist Promix BX in trays. Wet blotter paper remained spread over the seeds,and the plastic cover was kept on the germination tray to maintain high humidity conditions. (Pericarps were removed following stratification for the 1980 +/- treatment.) Acorns from the Lm stratification were placed onto moist Promix (following imbibition) in trays with the same configuration as for Hm seeds. Germination temperatures were controlled at 25/15 C (16/8hr) in a growth chamber. All germination tests were conducted in the dark. Germination counts were made every other day following first signs of germination. A seed was considered germinated once the elongating radicle showed positive geotropism. All germination tests were terminated after 30 days in the growth chamber.

Seedling growth

To determine the effect of pericarp and stratification on seedling growth, seeds from the 1980 collection were conditioned, stratified, and germinated as previously described. In fact, for each tray or jug of the germination study, there was a simultaneous, parallel tray or jug for the seedling growth study. Germination results were

recorded on these seed, also. Germinated seed were planted 2.5 cm deep in 1 liter plastic pots in moist Promix BX potting medium. The pots were placed in a growth chamber at 25/15 C (16/8 hr). All seedlings were grown in the dark and removed at 7,14,21,or 28 days for destructive sampling. Root length, shoot length, root dry weight, shoot dry weight, cotyledonary petiole dry weight and cotyledon dry weight were measured. The experiment was conducted on the three 1980 pericarp removal treatments and at eight intervals during Hm stratification and nine intervals during Lm stratification.

Adenylate analysis

The effect of Hm stratification and pericarp removal on the adenylate metabolism was determined on seed from the 1981 collection. Whole acorns were stratified according to the procedure previously outlined. Seed samples were removed following 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 weeks of stratification. Two five-acorn samples were taken from each of the two pericarp treatments (-/- and +/+) at each sampling interval. Embryos were rapidly diced (10 secs) and placed in liquid nitrogen. The seeds were ground with mortar and pestle under liquid nitrogen and the pulverized tissue put in a glass bottle. The bottle was labelled

and stored at -20 C. A preliminary test showed no differences between fresh and frozen tissue in extractability or recovery of adenylate compounds.

Measurement of adenylate metabolism during germination

To measure changes in adenylate energy during the germination period, 400 seed were stratified for 8 weeks. An 8 week stratification period synchronized the seeds so that the variation in rate and time of germination was minimized. Four trays of acorns (100 acorns/tray) were stratified for 8 weeks and then placed in the germinatoion chamber as above. One-half the acorns received pericarp treatment -/- and one-half the acorns remained intact (pericarp treatment +/+). Two samples of five acorns each were randomly collected from each tray at days 0, 5, 10, 15, 20, and 25 of the germination period. At collection, the seedlings (minus pericarp) were diced, frozen, pulverized by grinding and then stored in the freezer for later determination of the adenylates.

Extraction and quantification of adenylate compounds

The boiling water extraction method (Ching and Ching, 1972) was compared to the hot ethanol ATP extraction of St. John (1970). The preliminary tests showed the boiling

water to be more reproducible and to give higher sensitivity for acorn extracts. Ching and Ching (1972) and Stewart and Guinn (1969) also found that the boiling water method was better for ponderosa pine and cotton respectively.

The boiling water method of ATP, ADP and AMP extraction was as follows. Two grams fresh weight of the frozen tissue was placed in 25 ml of boiling, distilled water. The tissue was boiled for 8 min, allowed to steep for 3 min, and then 3 g of polyvinylpyrrolidone (PVP) was added and mixed to a slurry. The slurry was filtered by aspiration through #1 Whatman filter paper. The volume of the filtrate recovered was recorded and then brought to 15 ml volume with distilled water. The extract was stored for up to 3 hr before analysis or analyzed immediately.

Adenosine phosphate determinations.

One half ml aliquots of the cooled extraction solution were incubated at 37 C for 15 min in test tubes with each of the following mixtures (Ching and Ching, 1972).

1. ATP determination, 0.1 ml distilled water and 0.1 ml of the reaction buffer (0.1M TRIS, tris (hydroxymethyl) aminomethane, pH 7.75 with 0.5M magnesium acetate).

2. ADP and ATP determination, 0.1 ml of a solution containing 20 ug of pyruvate kinase (E.C. 2.7.1.40) (Sigma Chemical) and 500 nmoles of trisodium phospho(enol)pyruvate (Sigma,crystalline) and 0.1 ml of the reaction buffer.

3. AMP, ADP, and ATP determination, 0.1 ml of a solution containing 20 ug of pyruvate kinase, 500 nmoles of trisodium phospho(enol)pyruvate (Sigma,crystalline) and 20 ug adenylate kinase or myokinase (E.C. 2.7.4.3) (Sigma), and 0.1 ml of the reaction buffer.

The enzymes were desalted from the ammonium sulfate solution by microcentrifugation for one minute at 2000 rpm.

The luciferin-luciferase biological assay was used to quantify ATP, ADP and AMP. After reconstituting freeze dried firefly lantern extract (Sigma FLE-50) with 5 ml ice cold distilled water, the extract consisted of 0.05 M potassium arsenate and 0.02 M magnesium sulfate and 50 firefly lanterns. The firefly solution was then held for at least 16 hr in a cooler (2 C) in order to deplete the endogenous ATP within the extract. The firefly protein-enzyme (luciferin-luciferase) complex reacts with ATP in the presence of magnesium and oxygen and produces light. The intensity of the

light is porportional to the ATP concentration. The light emission was measured by a spectrophotometer and recorded on a strip chart recorder. The peak height produced from injection of firefly extract into seedling extracts was compared to peak heights of known ATP standards. Duplicate samples were determined on each extraction. After injecting 100 ul of firefly lantern extract (Sigma FLE-50), the maximum instantaneous peak height (mm) of light emission was used to quantify ATP, ADP and AMP. Peak heights have been used previously in luciferin-luciferase assays to measure adenosine phosphates (Aledort et al,1966; Ching and Ching, 1972). The light emitted was linearly correlated with ATP concentration between 5-400 uM ATP. With the spectrophotometer set to multiply light 10x, and the recorder set on the 1mV range, a peak height of 50 mm represented approximately 10 uM ATP. ATP was calculated from the peak height of reaction mixture 1, ADP concentration was determined from the difference in peak heights of mixtures 1 and 2, and AMP concentration from the difference between mixtures 2 and 3.

Calculation of ATP, total adenylates, and Energy Charge.

The amount of ATP per sample was estimated from regresssion equations that had been generated from standard curves for each vial (Table 1). Fresh ATP standards were

Table 1: Regression equations used to predict ATP concentrations in northern red oak acorns.[1]

Firefly extract vial	Coefficients		R^2
	b_0	b_1	
171	-3.4824	0.1255	.9998
191	-0.7255	0.1733	.9993
221	-0.2547	0.0919	.9978
241	-9.2479	0.4227	.9241
272	1.9029	0.0578	.9115
281	-0.9825	0.1029	.9747
282	1.2955	0.1090	.9072
091	5.1158	0.0577	.9809
092	6.9082	0.1225	.9877
271	-4.8675	0.0740	.9202
222	-2.4263	0.1018	.9908
301	-0.9454	0.1310	.9944
431	3.4990	0.0792	.9272
432	3.8114	0.2698	.9263
441	1.4995	0.2156	.9867
442	0.1203	0.1397	.9855
433	-1.6016	0.1584	.9085
051	0.0301	0.2442	.8346

[1]The model: $ATP = b_0 + b_1$ Pkht, where Pkht = peak height (mm) and ATP = adenosine triphosphate concentration (uM).

prepared for each batch of firefly extract, due to the high variability between vials of firefly extract. Each firefly solution (5 ml) permitted determinations for approximately 8 samples (including standards). The adenylate energy charge was calculated after Atkinson (1969) based on concentration of adenylates (umoles/g dry wt) in the seed tissue, where,

$$EC = \frac{[ATP] + .5[ADP]}{[ATP] + [ADP] + [AMP]}$$

The adenylate compounds were quantified as umoles per gram dry weight of acorn tissue. Moisture content of the acorn tissue was determined on aliquots of the pulverized samples at the time of extraction.

Statistical Analysis

All statistical analyses were carried out using Statistical Analysis Systems (SAS) programs (Helwig and Council, 1979).

Germination data.

Germination capacity was defined as the number of normal germinants at 28 days divided by the total number of seeds of the replicate. These germination capacities were transformed into the variable ARCGERM where

$$ARCGERM = \arcsin\sqrt{germcap}$$

Analysis of variance was then performed on the ARCGERM for the main effects of pericarp removal and weeks of stratification. Duncan's New Multiple Range test was used to determine significant mean differences. Peak values (Czabator, 1968; Bonner, 1977) were calculated to measure the speed of germination. Peak value is the highest quotient obtained from dividing the cumulative germination percent by the number of days of the incubation.

Seedling growth.

Means and standard errors were calculated for each seedling growth variable. Analysis of variance was performed on each variable to calculte F ratios for effects of pericarp removal and stratification time. Duncan's New Multiple Range test was used to determine significant differences among means. These analyses were conducted on data for etiolated seedlings after 14 and 28 days growth.

Two new variables were created from the basic measurements. A root length to shoot length ratio was calculated as,

Root/Shoot = root length/shoot length.

Axial dry weight was created by adding the dry weights of shoot and root and cotyledonary petiole. These variables gave a good index of the rapid changes occurring during the first 4 weeks of northern red oak growth.

Relative growth rates (RGR) were calculated from the mean dry weights for days 1, 7, 14, 21, and 28. The following equation was used (Radford, 1968),

$$RGR = \frac{\ln W'' - \ln W'}{t'' - t'}$$

where,

W = dry weight and

t = days as a continuous function.

Adenylate energy metabolism.

Means and standard errors were calculated for ATP, ADP and AMP concentrations. Also, means and standard errors were recorded for EC.

RESULTS

Germination

Influence of pericarp

Pericarp removal significantly increased northern red oak acorn germination. For seeds collected in 1980 and 1981, germination percentages (measured after 28 days incubation) were significantly higher when the pericarp was removed (Figure 1).

The highest germination percentage, when averaged over all stratification times, was 80% for the seeds in which the pericarp was intact during stratification but removed for the germination test (+/-).

Germination rates, as measured by peak value (PV), were slightly higher in seed with pericarps removed (+/- or -/-) than in seed with pericarp intact throughout (+/+) (Table 2).

The maximum difference between pericarp treatments occurred after 10 weeks of stratification when 70% of the acorns with pericarps removed prior to stratification (-/-) germinated in two days for a PV of 35.5. At week 12, PV for seed with pericarp treatment -/- was 13.9, 14.2 for pericarp treatment

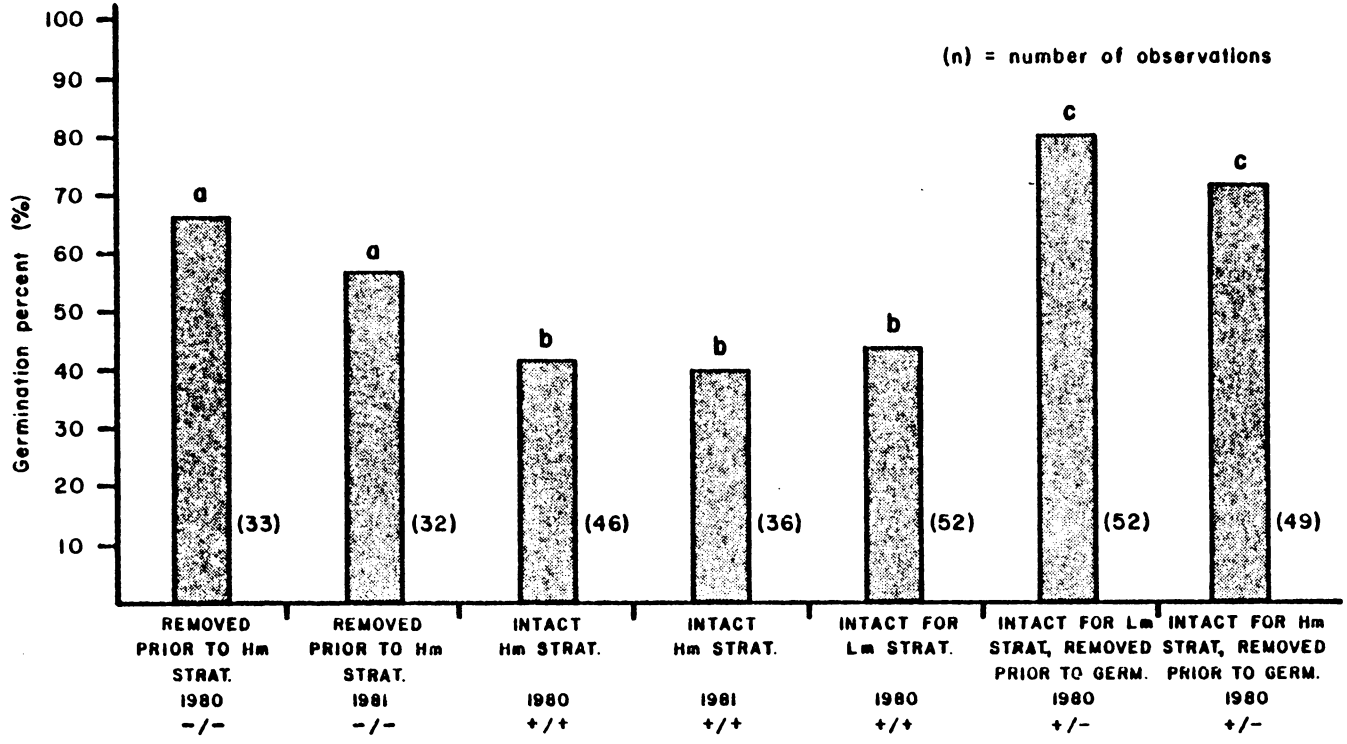

Figure 1: Germination percentage (after 28 days incubation) for northern red oak as affected by pericarp removal. Bars not followed by the same letter are significantly different at P .05 as determined by Duncan's New Multiple Range Test on arcsin transformations of germination percent. Numbers in parentheses equal n. Tests were conducted for two years except for Lm stratification.

Table 2: Peak values (PV[1]) for germination of northern red oak seed stratified for various times as affected by pericarp removal. Values in parentheses represent one $s_{\bar{x}}$.

PERICARP TREATMENT	STRATIFICATION TIME (weeks) 0	2	4	6	8	10	12	Total
	PV							
pericarp removed prior to stratification (-/-)	3.5 (.15)	1.9 (.27)	2.1 (.17)	4.0 (.09)	7.7 (.97)	35.5 (2.87)	13.9 (.73)	7.8 (1.61)
pericarp intact during stratification, removed prior to germination (+/-)	3.4 (.07)	1.9 (.29)	2.1 (.62)	4.7 (.16)	5.8 (.18)	7.5 (.49)	14.2 (.76)	5.2 (.73)
pericarp intact (+/+)	0.8 (.10)	0.6 (.04)	0.9 (.10)	3.2 (.25)	7.4 (.55)	13.0 (1.08)	7.2 (.76)	3.8 (.64)
Total	2.6 (.38)	1.4 (.23)	1.7 (.26)	4.0 (.20)	7.0 (.42)	18.6 (3.76)	11.7 (1.04)	

[1]Peak value (PV) is the highest quotient obtained when cumulative germination percent was divided by days of incubation.

+/- and 7.2 for acorns germinated with pericarp intact (+/+).

The fungicide applied on 1981 seeds had no effect on the germination percentages or germination rates regardless of pericarp treatment.

Influence of moisture content and stratification time

Low temperature (5 C) storage, i.e. stratification, of northern red oak seed increased germination significantly. Germination of both the high (70%) (Hm) and low (50%) (Lm) seed moisture treatments was significantly significantly ($P<.05$) increased by germination (Figures 2 and 3). There was no difference in the overall effect of Lm and Hm stratifcation for germination percentage or speed of germination. Both stratification moistures gave similar patterns. Comparisons of weeks 0, 4, 8, and 12 of Lm and Hm stratification showed that there were great similarities in germination at each interval. Only week 8 showed more than a 10% difference between the two moisture levels.

For each moisture treatment, germination declined during the first 4 weeks of stratification; germination was reduced from 40% to 30% between weeks 0 and 4. A rise in germinability then occurred between weeks 4 and 8. Another significant increase in germination was observed between 8 and 12 weeks of low temperature stratification.

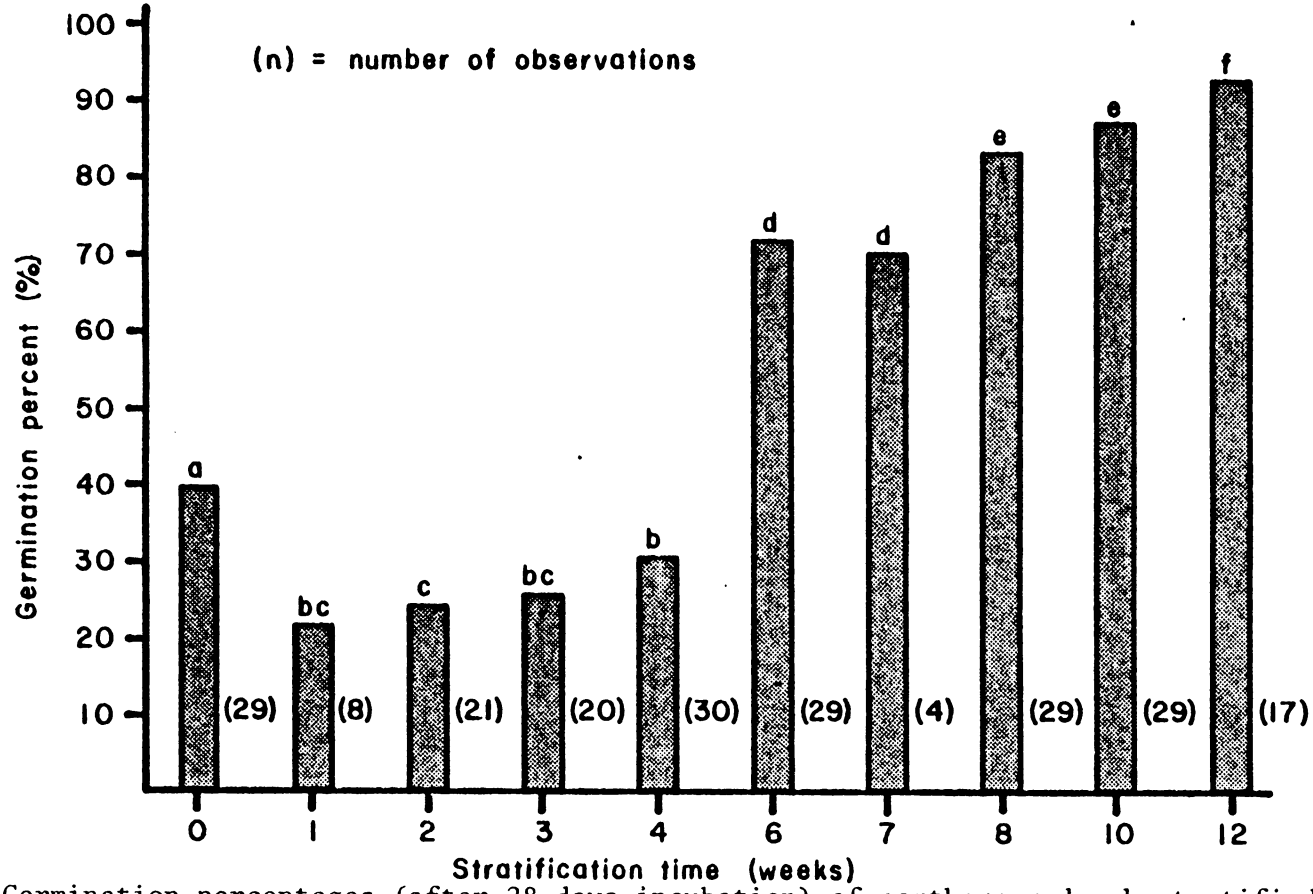

Figure 2: Germination percentages (after 28 days incubation) of northern red oak stratified at 70% moisture and 5 C. Values in parentheses equal n values for mean determinations. Bars not followed by the same letter are significantly different at P .05 as determined by Duncan's New Multiple Range Test on arcsin transformations on germination percentage.

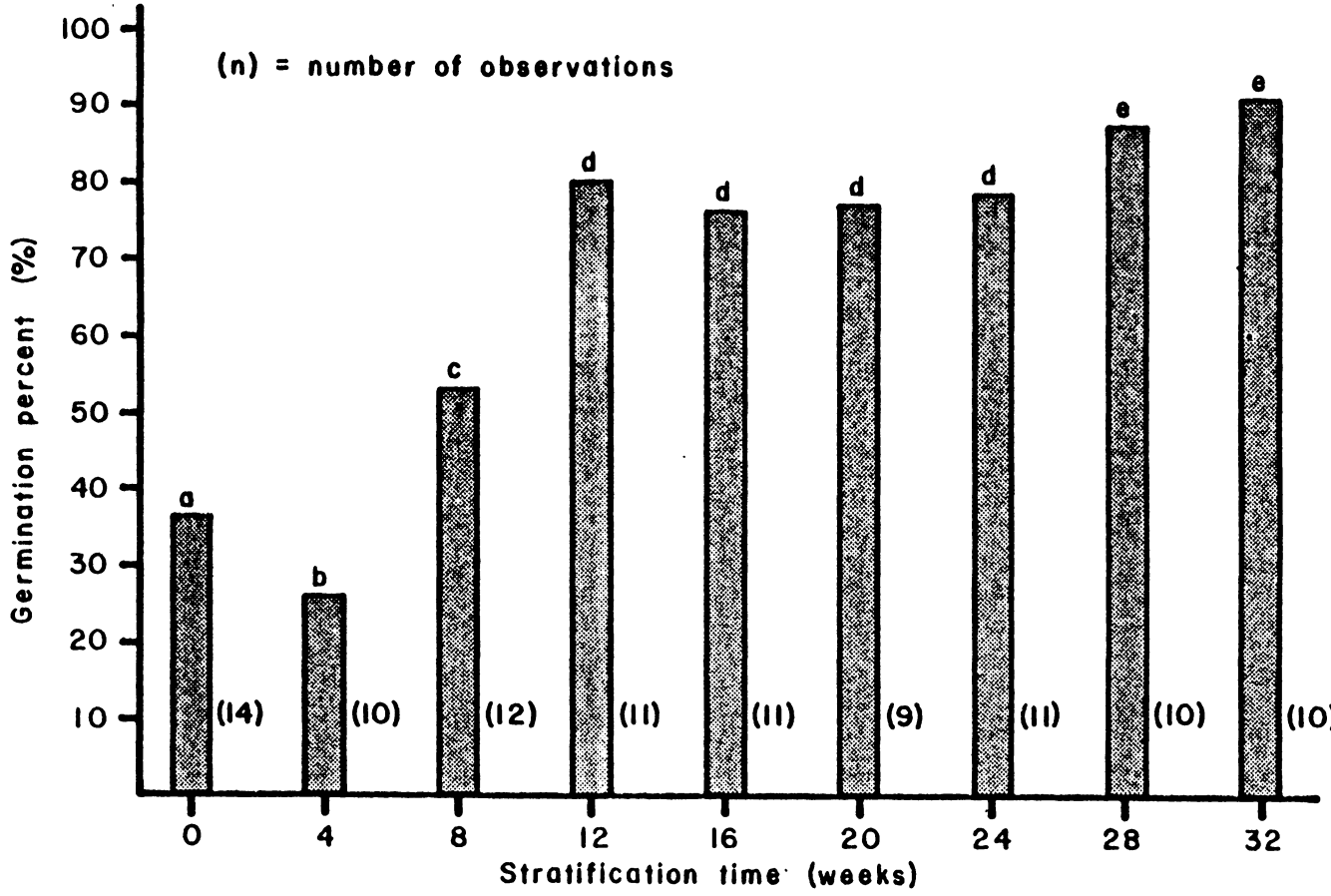

Figure 3: Germination percentages (after 28 days incubation) of northern red oak stratified at 50% moisture and 5 C. Values in parentheses equal n values for mean determinations. Bars not followed by the same letter are significantly different at P .05 as determined by Duncan's New Multiple Range Test on arcsin transformations of germination percentage.

A significant decrease in germination was found even prior to week 4 of Hm stratifcation (Figure 2). Germination dipped from 40% to 20-25% by week 1 and remained low for the next 3 weeks. There was a slight but significant increase by week 4 of Hm stratification. Germination increased greatly between 4 and 6 weeks of cold stratification from 30% to more than 70%. Additional significant increases in germination (to a high of 90%) occurred at weeks 8 and 12.

The initial decline and subsequent rise in germination during the first 4 weeks of stratification was mirrored in the germination rate of Hm-stratified seeds as measured by PV (Table 2). Between week 0 and 2, the PV decreased for all pericarp treatments and then increased between week 4 and 6. PV was generally highest at week 10.

Influence of seed year

There were no significant differences in germination or rate of germination (PV) between acorns collected in 1980 or 1981. The same responses to pericarp removal (treatments -/- and +/-) were noted for both collections (Figure 1). Similar trends during stratification, the early decline in germination percent and PV during weeks 1 to 4 followed by a rapid rise in germinability through week 12, were found in both years. Because there were no differences, the germination

results from 1980 and 1981 were pooled to increase sample size for statistical analyses.

Seedling Growth

Influence of pericarp

Following low moisture stratification (Lm), seeds with pericarp left intact and grown for 28 days in dark were taller than seedlings grown from acorns with pericarp removed (Table 3). Root length and shoot length were greater on intact (+/+) seedlings than on pericarp removed (+/-) seedlings; however there were no significant differences in the root/shoot ratios.

Pericarp removal did not significantly affect the shoot elongation of seedlings from acorns stratified under high moisture conditions (Table 3). Seedlings grown from acorns with the pericarp removed (-/-) and Hm stratified had a root/shoot length ratio closest to one (1.2), but this value was not significantly ($p<.05$) different from the other pericarp treatments (+/- and +/+).

The pericarp affected the relative growth rate (RGR) of the axis measured as dry weight accumulation (g/g/day), especially during the early period of Hm stratification (Figure 4).[5] With no stratification (week 0), seedlings from

[5]Relative growth rates (RGR) were determined from mean seedling root, shoot, and total axis dry weights at day 1, 7,

Table 3: Effect of pericarp removal[1] on northern red oak seedlings grown for 28 days averaged over all stratification times at low moisture, Lm (50%, d.w.) and high moisture, Hm (70%, d.w.) conditions. Means not followed by the same letter are significantly different as determined by Duncan's New Multiple Range Test ($P < .05$).

	PERICARP TREATMENT		
GROWTH VARIABLE	-/-	+/-	+/+
	----------Lm----------		
Root length (cm)		23.8 a	31.1 b
Shoot length (cm)		24.7 a	33.3 b
Root/Shoot ratio		1.4 a	1.1 a
	----------Hm----------		
	-/-	+/-	+/+
Root length (cm)	26.1 a	26.1 a	26.0 a
Shoot length (cm)	29.2 a	26.2 a	28.9 a
Root/Shoot ratio	1.2 a	2.0 a	1.6 a

[1]Pericarp treatments were (1) pericarp removed prior to stratification (-/-), (2) pericarp intact during stratification then removed prior to germination (+/-), and (3) pericarp intact (+/+).

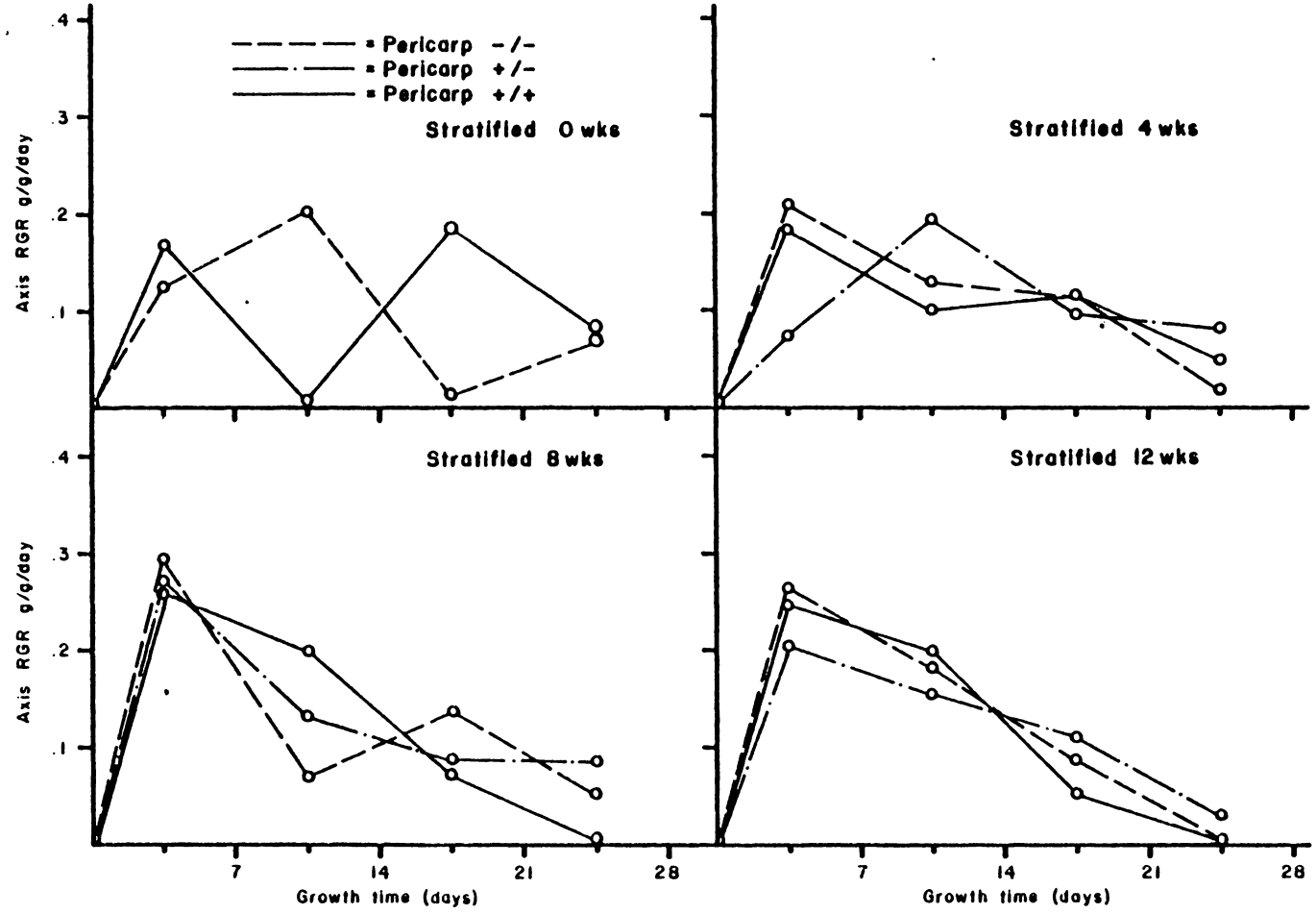

Figure 4: Effects of stratification and pericarp removal on the relative growth rate (RGR) of northern red oak axes during the first 28 days following germination. Pericarp treatments were, pericarp removed prior to stratification, -/-; pericarp intact during stratification then removed prior to germination, +/-; pericarp intact, +/+.

seed with pericarp removed (-/- or +/-) maintained a higher RGR for a longer period of time than did seedlings from intact acorns (+/+). Seedlings from pericarp removed treatments (-/- or +/-), however, flushed only once, while seedlings from intact (+/+) seed had two axial growth flushes. The first flush occurred between germination days 0 and 7, and the second flush between days 14 and 21. After 4 weeks of stratification, seedlings from pericarp removed seed (+/-) had their highest axial relative growth rate between days 7 and 14; whereas seedlings from pericarp treatments -/- and +/+ flushed during the first 7 days of germination. After 8 and 12 weeks of Hm stratification, there was little difference in the RGR of dark-grown oak seedlings. Seedlings from all pericarp treatments responded similarly by flushing during the first week, then declining in RGR through week 4.

14, 21, and 28. These values are meant only to characterize the seedling growth patterns. It should be noted that relative growth rates do not yield the same trends as absolute growth rates, and that RGRS indicate plant growth efficiency relative to the initial size of the plant. Analysis of variance on seedling growth measures indicated significant differences by pericarp treatment and week of stratification.

Influence of stratification

Generally, seedlings grown from stratified acorns, regardless of moisture content, grew taller and accumulated more axial dry weight with increasing stratification time. Tables 4 and 5 show the effect of Hm and Lm stratification, respectively, on root, shoot and axis growth after 14 and 28 days. After two weeks of stratification caused the root and shoot lengths of 14-day-old seedlings were significantly greater than non-stratified seedlings. Between weeks 8 and 10 of Hm stratification, the 14-day-old plants showed significant increases in root dry weight, shoot length, shoot dry weight, axis length and axis dry weight. Similar increases in shoot length, shoot dry weight and axis length were found in 28-day-old seedlings after 8 and 10 weeks of stratification. The first significant increases in growth of 28-day-old plants were observed after 4 to 6 weeks of Hm stratification. Major increases in root length, shoot length, shoot dry weight, axis length and axis dry weight (relative to seedlings from unstratified seeds) occurred during this period.

Subjecting seeds to low moisture stratification for up to 32 weeks (Table 5) produced growth responses that followed the same general pattern as those discussed for Hm stratification (Table 4). Stratification for longer than 12

Table 4: Effect of high moisture, Hm (70%, d.w.) stratification at 5 C on growth of northern red oak seedlings grown for 14 and 28 days after germination in the dark. Means not followed by the same letter are significantly different by Duncan's New Multiple Range Test ($P < .05$).

VARIABLE	GROWTH DAYS	STRATIFICATION TIME (weeks) 0	2	4	6	8	10	12
Root length (cm)	14	10.6 (b)	15.1 (a)	15.3 (a)	15.2 (a)	16.5 (a)	17.4 (a)	18.6 (a)
	28	21.4 (b)	19.6 (b)	21.2 (b)	26.0 (ab)	29.8 (a)	30.7 (a)	29.2 (a)
dry weight (g)	14	.07 (c)	.07 (c)	.07 (c)	.11 (b)	.11 (b)	.19 (a)	.13 (a)
	28	.18 (b)	.18 (b)	.18 (b)	.25 (b)	.33 (a)	.24 (b)	.21 (b)
Shoot length (cm)	14	1.4 (c)	5.3 (b)	2.4 (cb)	2.4 (cb)	3.7 (cb)	9.8 (a)	9.0 (a)
	28	12.9 (c)	19.4 (c)	19.3 (c)	28.6 (b)	30.9 (b)	41.2 (a)	37.0 (ab)
dry weight (g)	14	.05 (ab)	.03 (ab)	.01 (b)	.02 (b)	.03 (ab)	.07 (a)	.07 (a)
	28	.09 (c)	.17 (c)	.13 (c)	.28 (b)	.27 (b)	.38 (a)	.31 (ab)
Total axis length (cm)	14	12.0 (c)	20.5 (b)	17.8 (bc)	17.7 (bc)	20.3 (b)	27.3 (a)	27.6 (a)
	28	34.4 (c)	39.0 (c)	40.5 (c)	54.7 (b)	60.8 (b)	72.0 (a)	66.2 (ab)
dry weight (g)	14	.14 (c)	.13 (c)	.12 (c)	.17 (bc)	.17 (bc)	.31 (a)	.25 (a)
	28	.32 (c)	.39 (c)	.37 (c)	.57 (ab)	.65 (a)	.67 (a)	.56 (ab)
Root/Shoot length (cm)	14	6.8 (ab)	3.7 (cd)	7.6 (a)	6.3 (ab)	4.7 (bc)	2.1 (d)	2.4 (d)
	28	2.8 (a)	2.5 (ab)	2.4 (ab)	1.8 (bc)	0.9 (bc)	0.7 (c)	0.7 (c)

Table 5: Effects of low moisture, Lm (50%, d.w.) stratification at 5 C on growth of northern red oak seedlings grown for 14 and 28 days after germination in the dark. Means not followed by the same letter are significantly different by Duncan's New Multiple Range Test ($P < .05$).

VARIABLE	GROWTH DAYS	STRATIFICATION TIME (weeks) 0	4	8	12	16	20	24	28	32
Root length (cm)	14	10.6 (b)	18.0 (ab)	17.6 (ab)	17.6 (ab)	19.6 (ab)	20.6 (a)	21.0 (a)	-	14.8 (b)
	28	21.4 (b)	26.1 (ab)	23.8 (b)	30.0 (a)	32.1 (a)	24.8 (ab)	26.0 (ab)	24.2 (ab)	27.8 (ab)
dry weight (g)	14	.07 (b)	.07 (b)	.13 (ab)	.15 (a)	.13 (ab)	.15 (a)	.15 (a)	-	.12 (ab)
	28	.18 (b)	.24 (a)	.20 (ab)	.20 (ab)	.18 (b)	.22 (ab)	.29 (a)	.20 (ab)	.21 (ab)
Shoot length (cm)	14	1.4 (b)	2.2 (b)	1.9 (b)	6.3 (a)	8.6 (a)	9.0 (a)	10.3 (a)	-	9.2 (a)
	28	12.9 (b)	22.1 (ab)	16.8 (b)	28.1 (ab)	32.3 (a)	29.2 (ab)	29.2 (ab)	35.0 (a)	29.8 (a)
dry weight (g)	14	.05 (bc)	.01 (c)	.01 (c)	.05 (bc)	.06 (b)	.11 (a)	.09 (ab)	-	.08 (ab)
	28	.09 (b)	.16 (b)	.19 (b)	.26 (ab)	.22 (b)	.37 (a)	.31 (a)	.26 (ab)	.24 (ab)
Total axis length (cm)	14	12.0 (b)	18.9 (b)	19.6 (b)	24.0 (ab)	28.2 (a)	29.6 (a)	31.3 (a)	-	24.1 (ab)
	28	34.4 (b)	51.3 (ab)	40.6 (b)	58.1 (ab)	64.4 (a)	54.1 (ab)	55.3 (ab)	59.2 (a)	57.7 (ab)
dry weight (g)	14	.14 (bc)	.10 (c)	.18 (bc)	.23 (ab)	.22 (ab)	.29 (a)	.27 (a)	-	.23 (ab)
	28	.32 (c)	.44 (b)	.44 (b)	.49 (ab)	.44 (b)	.63 (a)	.67 (a)	.49 (ab)	.49 (ab)
Root/Shoot	14	6.8 (a)	7.3 (a)	7.8 (a)	3.6 (b)	3.1 (b)	2.7 (b)	2.0 (b)	-	2.3 (b)
	28	2.8 (ab)	3.1 (a)	2.1 (ab)	1.4 (b)	1.0 (b)	1.0 (b)	1.0 (b)	0.7 (b)	0.9 (b)

weeks had little additional effect on seedling growth. In some cases, extended Lm storage resulted in lower values. The highest values for all growth parameters except root/shoot ratio occurred between weeks 16 and 24. There appeared to be a declining trend in the vigor of seedlings after 28 weeks of Lm stratification, but this decline was not significant. The major increases in root, shoot, and axis length of 28-day-old seedlings occurred from seeds that underwent between 8 and 16 weeks of Lm stratification. Root, shoot, and axis reached a maximum in seedlings developed from seeds stratified for 16 weeks, while dry weight accumulation in the root, shoot, and axis peaked with seedlings whose seed had been stratified a month longer at week 20.

Significant decreases in the root to shoot length ratio occurred after Hm and Lm stratification treatments (Table 4 and 5). The decline occurred for 28-day-old plants developed from seeds with 6 weeks of Hm stratification. For Lm stratification, a significant reduction was evident between week 4 and 16. After reaching a value of about one at 12 weeks of Lm stratification and at 8 weeks of Hm stratification, the root/shoot ratio for 28-day-old seedlings showed some tendency to decline with extended periods of stratification.

Relative growth rates were calculated from root and shoot dry weights for Hm stratification for 0, 4, 8 and 12

weeks (Figures 5 and 6). Generally, etiolated seedlings accumulated more dry matter in the root during the first week of growth. There was little shoot growth during the first week and then during the 2nd or 3rd weeks shoot RGR increased. The highest shoot RGRs were found on seedlings developed from acorns with 4 to 8 weeks of HS stratification. There was only one growth flush for root and shoot growth on plants from seeds stratified for 8 and 12 weeks; whereas, for 0 and 4 weeks of cold treatment, the growth tended to be more episodic, especially for roots. For example in Figure 5a and 5b, the root RGR shows two distinct surges; one between day 0 and 7, and another between day 14 and 21. These curves show generally lower RGRs from partially afterripened acorns. The curves in Figure 6 show that roots and shoots were more vigorous on seedlings from seed that had been stratified for 8 or 12 weeks.

Adenylate energy metabolism

Adenylate response to stratification and pericarp removal

Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) concentrations of acorns fluctuated during stratification (Figures 7 and 8). All nucleotides were at their maximum observed value prior to stratification with 4.3 to 5.0 umoles/g dry wt. This 0

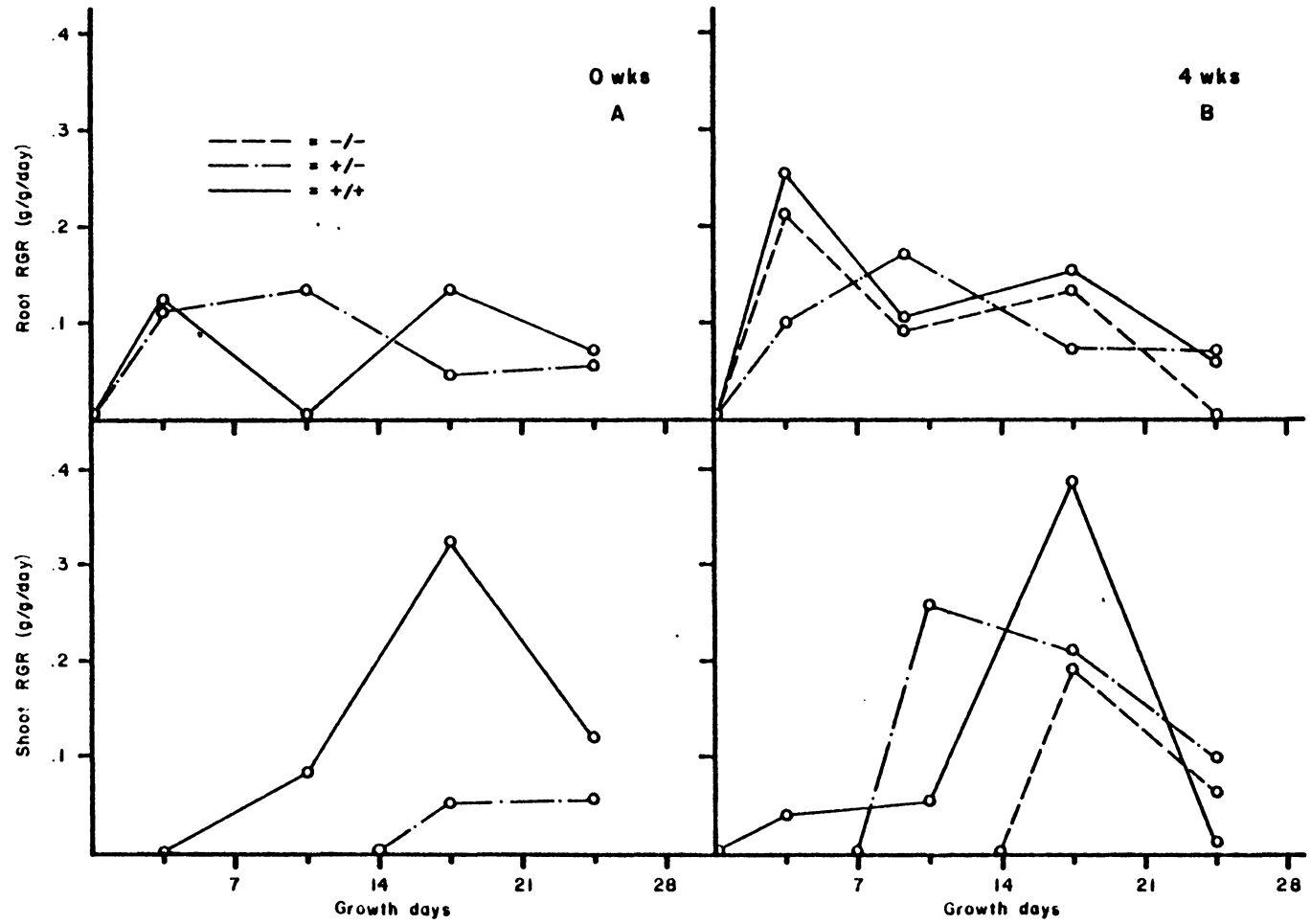

Figure 5: Effects of stratification (Hm) and pericarp removal on the relative growth rates (RGR) of northern red oak roots and shoots during the first 28 days growth. Acorns were stratified 0 (A) and 4 (B) weeks and pericarp treatments were, pericarp removed prior to stratification, -/-; pericarp intact during stratification then removed prior to germination, +/-; pericarp intact, +/+.

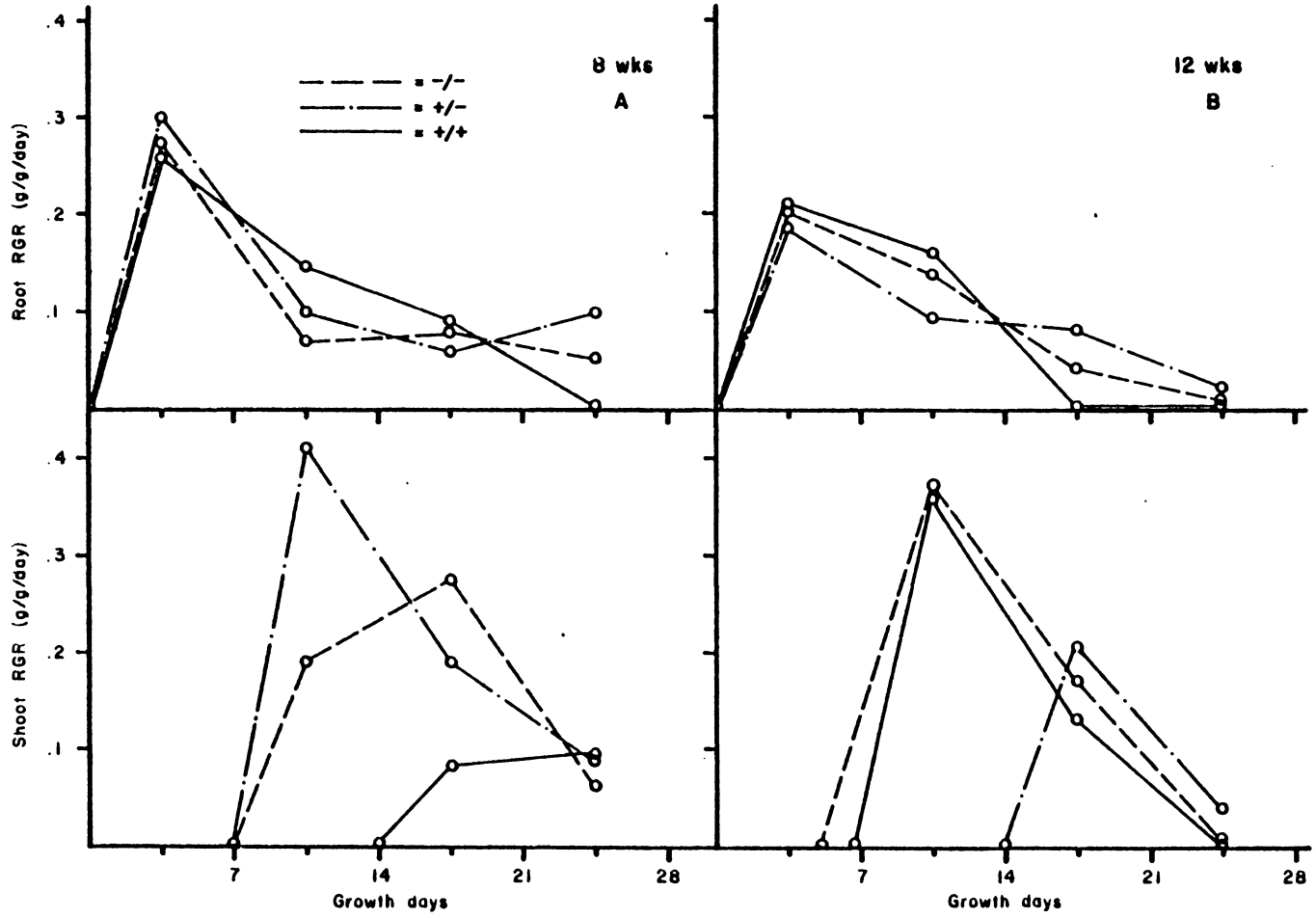

Figure 6: Effects of stratification (Lm) and pericarp removal on the relative growth rates (RGR) of northern red oak roots and shoots during the first 28 days growth. Acorns were stratified 8 (A) and 12 (B) weeks and pericarp treatments were, pericarp removed prior to stratification, -/-; pericarp inact during stratification then removed prior to germination, +/-; pericarp intact, +/+.

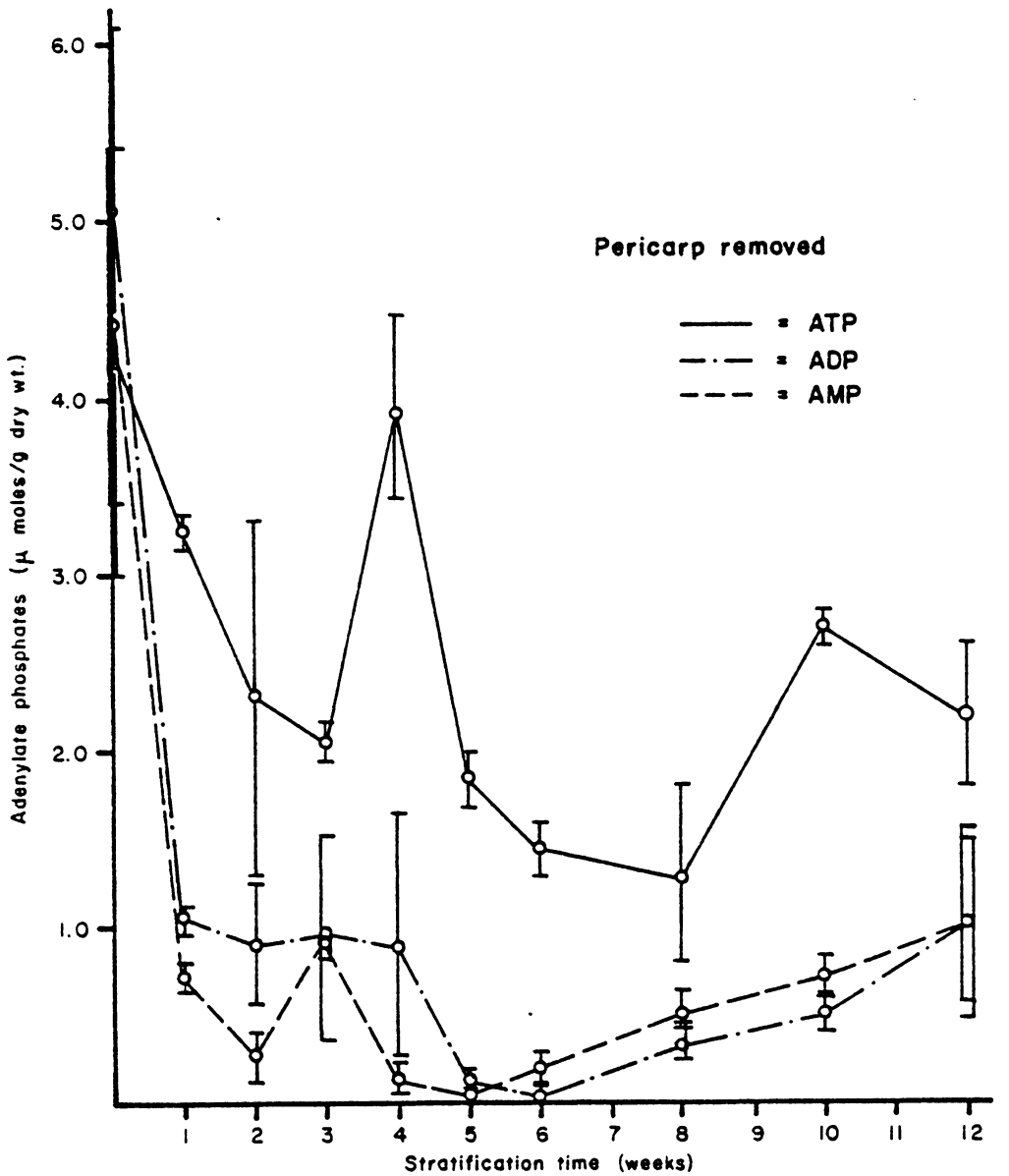

Figure 7: Adenosine phosphate levels during 12 week stratification (5 C) of northern red oak acorns with pericarp removed. Verticle bars represent $\pm s_{\bar{x}}$. Abbreviations: ATP (adenosine triphosphate), ADP (adenosine diphosphate), AMP (adenosine monophosphate).

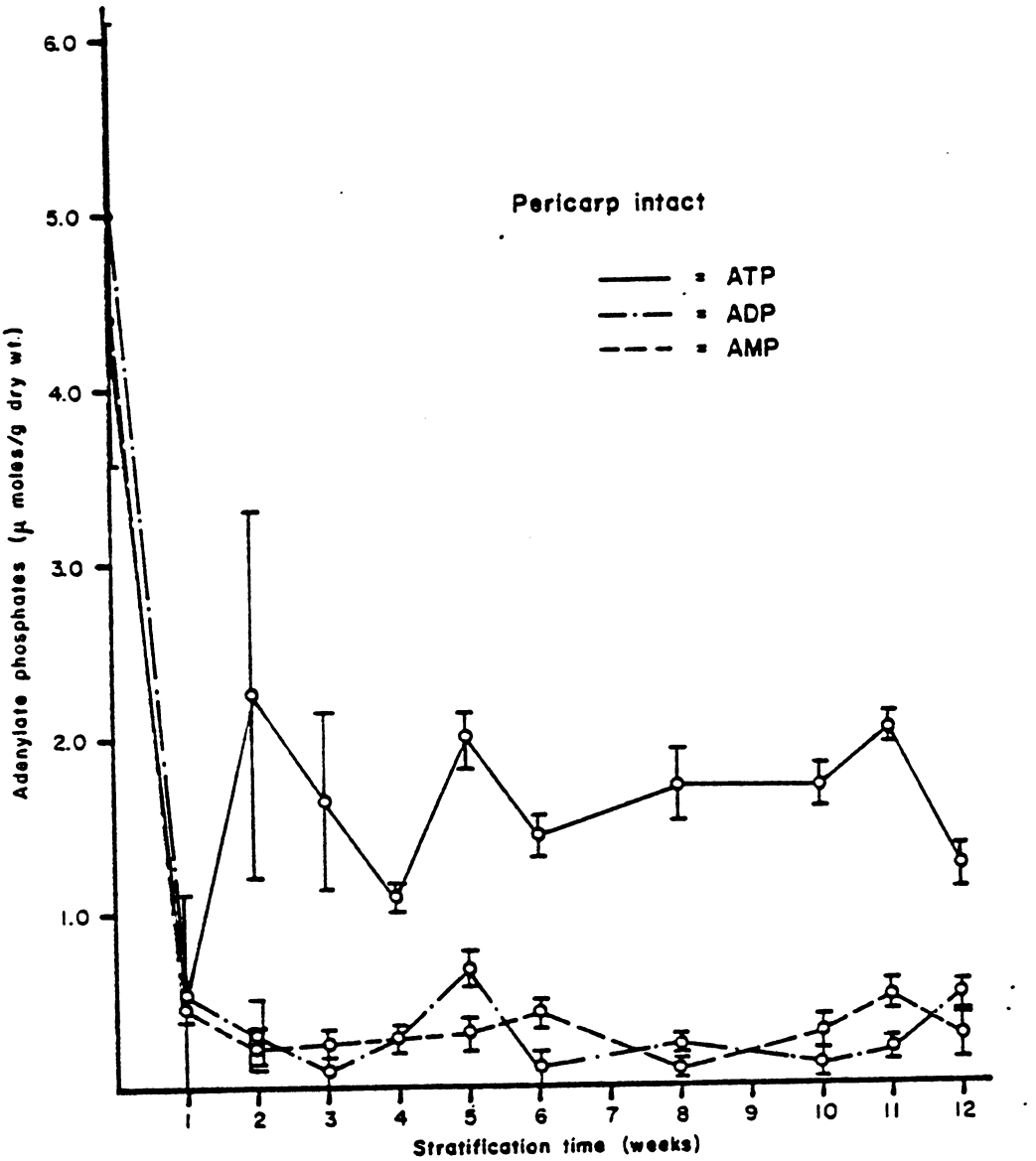

Figure 8: Adenosine phosphate levels during 12 week stratification (5 C) of northern red oak acorns with pericarps intact. Verticle bars represent $\pm$ $s_{\bar{x}}$. Abbreviations: ATP (adenosine triphosphate), ADP (adenosine diphosphate), AMP (adenosine monophosphate).

time measurement was made 12 to 24 hours after imbibition of freshly harvested acorns. After only 1 week at 5 C, these initially high levels of all the energy-rich adenylates declined sharply in intact acorns (50%, 78%, and 84% of week 0 values for ATP, ADP, and AMP, respectively). When acorns were stratified with the pericarp removed (Figure 7), ATP decreased more slowly for 3 weeks and then increased sharply (3.9 umoles/g dry wt) at week 4. ATP dropped after week 4 and reached the lowest level at week 8 and increased at week 10. ADP and AMP of acorns with pericarps removed dropped more or less steadily through week 5. The ADP levels increased again at week 8 then remained stable through week 12. AMP levels were lowest (0.2 umoles/g dry wt) at week 5, and then increased continuously through week 12.

After the initial high levels at week 0, the major peaks of ATP for acorns with pericarp intact (+/+) were at weeks 2 and 5, with a small peak at week 11 (Fig. 8). ADP and AMP occurred sychronously with ATP peaks at weeks 5, 8, and 12 for ADP while AMP peaked at weeks 6 and 11. Except for week 3, ATP increases followed surges in ADP and AMP. For example, AMP increased at week 6, ADP at week 8 and ATP at week 11.

The balance of the adenylate energy system within the acorn was distinctly affected by stratification. Regardless

of pericarp treatment EC showed two major increases during stratification (Figure 9). EC initially rose from .49 to .77 between 0 and 2 weeks of cold treatment for acorns with pericarp removed (-/-) and from .49 to .82 for intact seed. The second next peak in EC occurred at week 5 (.92) with pericarp removal and at week 8 (.88) with pericarp intact. Energy charge decreased slightly or remained stable at (.73) through the remainder of stratification with acorns of each pericarp treatment.

Changes in adenylates during germination

After 8 weeks of low temperature stratification, acorns with pericarps removed (-/-) and pericarp intact (+/+) were placed in warm temperatures (15/25 C, 8/16 hr); and the ATP, ADP and AMP concentrations of germinants were measured at 5 day intervals for 25 days (Figures 10 and 11). Levels of ATP for seedlings with the pericarp removed doubled (from 1.8 to 3.6 umoles/g dry wt) from germination date (as measured by radicle emergence) to day 5. ATP declined sharply to 0.9 umoles/g dry wt between day 20 and 25 (Figure 10). Concurrent with the initial rise in ATP was a decline in ADP from day 0 to day 10. ADP levels remained constant through the remainder of the growth period near 0.5 umoles/g dry wt. AMP levels increased from 0.2 to 0.9 umoles/g dry wt at day

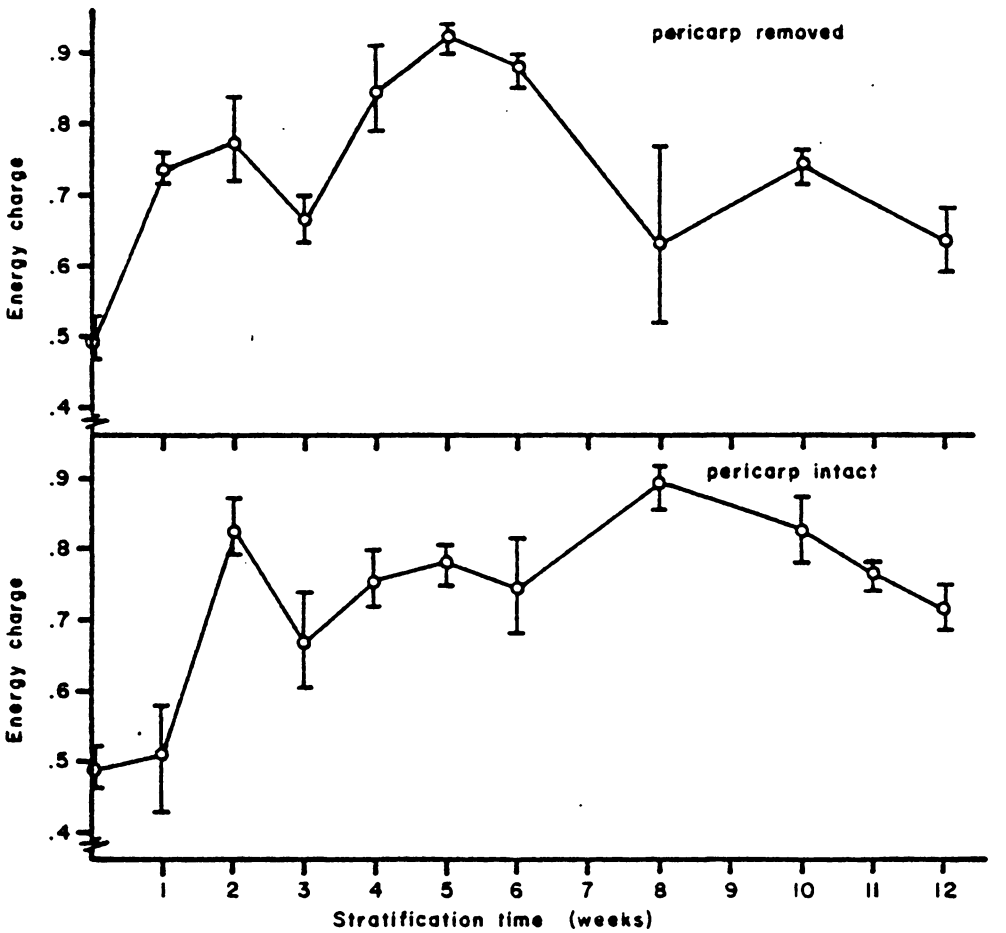

Figure 9: Effects of stratification (5 C) on energy charge, EC, of northern red oak acorns with pericarp removed and pericarp intact. Verticle bars represent $\pm s_{\bar{x}}$. EC was calculated as [ATP] + 0.5[ADP]/ [ATP] + [ADP] + [AMP].

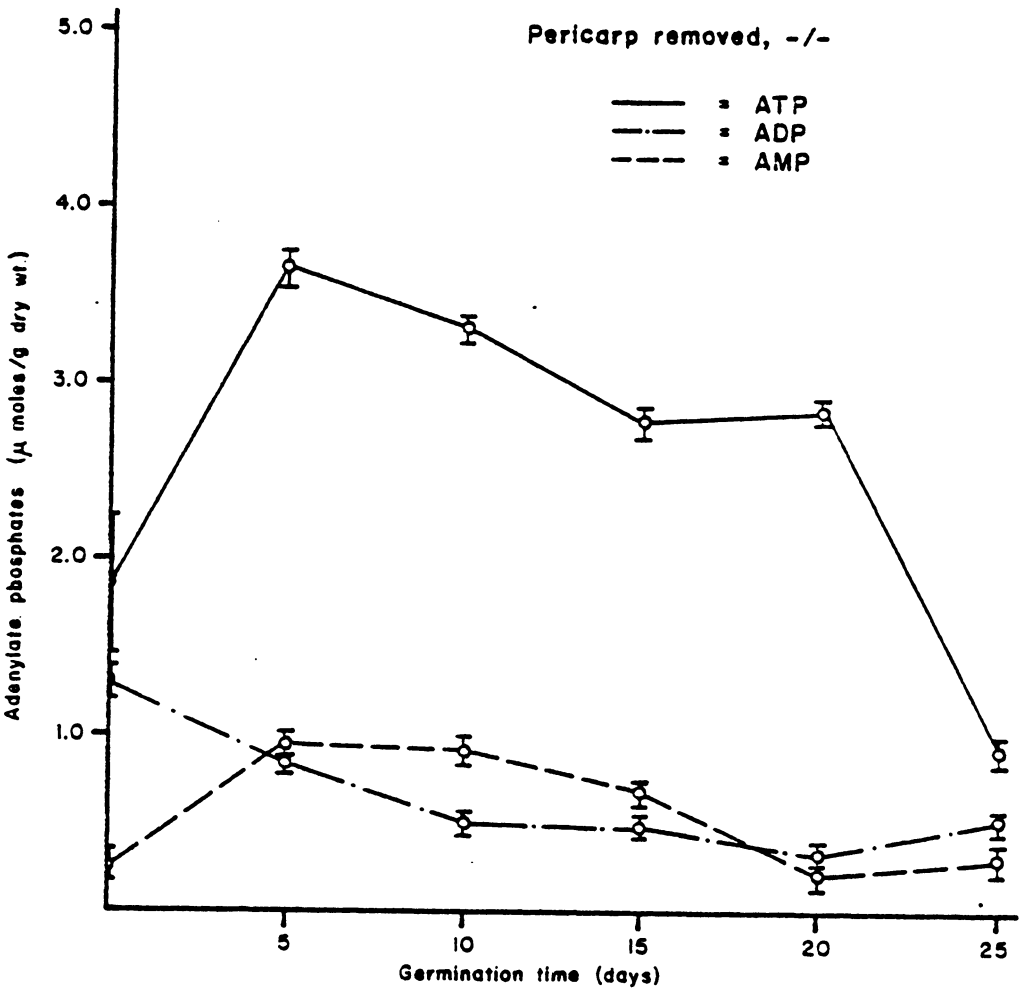

Figure 10: Adenosine phosphate levels through first 25 days of germination of northern red oak. Acorns were stratified 8 weeks and germinated with pericarp removed. Vertical bars represent + $s_{\bar{x}}$. Abbreviations: ATP (adenosine triphosphate), ADP (adenosine diphosphate), and AMP (adenosine monophosphate).

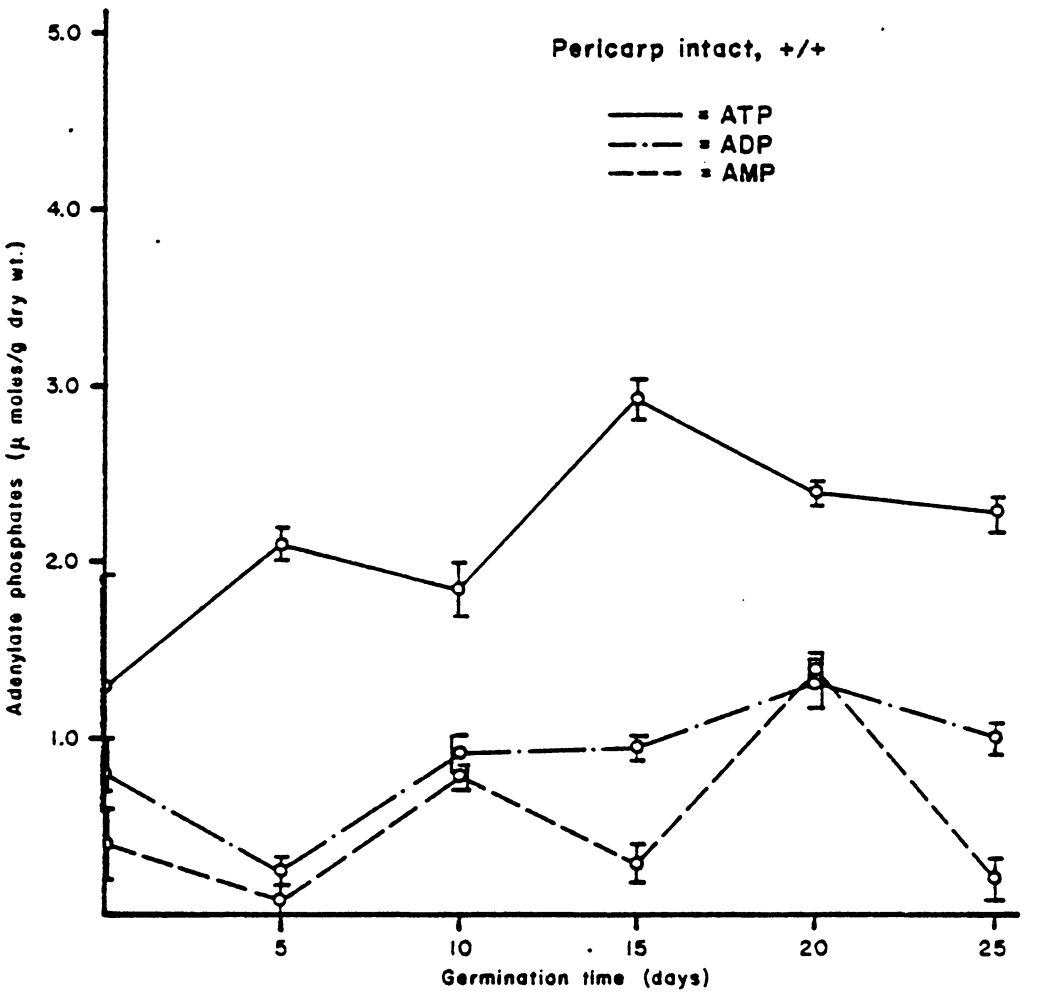

Figure 11: Adenosine phosphate levels through first 25 days of germination of northern red oak. Acorns were stratified for 8 weeks and germinated with pericarp intact. Verticle bars represent $\pm s_{\bar{x}}$. Abbreviations: ATP (adenosine triphosphate), ADP (adenosine diphosphate), and AMP (adenosine monophosphate).

5, remained stable for 10 days, and then declined at day 20 to about 0.2 umoles/g dry wt .

For acorns germinated with pericarp intact (+/+), the levels of ATP generally increased through the first 15 days of growth, then declined through day 25 from 2.9 to 2.3 umoles/g dry wt (Figure 11). Meanwhile, levels of ADP and AMP decreased at day 5, and then increased at day 10. After day 10, ADP levels remained constant near 1.0 umoles/g dry wt, while AMP concentration decreaased to 0.3 umoles/g dry wt. AMP concentration increased significantly at day 20 to 1.3 umoles/g dry wt while the ADP levels remained nearly constant. AMP decreased between days 20 to 25 from 1.3 umoles to 0.2 umoles/g dry wt.

Comparision of EC and germinability during stratification

Energy charge and germination following stratification of acorns with pericarp removed (-/-) and pericarp intact (+/+) are compared graphically in Figures 12 and 13. With the pericarp removed, EC increased at week 2 of stratifcation, then declined slightly, and increased again at week 5. This increase and decrease of the adenylate energy system was accompanied by decreased germinability from week 0 to week 4 and then a sharp rise in germination at week 6. After week 6 germination remained high (90%) through the remainder

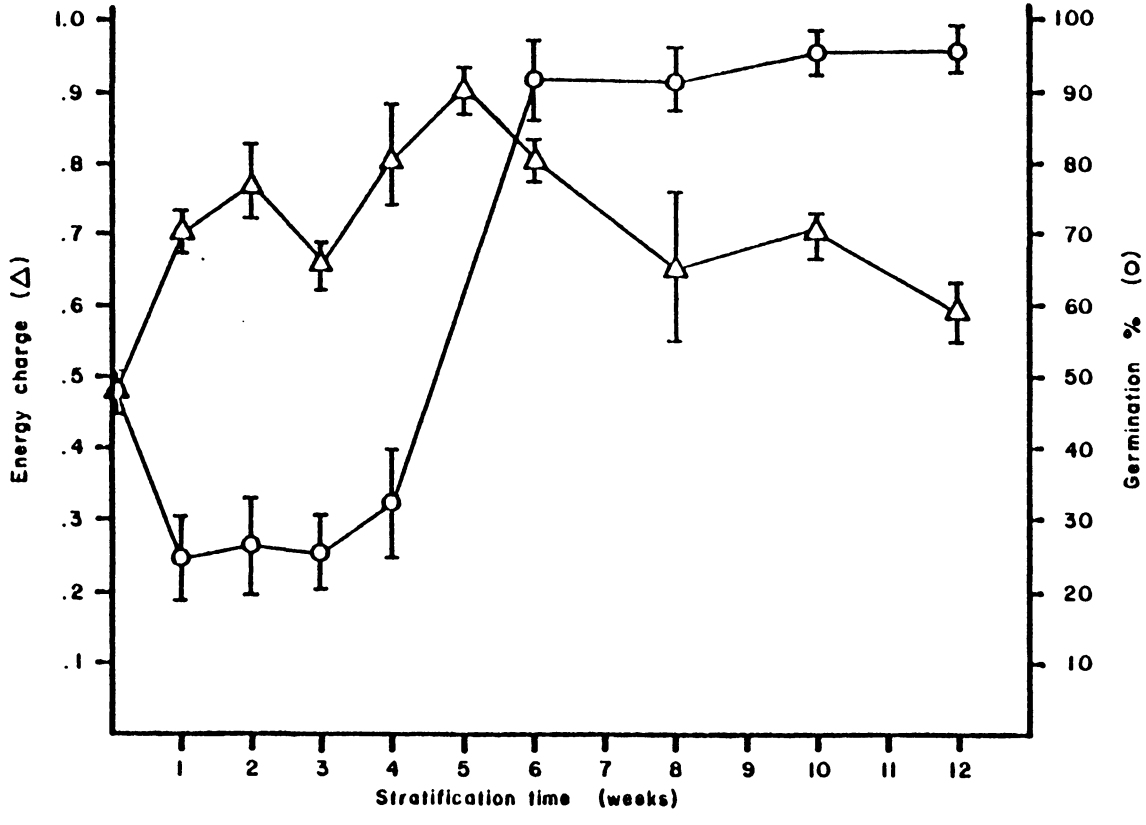

Figure 12: Relationship between energy charge, EC, and 28 day germination percentage for northern red oak acorns during 12 weeks of stratification (5 C) with pericarps removed. EC was determined as [ATP]+ 0.5[ADP]/ [ATP]+ [ADP] + [AMP].

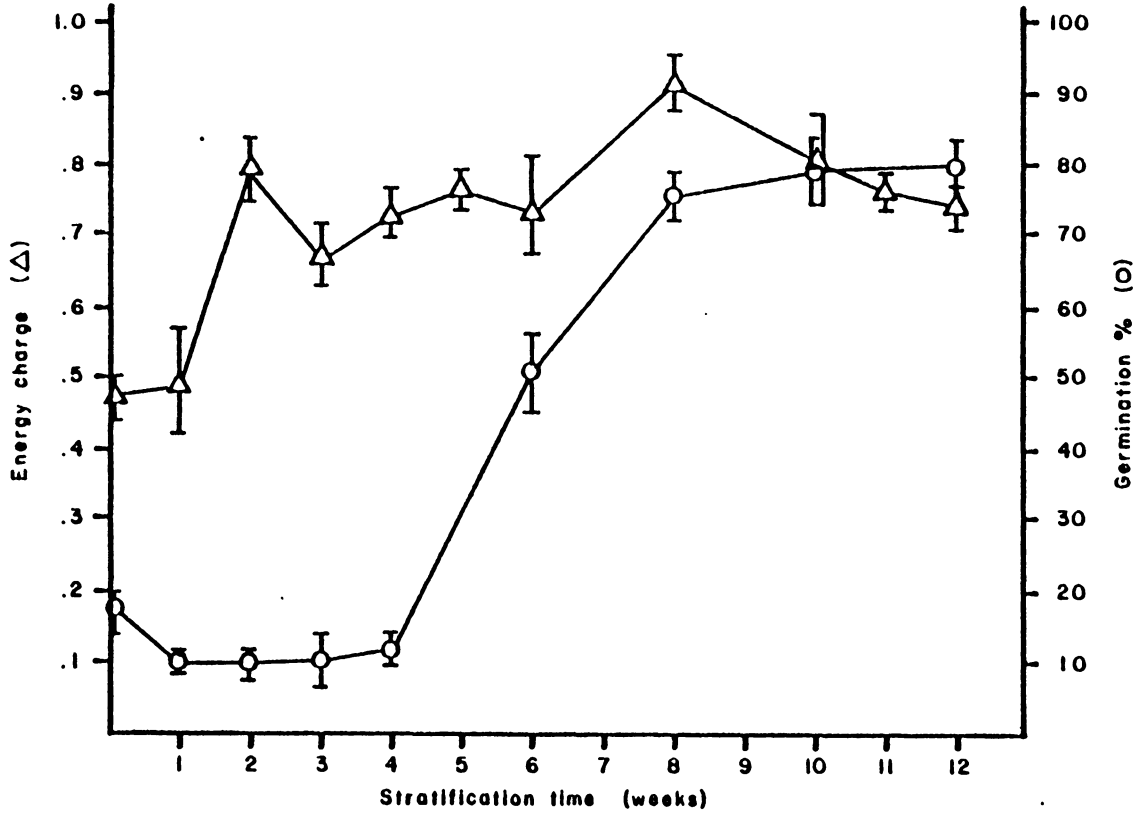

Figure 13: Relationship between energy charge, EC, and 28 day germination percentage for northern red oak acorns during 12 weeks of stratification (5 C) with pericarps intact. EC was determined as [ATP] + 0.5[ADP]/ [ATP] + [ADP] + [AMP].

of the 12 week stratification, while the EC stabilized around 0.70. Similar patterns were observed for EC and germination of seed stratified and germinated with pericarp intact (+/+) (Figure 13). In this case, the second rise in EC did not occur until between week 6 and 8; an increase in germinability was observed during the same interval.

Relationship between ATP and seedling relative growth rate

Rate of root and shoot dry matter mobilization (RGR) from the reserves of the cotyledons might bear relation to the changes in ATP concentration during eticlated growth. To make such comparisons, seeds stratified for 8 weeks with pericarp removed (-/-) or pericarp intact (+/+) were germinated for 28 days. As shown in Figure 14, the RGR of roots and shoots of seedlings with pericarp removed appeared to parallel ATP concentrations. There is an initial rise in ATP from day 0 to 5. The RGR of roots peaks during week 1 then declines rapidly. The RGR of shoots reached a maximum in the third week; thereafter RGR and ATP levels began to decline. This synchrony between ATP concentrations and growth of roots and shoots was also pronounced in acorns with pericarp intact (Figure 15). A rapid increase in root RGR during week 1 (.2 g/g/day) was followed by a slower continuous decline. The increase and decrease in RGRs of roots coin-

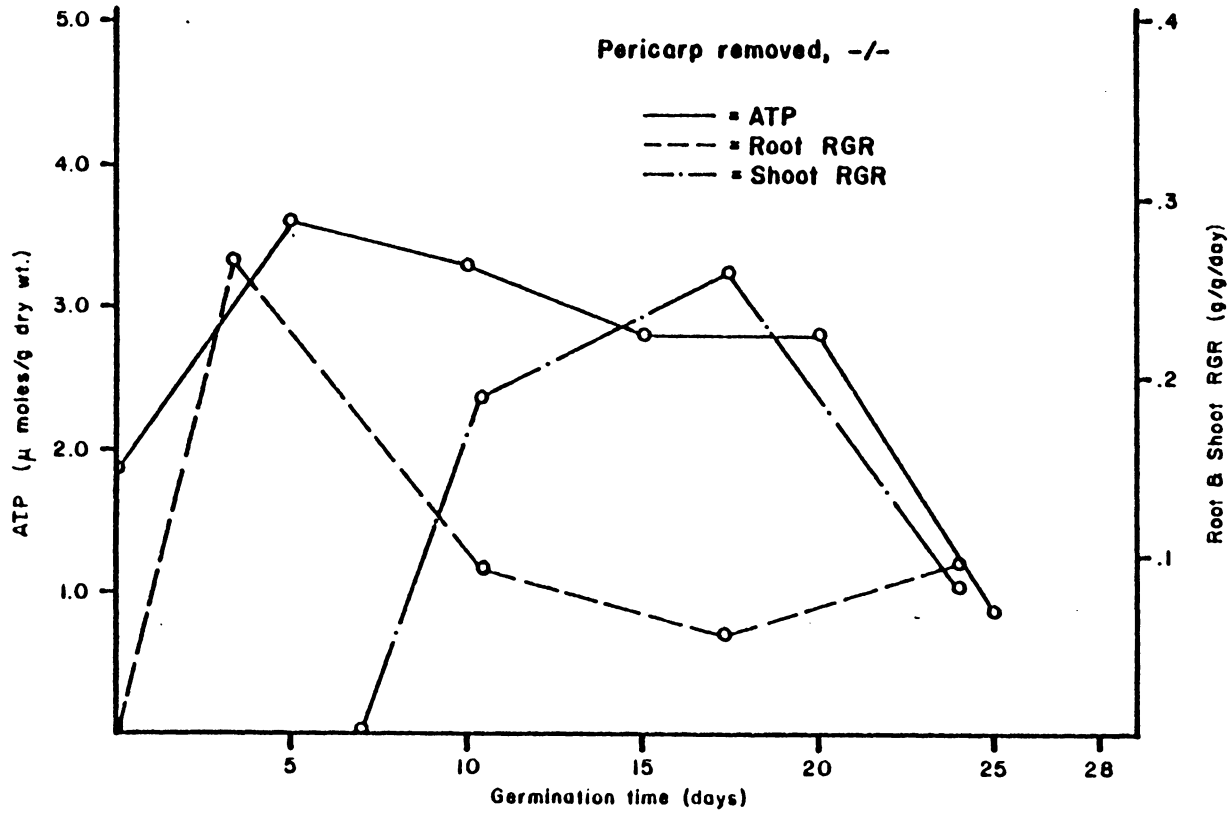

Figure 14: Relationship between the relative growth rates (RGR) of root and shoot dry weight and levels of adenosine triphosphate (ATP) during 28 days germination and growth of northern red oak seedlings from 8 week stratified seeds with pericarp removed.

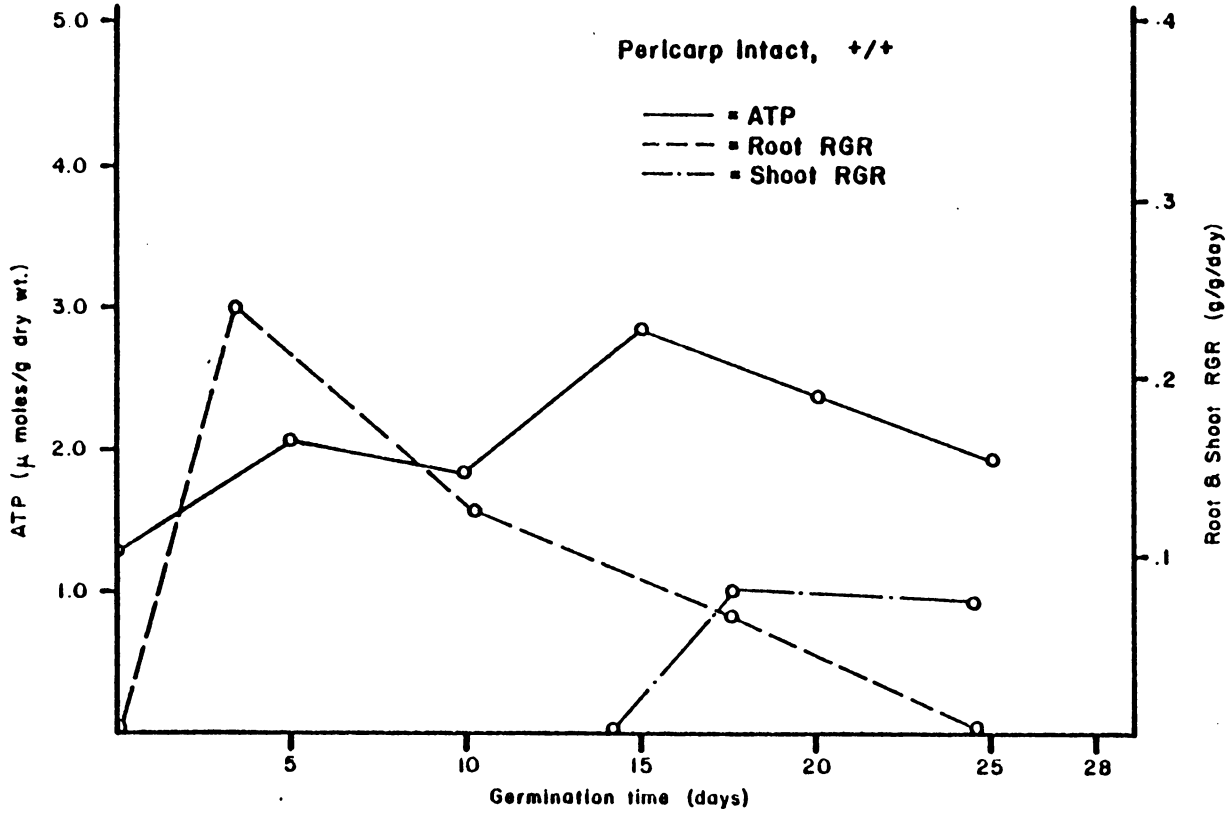

Figure 15: Relationship between the relative growth rates (RGR) of root and shoot dry weights and the levels of adenosine triphosphate (ATP) during 28 days germination and growth of northern red oak seedlings from 8 week stratified seeds with pericarps intact.

cided more or less with a peak ATP levels at day 5 (2.9 umoles/g dry wt). A second increase in ATP levels preceded slightly the burst of shoot growth. ATP levels and RGRs both were reduced toward the end of the 4 week germination period.

DISCUSSION

Dormancy and Germination

Role of pericarp

Certainly, the thick hard outer covering of the acorn, the pericarp, must have a vital role in oak survival and germination under natural conditions. Prevention of rapid water loss from the embryo and maintenance of the integrity of the young sporophyte are two probable purposes of the pericarp. However, the biochemical or physiological role of the pericarp in regulating dormancy in red oak is unclear.

Korstian (1927) found that the removal of the pericarp in northern red oak seeds prior to greenhouse planting did not break dormancy. He concluded that, since pericarp removal only hastened "afterripening" slightly, dormancy in red oak was inherent in the embryo itself. This embryo dormancy hypothesis was supported by Farmer (1974) based on experiments with cracked acorns. Farmer found that cracking northern red oak acorns did not greatly stimulate germination of non-chilled or prechilled acorns. He concluded that dormancy in northern red oak seeds was due to conditions within the embryo. Considering this information, it is

interesting that the rules for germination tests of Quercus spp. require that the pericarp and testa (single- layered seedcoat) be removed; but no stratification is required (ISTA, 1976). This protocol implies that, when the pericarp is removed, so is the factor causing germination delays. If the embryo is entirely responsible for dormancy in red oaks, perhaps the testing criteria should be modified to include a prechilling period with no need for pericarp removal.

Experiments in this study indicated pericarp removal significantly increases total germination (Figure 1) and germination rate (Table 2) when compared to intact acorns. Acorns with the pericarp removed germinated faster and more frequently (higher percentage at 28 day) than acorns with the pericarp intact. Removal of the pericarp allowed germination of about 55% in freshly-collected northern red oak acorns versus 10% in intact seeds. Johnson (1979) reported 60% germination for Q. nuttalli (Nuttall oak) at collection date with pericarp removed. These results suggests that the pericarp has a definite role in retarding germination.

Bonner (1970) conducted tests on the role of pericarp in gas exchange and water absorption by the embryo and concluded that the pericarp plays a strong part in the dormancy of several bottomland red oak species. He hypothesized that the most likely influence of the pericarp was in reducing

oxygen availability. Korstian (1927) assumed the pericarp of northern red oak to be permeable to gases and water, since its removal had little effect on germinability. In this study, however, acorns in this study with pericarps removed prior to stratification (-/-) did not germinate as rapidly or fully as those acorns with pericarps intact during stratification and removed prior to germination (+/-). This suggests that pericarps are perhaps important in some aspect of the afterripening process. (During afterripening, several biochemical processes take place; one of the most notable is the conversion of lipids to soluble carbohydrates (Korstian, 1927; Brown 1939; Vozzo, 1974).) On the other hand, this research suggests that pericarp removal prior to germination (+/-) results in faster and more complete germination; this enhancement of germination may be due to increased oxygen availability or to removal of some inhibitory substance in the pericarp.

Vozzo (1981) recently described microbial activity within the inner walls of the pericarp of water oak. Fungi within the endocarp and mesocarp layers of the pericarp may be associated with the rupturing of the pericarp leading to germination. He further suggested that there could be a symbiotic relationship between the acorn and fungus: the seed providing food for the fungus and the fungus improving

gas exchange for the embryo by rupturing the pericarp. If this fungal-acorn interaction does exist, the role of the pericarp in dormancy of water oak may be an ecological development rather than a purely biochemical or physiological factor.

Jones and Brown (1968) working with Q. falcata var. pagodafolia (cherrybark oak) and northern red oak, tested the pericarp's effect on delayed germination. Pericarp sections from various regions of the acorn were removed and differences in germination capacities due to each treatment were determined. Their results strongly indicate that the pericarp was the cause of red oak dormancy in these two red oak species, and that cell expansion during low temperature stratification allowed for the pericarp to crack and germinate. One does not have to invoke a single common cause for dormancy in the whole subgenus, however. Indeed, the variety of ecological niches occupied by members of this subgenus suggests that some very different mechanisms of dormancy must be present.

Role of stratification in breaking dormancy

Cold temperature preconditioning is a requirement of more than 600 woody plant seeds for adequate germination (USDA, 1974). Germinability of northern red oak acorns

greatly increases between the second and third months of stratification (Farmer, 1974). Although stratification definitely increases the germination of northern red oak, there is controversy as to the need for prechilling acorns prior to testing. International Rules for Testing Seeds (ISTA, 1976) recommend that acorns not be stratified for germination, but merely that the pericarp removed. Based on the present work and others, these recommendations should be modified since red oak acorns germinate much more rapidly and completely with 8 to 10 weeks of low temperature stratification.

Red oak seed have been kept viable in storage for up to 5 years at temperatures just above freezing and with moisture contents no less than 45% (Bonner, 1977). Brown (1939) reported optimal storage temperatures for northern red oak of 12.5 to 10 C based on faster germination after 3 months. The recommended storage temperature for most cold requiring seed (USDA, 1974; ISTA, 1976) including red oak (USDA, 1977; Bonner, 1975) is 5 C.

Several reports have stressed the importance of maintaining red oaks seeds at moisture contents greater than 45% to maintain viability (Roe, 1946; Holmes and Buszwicz, 1955; Krajicek, 1968; Bonner, 1970, 1973). In the present study there were no differences in germination or seedling growth

following cold stratification at 70% or 50% moisture. These results indicate that there was enough water in the 50% seed moisture treatment to carry out any necessary dormancy-breaking biochemical reactions. In a similar study, Farmer (1974) found that there were no differences in "stratification" at 82% and "storage" at collection moisture levels of 62%. He based these conclusions on germination and seedling growth.

Possible role of stratification on deepening of dormancy

Dormancy onset usually occurs during the last month of maturation for most tree seed (USDA, 1974a). During this process, growth inhibitor concentrations increase rapidly in some species including members of the red oak group while growth promotors decrease in activity (Amen, 1968; Szczotka, 1970; Hopper, 1979). Another important process that occurs during the last weeks prior to abscission is the conversion of food reserves to the less available but more efficiently stored form of lipids. When abscission occurs, the seed is usually fully dormant (presumably) and requires an afterripening period. During afterripening, growth hormone balance shifts in favor of promoting substances (Amen, 1968; Hopper, 1979) and lipids are converted to soluble carbohydrates (Brown, 1937; Vozzo, 1974).

In this study, freshly collected seed germinated more completely and faster than seed that had been stratified for 1 to 4 weeks (Figure 2 and Table 2). Such a deepening of dormancy has not been previously reported for northern red oak, nor any other red oak species. This negative germination response was seen at both seed moisture levels in all pericarp treatments, and in both years (two different collection sites).

There may be several explanations for this deepening of dormancy. Two seem more likely. The first is that the cold temperatures may initiate biochemical reactions leading to deeper dormancy. Such reactions, once completed, might then give significant control over germination. In nature, the ecological significance of such mechanisms is clear. After the acorn falls, low night temperatures of late fall would initiate this deepening of dormancy, thus preventing germination. In this way, the oak seeds may adapt to temperature fluctuations associated with late autumn and remain dormant. A second possible cause for reduced germination after 1 to 4 weeks of stratification is that some acorns were immature and, therefore, not dormant when collected. Plumb (1980) found that two California red oaks (*Q. kelloggii* and *Q. wislizenii*) will germinate as early as August when collected green. Although acorns were collected from the ground

in the present study, unripe acorns may have been present. According to this hypothesis, the passage of time (at any temperature) could permit continued development of the fruit and fuller expression of dormancy. Further research might verify this hypothesis. Harvesting and germinating northern red oak seed periodically from September through October should establish the actual time of dormancy onset.

Response of adenylates to stratification

Energy metabolism of northern red oak acorns fluctuated during the 12 weeks of wet stratification (Figure 8). The low energy charge (EC) of .49 at collection perhaps indicated a relatively low level of available energy in the recently rehydrated system. Low energy charge is common for quiescent (dry) seeds as reported for ponderosa pine (Ching and Ching, 1972) and pea (Brown, 1965). According to Chapman et al (1970), EC values below .5 indicate senescence and little chance for recovery. These critical levels were determined for prokaryotic (bacterial) cells, however, and eukaryotic cells of higher plants may have a different energy metabolism. Furthermore, vacuoles in plant tissue and compartmentation of some nucleotides may create a dilution effect on the EC values (Ching and Ching, 1972).

The low EC values in freshly harvested acorns contrast with the relative high levels of ATP, ADP and AMP simultaneously observed (Figure 7). These high nucleotide concentrations may result from increased activity of hydrolytic enzymes during imbibition. Imbibition initiates many biochemical activities such as respiraton (Brown, 1939; Ching,1973), adenylate kinase activity (Bomsel and Pradet, 1968; Ching and Ching, 1972); phytase activity (Hall, 1966), catylase activity (Ching, 1973), and hydrolysis of growth regulators to free forms (Khan, 1977). Levels of adenylates observed after imbibition may suggest that a rapid mobilization of energy reserves is made available for the activation of such hydrolytic enzymes. Adenylate nucleotides can increase dramatically within 2 to 4 hours of imbibition in other seed, probably through glycolysis (substrate phosphorylation), fatty acid oxidation, and respiration (oxidative phosphorylation) (Ching, 1973).

A synchronization in ATP, ADP and AMP levels was evident during the course of stratification. The levels of ATP seemed to increase concurrently with either no change or a decline in ADP or AMP. The activity of adenylate kinases may have been high in northern red oak, similar to observations in lettuce (Bomsel and Pradet, 1968). Adenylate kinase enzymes regulate the phosporylation of AMP and ATP to

ADP. ADP may then be oxidatively phosphorylated to ATP. Such temporal changes in adenylates suggest that energy-requiring and energy-yielding reactions occur during low temperature in preparation for germination. With the exception of timing, the removal of the pericarp contributed little in changing the energetics of the adenylate system during stratification of northern red oak acorn.

Two major peaks of adenylate energy activity occurred as indicated by EC during stratification (Figure 9). The first major activity was evident during week 2. EC rose from 0.5 to 0.8. This rise in energy status coincides with an increased respiratory activity and oxygen consumption observed by Brown (1939) during the early period of low temperature storage. Brown's explanation for this increased rate in respiration was an increased conversion of lipid to carbohydrates, a process that requires excess oxygen. Korstian (1927) reported a significant decrease in acorn lipid content during storage of northern red oak. Further, the respiratory quotient (R.Q.) of 0.6-0.7, observed by Brown suggested that lipids act as substrate for respiration early in stratification. Results from Brown's study correlate well with the EC data earlier discussed (Figure 9). Oxidation of lipids would yield energy and this may well explain the increased EC and ATP levels at week 2 of stratification.

Although energy appeared available at 2 weeks of stratification, the acorns did not germinate. In fact during this period, the acorns were in their deepest dormancy. Presumably, ripening (afterripening) is essential for germination and growth.

Although the EC values were high after 2 weeks of stratification, concurrent germination was minimal (Figure 12 and 13). Subsequently (after 5 weeks for pericarp removed seed (-/-) and 8 weeks for pericarp intact seed (+/+)) the EC again peaked and the highest values for germination (90%) were observed. The more rapid return to high EC value for acorns with pericarp removed may be due to more rapid oxidative phosphorylation. The energy charge remained stable throughout the remainder of stratification as did the germination. A 7-fold increase in EC during two week stratifcation of ponderosa pine has been reported (Ching and Ching, 1972), but no increases were observed during stratification of maple seed (Simmonds and Dumbroff, 1974).

Seedling Growth

Role of pericarp

There were no significant differences in 28-day-old seedling root or shoot lengths associated with pericarp removal following 8m stratification (Table 3). However, the

removal of the pericarp resulted in significantly lower seedling dry weights. This reduction of dry weight may result more directly from the removal of a portion of the cotyledons (necessary for the rapid removal of pericarps). Similar results were recently found in a study of northern red oak seedlings in Michigan.[6] That work attempted to simulate losses of cotyledonary material due to rodents and weevils, and to determine the effect such losses would have on germination and seedling growth. Removal of less than 25% of the cotyledon resulted in decreased growth of etiolated and light-grown plants. This cotyledon effect may be due to a wounding response, a loss of food stuff, or reduction of some growth regulator. However, food reserves from cotyledons have been shown to partially sustain seedlings for over 100 days (Hopper, unpublished data). Thus, some food materials were partially present at the end of 28 days even with the pericarp and some cotyledonary tissue removed.

The effect of pericarp removal on seedling RGR was most pronounced early in stratification. Relative growth rates were more episodic after 0 or 4 weeks than after 8 or 12 weeks of stratification (Figure 4). These responses probably indicate a confounding of the growth process with dormancy factors. Seedlings developed from non- or

---

[6]Personal communication with , University of Michigan-Ann Arbor, 1/25/82.

partially-stratified acorns with pericarp removed grew more efficiently than seedlings with pericarp intact, perhaps indicating oxygen availability is a limitation in intact acorns. Seedlings with pericarp removed after stratification (+/−) exhibited a delay in axis dry weight accumulation with less than 4 weeks of stratification (Figure 4). This may be a wounding response from fruit coat removal. Since relative growth rate of seedlings developed from seeds with intact pericarps (+/+) peaked a week earlier, there is no apparent advantage to the removal of the pericarp. On the other hand, those seed with pericarp removed prior to stratification (−/−) exhibited higher RGRs; ; they may have stabilized from any reaction to wounding by the time the seedling growth test was conducted. These results were corroborated by Allen and Farmer (1976) who reported that pericarp removal after stratification had no effect on shoot growth of the red oak species, Q. *ilicifolia* (bear oak).

Influence of stratification on growth

Acorn stratification has a significant and positive effect on seedling growth. Some parameters showed a positive response to stratification after only 14 days of growth (Table 4 and 5). The differential response to stratification was observed for 14-and 28-day-old plants. Others have

shown that stratification affects seedling growth (Farmer, 1976; Bonner, 1977; Perry, 1981), but the length of time that these effects remain during further seedling/sapling development is unknown. Sluder (1979) reported for coniferous plants that taller seedlings grew faster, remained taller and had a better chance of survival than smaller seedlings. Perry (1981) suggested that stratification effects on growth rates would still be evident as late as 15 years in a loblolly pine plantation. Such an effect could significantly alter nursery practices and genetic seletions of conifers and hardwoods. Moreover, stratification length that gives the fastest germination and best growth of seedlings may produce highest yields in plantations. Data from the present study indicate that 8 to 10 weeks of low temperature stratifcation results in the highest RGR for northern red oak seedlings (2.7 axial g/g/day) (Figure 4).

Moisture contents during Hm and Lm stratification had a minimal effect on seedling growth, except that Hm stratification tended to produced larger seedlings in shorter stratification times. During Hm stratification, the seed were spread in single layers under wet paper towels perhaps allowing for better aeration of acorns. (In Lm stratification the acorns were stored dry in plastic jugs.) Also, 70% moisture may have permited more active metatclism and thereby hasten the afterripening process.

In both Hm and Lm stratification, longer periods (12 weeks and 32 weeks respectively) produced somewhat smaller plants than 8 and 20 weeks of stratification (Table 4 and 5). Marshall (1981) found that 8 to 12 weeks of stratification of northern red oak seeds was better than 12 to 14 weeks in resultant seedling growth. Farmer (1974) found for northern red oak that maximum shoot growth occurred after 10 weeks of stratification, and that significantly less growth occurred after 16 weeks. Long periods in storage leads to deteriorative processes that result in reduced germination and growth in soybeans (Egli et al., 1979). There may be an optimal stratification length for northern red oak; germination and resultant seedling growth are perhaps reduced with shorter periods due to incomplete afterripening. If the effects of stratification on seedling growth could be expressed for 10 to 15 years, then very careful attention to preconditioning seed with optimal stratification periods is warranted.

Several biochemical and morphological changes occur during the first 2 months of cold stratification. Vozzo (1975) found an increase in mitochondrial number and size for stratified water oak embryos. The hormonal balance for water oak acorns changes at 5 to 6 weeks of cold stratification from inhibitor control to promotor control (Hopper,

1979). Dury (1977) concluded that gibberellic acid levels significantly increased after 5 weeks of stratification for northern red oak, thus preparing the acorn for germination and growth. Better mitochondrial organization has been associated with increased seed and seedling vigor in corn (McDaniel and Sankissia, 1968) and barley (McDaniel, 1969). Brown (1939) and Vozzo (1978) reported that in red oak seeds lipids were converted to soluble carbohydrates during the first weeks of stratification. Ching and Ching (1972) referred to such processes in dormant seed as producing a "readiness to germinate". Results from the present study support these theories. As stratification in northern red oak seed proceeded, germination and seedling growth was poor during the first 3 weeks, presumably when biochemical and morphological changes occur (Figure 2 and Table 4). After the first month of stratification, the seed germinated faster (Table 2) and seedlings grew better (Figures 4, 5 and 6).

Associated with stratification were alterations in shoot growth patterns. Normally, root growth surges first, prior to shoot growth (Figure 5 and 6). Root growth parameters provided excellent indicators for the effect of stratification, since the radicle is the first organ to emerge and began to differentiate. Inferior growth due to factors associated with dormancy may be manifested in root growth as

observed in this study. Allen and Farmer (1979) reported that cummulative shoot growth was not affected by stratification, but that leaf number and leaf area increased with stratification. These results suggests that stratification may remove a block to additional development of leaves in the embryo.

After 8 weeks of stratification, epicotyl elongation began by 14 days as opposed to a 21-day delay for seed stratified only 2 to 4 weeks (Figures 5 and 6). The readiness to germinate and grow in acorns is important, especially in its establishment of a balanced shoot and root system. The root/shoot ratio approaches one only after 8 weeks stratifcation; whereas, seed stratified for shorter periods developed into seedlings having most of the growth in long roots with root/shoot ratios as high as 5 (Table 4). Seedlings with root/shoot ratios near one have an excellent chance for survival. Root/shoot ratios were used as an indicator of field growth and survival for bareroot seedlings (Mustanoja and Leaf, 1965).

Response of ATP and EC to growth

ATP provides the metabolic energy for enzymes used in hydrolysis of reserve food. Eight weeks of stratification synchronized acorns within a lot so that they would rapidly

and uniformly germinate. Adenosine phosphate levels and EC exhibited lag, exponential, and stationary phases of growth (Figures 14 and 15). Chapman et al (1968) determined critical values of EC for bacteria to be greater than .8 during active growth , between .5 and .7 during stationary growth maintaining viability, and below .5 upon starvation and death. The values for germinating and growing northern red oak were more closely related to those observerd for sycamore cell culture (Brown and Short, 1969) and ponderosa pine (Ching and Ching, 1972). For sycamore cell cultures, the EC was .66 during active growth and .81 at a stationary phase. Similarly for ponderosa pine, EC was .85 during stratification (stationary phase) and .65 to .75 during early germination (active growth). The energy status of northern red oak seedlings fluctuated similarly depending on the relative growth rate. Thus energy status between bacteria cells and higher plants may be very different.

ATP levels have been related to seedling injury and growth. Stewart and Guinn (1969) reported that chilling injury of cotton seedlings was reflected by a depletion of ATP soon after the injury. According to Ching (1972), the temporal rise and fall of ATP reflects a coordination of metabolic activity. She further described tests for seed vigor using ATP as a primary biochemical index (Ching,

1973;1975). Free nucleotide patterns in growing tissue provide excellent indicators of metabolism as suggested by Brown as early as 1962. He related germination of pea to increases and decreases of adenylate nucleotides. The rationale for using ATP levels for indexing seed vigor finds support in studies by several workers who have found significant differences in the energy status for various genotypes of crimson clover (Ching,1975), oats (McDaniel,1976), barley (McDaniel, 1969), peanut (Crompton et al, 1980) and corn (McDaniel and Sarkissa, 1968). McDaniel (1976) recently concluded that adenylate energy systems was significantly and positively correlated (r=.77) withyield potential of wheat. Using ATP and EC to index the state of dormancy in northern red oak in a manner likewise to those suggested for other systems may be useful. The results presented here indicate that germination, seedling growth, and EC may be fundamentally related to the degree of seed dormancy. As dormancy is overcome by stratification, changes in germination, growth, and seed energetics follow.

Ripeness to Germinate

Several changes occur in northern red oak acorns during the first 4 to 6 weeks of stratification. This period of biochemical and physiological changes may be defined as the

acorn's "ripeness to germinate". These changes ultimately promote the germinability and subsequent growth of the oak seed, but the initial effect is to reduce germination. As the acorn stratifies, stored lipids are converted to soluble carbohydrates, and these sugars are transported into the growing embryonic axis. This conversion occurs during the first 2 to 3 weeks of storage and coincides with an increase in energy charge. Bioenergically, the acorn has the capability to germinate and grow at collection, but until the "ripeness to germinate" changes occur, germination and growth is impaired.

CONCLUSIONS AND RECOMENDATIONS

The degree or depth of dormancy and early seedling growth in northern red oak acorns was influenced by the pericarp and exposure of the acorns to cold temperature. In freshly-collected seeds, pericarp removal increased germination about five-fold (55% versus 10% in intact seeds), while stratification for 6 to 8 weeks significantly increased germinability of intact acorns and naked seeds to 90%. International Seed Testing Asscciation (ISTA) rules for testing Quercus require pericarp removal but no chilling period. Since stratification had a significant effect on germination above and beyond pericarp removal, ISTA rules for testing Quercus should be revised to include a requirement for red oaks to be stratified with pericarp intact. Futhermore, the rules should distinguish between the two subgenera of Quercus since red oaks (subgenus Erythrobalanus) and white oaks (subgenus Leucobalanus) have very different germination systems. As a minimum rules should require that northern red oaks seeds be stratified for at least 8 weeks prior tc testing.In such a procedure the pericarp does not have tc be removed to acheive maximum germination. This would simplify

testing by avoiding the time-consuming pericarp removal procedures.

A deepening of dormancy was observed during the first 4 weeks of stratification. Freshly-collected seeds germinated significantly faster and more completely than did seeds that had been stratified from 1 to 4 weeks. This response was seen at two seed moisture levels (50% and 70% d.wt.) and in two years (at two collection sites). This observation has not been reported previously for northern red oak, nor any red oak species. Additional study on the timing of dormancy onset is needed. It is not known, for example, if dormancy would have been lower from earlier collection nor if cold temperatures are needed to cause the increased dormancy following harvest.

Although pericarp removal had little effect on seedling growth, stratification had a positive effect on growth and relative growth rates. Roots and shoots had a greater dry weight and length with 8 to 12 weeks of stratification. If these effects of stratification on future seedling growth could be expressed for 10 to 15 years, as observed for conifers (Perry, 1981), then careful attention to optimal stratification periods is warranted.

There were no diferences in seedling growth from high and low seed moisture stratification conditions. From this

study there appeared to be no advantage on germination and seedling growth from increased seed moisture levels during stratification. Since water was not a limiting factor, it is recommended that northern red oak seeds be stored at 50% moisture and 5 C. Prior to germination seeds should be imbibed for 52 hours.

Energy metabolism as measured by adenylate phosphates and by EC fluctuated markedly during 12 weeks of stratification. The EC indicated two peaks, one between 1 and 3 weeks and again between 6 and 10 weeks. The EC of 0.7 to 0.8 during the second and third weeks probably related to oxidation of lipids. The second surge in EC during the eighth week was concurrent with an increase in germinability. EC may be useful indexing the degree of dormancy and seedling vigor.

In short, the dormancy of northern red oak appears to be regulated by the pericarp and internal blocks within the embryo. The blocks within the embryo may be overcome with 6 to 10 weeks of stratification. During that time, physiological changes within the seed (afterripening) create a condition refered to as "ripeness to germinate". These afterripening changes allow for more rapid seedling growth. Thus a more competent seedlot in terms of high germination capacity and fast germination rate will produce more competent seedlings in terms of larger and faster growth.

LITERATURE CITED

Amen, R.D. 1968. A model of seed dormancy. Bot. Rev. 34: 1-31.

Anderson, J.A. 1977. Adenylate metabolism of embryonic axes from deteriorated soybean. Plant Physicl. 59: 610-614.

Anderson, J.D. 1977a. Responses of adenine nucleotides in germinating soybean embryonic axes to exogenously applied adenine and adenosine. Plant Physiol. 60: 689-692.

Atkinson, M.R. and R.K. Morton. 1959. In: Comparative Biochemistry Vol II. ed by M. Florkin and H.S. Mason. Academic Press, N.Y.

Barton, L.V. 1953. Seed storage and viability. Contrib. Boyce Thompson Institute 17: 87-103.

Barton, L.V. and J.L. Bray. 1967. Biochemical studies of dormancy and after-ripening of seeds . IV. Further studies on changes in contents of some amino acids and organic acid. Contri. Boyce Thompson Institue 23: 311-318.

Bonner, F.T. 1974a. Determining seed moisture in Quercus. Seed Sci. and Tech. 2: 399-405.

Bonner, F.T. 1974b. Chemical components of some southern fruits and seeds. USDA-Forest Service Res. Note SC-183, p. 3.

Bonner, F.T. 1975. Storage and stratification recommendation for pecan and shagbark hickory. Tree Planters Notes 27: 3-5.

Bonner, F.T. 1973. Storing red oaks acorns. Tree Planters' Notes 24: 12-13.

Bonner, F.T. 1976. Maturation of Shumard and white oak acorns. Forest Sci. 22: 149-154.

Bonner,F.T. 1970. Storage of acorns and other hardwood seed -- Problems and possibilities. Proc Southeastern Nurserymen's Conference. USFS, State and Private Forestry, pp 77-82.

Brown,J.W. 1939. Respiration of acorns as related to temperature and after-ripening. Plant Physio. 14: 621-645.

Brown, E.G. 1962. The acid scluble nucleotides of mature pea seeds. Biochem. J. 85: 633-640.

Brown,E.G. and K.C. Short. 1969. The changing nucleotide patterns in sycamore cells during culture in suspension. Phytochemistry 8: 1365-1372.

Brown,E.G. and J.L.Wray. 1968. Correlated changes of some enzyme activities and cofactor and substrate contents of pea cotyledons tissue during germination. Biochem. J. 108: 437-444.

Chapman,A.G., L.Fall and D.E.Atkinson. 1971 Adenylate energy charge in Escherichia coli during growth and starvation. J. Bacteriol. 108: 1072-1086.

Ching,T.M. 1972. Metabolism of germinating seeds. In: Seed Biology Vol. II ed by, T.T.Kozlowski. Academic Press,N.Y.: 220-276.

Ching,T.M. 1973a. Biochemical aspects of seed vigor. Seed Sci. and Tech. 1: 73-88.

Ching,T.M. 1973b. Adenosine triphosphate content and seed vigor. Plant Physiol. 51: 400-402.

Ching,T.M. 1975. Temperature regulation of germination in crimson clover seeds. Plant Physiol. 56: 768-771.

Ching,T.M. and K.K.Ching. 1972. Changes in adenosine phosphates and adenylate charge in germinating ponderosa pine seeds. Plant Physicl. 50: 536-540.

Ching,T.M. and J.M.Crane. 1974. Adenylate energy pool and energy charge in maturing rape seeds. Plant Physicl. 54: 748-751.

Ching,T.M. and H.Danielson. 1972. Seedling vigor and adenosine triphosphate levels of lettucs seeds. Proc. Ass. Off. Seed Anal. 62: 116-124.

Ching,T.M. and K.E.Kronstad. 1972. Varietal differences in growth potential, adenylate energy level, and energy charge of wheat. Crop Sci. 12: 785-789.

Ching,T.M. and I. Schoolcroft. 1968. Physiological and chemical differences in aged seeds. Crop Sci. 8: 407-409.

Copeland,L.O. 1976. Principles of Seed Science and Technology. Burgess Publ. Co., Minneapolis, Minn. 369 pp.

Crocker,W. 1948. Growth in Plants. Reinheld, N.Y. 459 pp.

Crocker,W. and L.V.Barton. 1953. Physiology of seeds. Chron. Botanica, Waltham, Mass. 267 pp.

Crompton,C., J.C.Wynn and R.P.Patterson. 1978. Calcium content, energy level and seed vigor in peanuts. Crop Sci. 18: 736.

Dury,C.D. 1977. Growth hormones and their relationship to dormancy in Quercus. Ph.D. Dissertation, Virginia Polytechnic Institute and State University.

Fenton,R.H. and E.I.Sucoff. 1965. Effects of storage treatments on the ripening and viability of Virginia pine seed. USDA-FS Res. Note NE-31,6p.

Flemion,F. 1933. Dwarf seedlings from non-afterripened embryos of Rhodotypos kerriodes. Contrib. Boyce Thompson Instit. 5: 161-165.

Fleamion,F. and J. Beardow. 1965. Production of peach seedlings from non-chilled seeds. II. Effect of subsequent cold periods on growth. Contrib. Boyce Thompson Instit. 65: 101-113.

Garrard,L.A. and R.H. 1963. Development of seedling from non-afterripened seeds of 'Lovell' peach. Proc. of Florida State Hort. Soc. Miami, Nov. 4-7,1963, pp. 387-393.

Haferkamp,M.R.,G.L.Jordan and K.Matsuda.1977. Physiological development of Lehman lovegrass seeds during the initial hours of imbibition. Agron. J. 69:295-299.

Helwig,J.T. and K.A.Council, eds.1979. SAS Users Guide, 1979 edition. SAS Institute, Raleigh,N.C. 494 pp.

Holmes,G.D. and G.Buszewicz.1955. Longevity of acorns with several storage methods. Great Britain Forestry Commission Report on Forest Research 1954/55, p 88-94.

Hopper,G.M. 1979. Endogenous growth regulators in Quercus nigra L. seed. Unpublished M.S. Thesis, Mississippi State University.

Janerette,C.A.1978. An in vitro study of seed dormancy in sugar maple. For Sci. 24:43-51.

Johnson,R.L.1975. Natural regeneration and development of Nuttall oak and associated species. USDA-FS Res. Pap. SO-1-4:12.

Johnson,R.L.1979. A new method of storing Nutall oak acorns over winter. Tree Planters' Notes 30:6-8.

Jones,L. and C.L.Brown.1966. Cause of slow germination in cherrybark and northern red oak. Pro. Asso. Off. Seed Anal. 56:82-88.

Kao,C. and K.S.Rowan.1970. Biochemical changes in seed of Pinus radiata D. Don during stratification. J.Exp.Bot. 21:869-873.

Khan,A.A.1977. Seed dormancy: changing concepts and theories. In: The Physiology and Biochemistry of Seed Dormancy and Germination. ed.by A.A. Khan. North-Holland Pub.Co. Amsterdam, N.Y.pp 447.

Koller,D.1972. Environmental control of seed germination. In: Seed Biology Vol. II ed. by,T.T.Kozlowski. Academic Press, N.Y., pp. 2-93.

Korstain,C.F.1927. Factors controlling germination and early survival in oaks. Yale Uni. School of Forestry. Bull 19: 115 pp.

Krajicek,J.E.1968. Acorn moisture content critical for cherrybark oak germination. USDA-FS Res. Note AC-63.

Lewak,S. and R.M.Rudnicki.1977. Afterripening in cold-requiring seeds. In: The Physiology and Biochemistry of Seed Dormancy and Germination. ed. by, A.A.Khan. North-Holland Pub. Co., Amsterdam, N.Y.: 447 pp.

Logan,K.T. and D.F.W.Pollard. 1976. Growth acceleration of tree seedlings in controlled environments at Petawawa Forest Exp. Sta. Info. Rep. PS-X-62: 11 pp.

Mayer,A.M. 1977. Metabolic control of germination. In: The Physiology and Biochemistry of Seed Dormancy and Germination. ed. by. A.A.Khan. North-Holland Pub.Co., Amsterdam,N.Y.: 447.

McDaniel,R.G. 1969. Relationship of seed weight, seedling vigor and mitochondrial metabolism in barley. Crop Sci. 9:823-827.

McDaniel,R.G. 1973. Genetic factors influencing seed vigor: biochemistry of heterosis. Seed Sci. and Tech. 1: 25-50.

McDaniel,R.G. 1976. Annual research report. American Seed Trade Ass. 92nd Convention. Houston, TX.1975. In: Search 13: 1-7.

McDaniel,R.G. and I.V.Sarkissa. 1968. Mitochondria heterosis in maize. Genetics 59: 465-475.

McLemore,B.F. and J.P.Barnett. 1967. Effective stratification of spruce pine seed. Tree Planters' Notes 18: 1-2.

McDermott,J.J. 1941. A physiological study of afterripening in acorns. Ph.D. Dissertation. Duke University.

Nikolaeva,M.G. 1968. On the hormonal nature of the regulation od deep dormancy of seeds. Inter. Symp. on Seed Physiology of Woody Plants. 39-44. Inst. of Dendrology and Kornick Arboretum of the Polish Academy of Sciences, Kornick.

Nikolaeva,M.G. 1969. Physiology of Deep Dormancy in Seeds. Nat. Sci. Found. Wash.,D.C. 220 pp.

Olney,H.O. and B.M.Pollock. 1960. Studies of rest period. II. Nitrogen and phosphorus changes in embryonic organs of afterripening cherry seed. Plant Physiol. 35: 970-975.

Osborn,D.J. 1973. Nucleic acids and seed germination. In: The Physiology and Biochemistry of Dormancy and Germination, ed. by A.A.Khan. North-Holland Pub. Co., Amsterdam, N.Y.: 447 pp.

Parrish,D.J. and A.C.Leopold. 1978. On the mechanism of aging in soybean seeds. Plant Physiol. 61: 365-368.

Plumb,T.R. 1979. Collecting, storing and germinating acorns of southwestern oaks. In: Workshop on seedling physiology and growth problems in oak planting. Columbia, Mo., Nov. 6-7,1979. USDA-FS Teh Rep. SC-62.

Radford,P.J. 1967. Growth analysis formulae-Their use and abuse. Crop Sci. 7: 171-175.

Roberts,E.H. 1969. Seed dormancy and oxidation processes. Symp. Soc. Exp. Bot. 23: 161-192.

Roe,E.I. 1946. Viability of acorns. Amer. Nurseryman 8:24-26.

Reich,P.B., R.C.Teskey, P.S.Johnson, and T.M.Hinckley. 1980. Periodic root and shoot growth in oak. For. Sci. 26: 590-598.

Santarius,K.A. and U.Heber. 1965. Changes in the intracellular levels of ATP,ADP, AMP, and Pi and regulatory function of the adenylate system in leaf cells during photosynthesis. Biochim. Biophys. Acta 102: 39-54.

Shen,L.C., L.Fall, G.M.Walton and D.E.Atkinson. 1968. Interaction between energy charge and metabolic modulation in the regulation of enzymes of amphibolic sequences. Phosphofructokinase and pyruvate dehydrogenase. Biochem 7: 4041-4045.

Simmonds,J.A. and E.B.Dumbroff. 1974. High energy charge as a requirement for axis elongation in response to gibberrellic acid and kinetin during stratification of Acer saccharrum seeds. Plant Physiol. 53: 91-95.

Sluder,E.R. 1979. The effects of seedling size on survival and growth of loblolly pine. Tree Planters' Notes 30: 25:28.

Smith,D.Wm. and N.E.Linnartz. 1980. The southeastern hardwood region. p. 145-230. In: J.W.Barrnett (ed.) Regional Silviculture of the U.S. 2nd edition. John Wiley and Sons. N.Y.,N.Y.

Soljanik,I. 1968. Producing seedlings from unripe forest seed. Serbian Ass. of For. Eng. and Tech. T167-S8012.

Southern Forest Resource Analysis Comm. 1969. The South's Third Forest... How can it meet future demands. Forest Farmers Ass., Atlanta,Ga.: 111pp.

Stokes,P. 1953. The stimulation of growth by low-temperature in embryos of Heracleum sphodylium L. J. Exp. Bot. 4: 222-234.

Stone,E.B. 1957. Embryo dormancy and embryo vigor of sugar pine as affected by length of storage and storage temperature. For. Sci. 3: 357-371.

Stone,E.C. and J.W.Duffield. 1950. Hybrids of sugar pine by embryo culture. J. For. 48: 200-201.

Stewart,J.M. and G.Guinn. 1969. Chiling injury and changes in adenosine triphosphate of cotton seedlings. Plant Physiol. 44: 605-608.

St. John,J.B. 1970. Determination of ATP in Chlorella with the luciferin-luciferase enzyme system. Anal. Biochem. 37: 409-416.

Thaplyal,R.C. and B.N.Guta. 1980. Effect of seed source and stratification on the germination of deodar seed. Seed Sci. and Tech. 8: 145:150.

USDA. 1974a. The outlook for timber in the United States. Forest Res. Rep. 20: 374.

USDA. 1974b. Seeds of Woody Plants In the United States. FS-hbk. 450.

USDA. 1978a. The forest resources of West Virginia. FS Res. Bull. NE-56.

USDA. 1978b. Virginia's timber, 1977. FS Res. Bull. SE-44.

USDA. 1980. Reveiw draft of an analysis of the timber situation in the United States 1952-1980. Report by U.S. Forest Service: 541 pp.

Villiers,T.A.1972. Seed dormancy. In: Seed Biology Vol. II, ed. by T.T.Kozlowski. Academic Press,N.Y.: 220-276.

Vogt,A.R.1970.Effect of gibberellic acid on germination and initial seedling growth of northern red oak. For Sci. 16:453-459.

Vozzo,J.A.1975. Anatomic observations of dormant, stratified and germinated Quercus nigra embryos. Phytomorphology 23: 245-255.

Vozzo,J.A.1975.Germination analyses of excised embryo cylinders and whole acorns of water oak. USDA-FS Res. Note SO-153, 3 p.

Weaver,G.H. and L.F.Hough.1959. Seedling growth studies of early ripening peaches. I. Interrelationships between embryo maturity, growth substances and seedling growth. Amer. J. Bot. 46:718-724.

Williams,S.G.1970. The role phytic acid in the wheat grain. Plant Physiol. 45:376-381.

Woods,J.H. and G.M.Blake.1981. Effects of stratification of Pinus ponderosa (var. Scopulorum Englem.) seed from Colstrip, Montana, USDA Res. Note 18, Montana Forest and Conservation Exp. Station, Missoula, Montana.

The vita has been removed from the scanned document

SEED DORMANCY AND GERMINATION OF NORTHERN RED OAK

by

George Martin Hopper

(ABSTRACT)

Northern red oak (Quercus rubra L.), a valuable timber species in the eastern United States, has a delayed growth in the early years of establishment. Freshly harvested northern red oak seed exhibit dormancy that may be broken by stratification or pericarp removal. In this research, germination, seedling growth and adenylate energy metabolism of northern red oak with pericarp removed and intact was measured during stratification (5 C) for two consecutive years. Two seed moisture levels (50% and 70% d.w.) during stratification were tested on intact acorns, pericarp removed seeds and acorns intact during stratification and then the pericarp removed prior to germination. Pericarp removal increased germination five-fold at harvest (from 10% to 55%), but almost half the naked seeds were still dormant. There was a deepening of dormancy during the first 4 weeks of stratification; but, stratification for 6 to 8 weeks significantly increased germination and germination rate. Etiolated seedlings grew taller and faster from acorns that had been stratified 8 to 12 weeks than from acorns with no or only 4 weeks of stratification. Significant increases in

root, shoot, and axial dry weights and lengths, and root/shoot ratios were observed as early as 14 days after germination. Pericarp removal had no significant effect on seedling growth. Relative growth rates of seedlings were compared by pericarp treatment and stratification time. There were no significant differences in germination or seedling growth between 50% and 70% seed moisture content. Adenylate (ATP, ADP, AMP) levels were measured using the luciferin-luciferase assay. Energy charge (EC) increased during stratification at 2 weeks when germination was low. Thereafter EC decreased before increasing at 8 weeks of stratification. This second rise in EC was concurrent with an increase in germinability. ATP concentrations during the 28 day growth time appeared to be associated with surges in relative growth rates of roots and shoots.