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Embryo culturing methodology and investigation of seed dormancy mechanisms in Mountain Silverbell (Halesia Carolina L.)

Paul Conrad Durr

To the Graduate Council:

I am submitting herewith a thesis written by Paul Conrad Durr entitled "Embryo culturing methodology and investigation of seed dormancy mechanisms in Mountain Silverbell (Halesia Carolina L.)." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Edward R. Buckner, Major Professor

We have read this thesis and recommend its acceptance:

Otto J. Schwarz, John Day

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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104

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We have read this thesis
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John W. Day

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Accepted for the Council:

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EMBRYO CULTURING METHODOLOGY AND INVESTIGATION OF SEED DORMANCY MECHANISMS IN MOUNTAIN SILVERBELL (*HALESIA CAROLINA* L.)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Paul Conrad Durr

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ABSTRACT

Desirable wood properties once made mountain silverbell (*Halesia carolina* L.) a favored species among loggers in the southern Appalachians. Showy flowers, disease resistance, and handsome form continue to make it a desirable ornamental. Delayed and erratic germination over a one to two year period, plus a poorly understood dormancy mechanism, may be responsible for limiting its commercial availability. Prolonged warm and cold stratification has been recommended as a presowing treatment, but it is not clear whether such treatments relieve dormancy by overcoming hardseededness, inhibiting chemicals, or morphological and physiological impediments within the embryo itself.

During the years 1982-84, a study was undertaken to find means of hastening germination, and to provide for a clearer understanding of dormancy. Various conventional sacrification procedures were used to quickly remove hard seed coverings, but these failed to induce germination. *In vitro* culturing of excised surface sterilized embryos, however, proved highly successful, yielding 100 percent germination within 7 days.

Later experimentation showed that morphological and physiological dormancy factors were absent at the time of fruit abscision in mid autumn. Exogenously applied extracts from fruit and seed layers also did not inhibit germination. The primary impediments

appeared to center on the hardened endocarp which physically restricted water availability to the quiescent embryo for several months, and then mechanically prevented embryo expansion once hydration occurred.

Overwintering on the forest floor (or artificial stratification designed to mimic overwintering) gradually overcomes physical and mechanical barriers of hardseededness, especially under moist, non-sterile conditions. Embryo excision and culture expedites this process so that seedlings suitable for out-planting can be obtained within 1 year.

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CHAPTER I

INTRODUCTION

Records of early logging in the southern Appalachians indicate that mountain silverbell (Halesia carolina L.) was once a valuable timber tree. Desirable wood properties such as stability, figured grain, and a high degree of workability made it a favored species among local furniture manufacturers (Sudworth, 1897; Ayres and Ashe, 1905; Holmes, 1911). More recent studies indicate that the wood may also be well suited for light construction, veneer, and pulp production (Peattie, 1950; Harrar, 1967).

The combination of showy flowers that appear with the developing leaves in the spring, a high degree of disease and insect resistance, and handsome form, make mountain silverbell a valuable ornamental. These features, plus a relatively rapid growth rate under favorable conditions, suggest that intensive management of this species for horticultural and timber uses might be profitable (Keeler, 1912; Lemmon, 1955; Dirr, 1977).

Despite the tree's apparent commercial value, mountain silverbell has not been widely utilized in recent times. Propagation difficulties both with seeds and cuttings have limited its availability as an ornamental, while a restricted natural occurrence in the little-managed Appalachian hardwood region precludes its more common usage for forest products.

Previous studies and nursery experience indicate that up to 2 years are required to establish a seedling suitable for outplanting. Low germination percentages and erratic germination rates over a prolonged period make propagation from seed troublesome and expensive. A very hard endocarp (Jack, 1894) and a poorly understood stratification requirement (Giersbach and Barton, 1932; Wyman, 1971; Bonner and Mignery, 1974; Dirr, 1983) have been suggested as reasons for these difficulties, but it is not yet clear whether delayed seed germination is due solely to physical obstructions or whether other constraints may also be involved.

Vegetative regeneration of silverbell using soft or semihardwood cuttings in conjunction with root-promoting compounds such as indolebutyric acid (IBA) and napthaleneacetic acid (NAA) has generally been effective (Doran, 1957; Halward, 1965). Although this is the most common means of propagation, the limited number of commercial growers who handle silverbell report varying degrees of success in winter hardening the rooted cuttings. The stems often split or are killed back unless they are given careful protection during the winter months. These problems in vegetative regeneration, like those from seed, make propagation from cuttings somewhat tenuous. Furthermore, the availability of suitable cutting material may be restricted in certain areas and at certain times of the year.

Until the dormancy mechanisms controlling germination are better understood, and more practical and expedient propagation methods found, mountain silverbell will likely have limited use in either forest or horticultural applications.

CHAPTER II

LITERATURE REVIEW

Taxonomic Considerations

The silverbells (*Halesia* spp.) are components of the Styracaceae, a family containing 13 genera and approximately 150 species. The majority of the species in this family are distributed in the temperate, subtropical, and tropical regions of Africa, South and Central America, southeastern United States, southeastern Asia, and the Mediterranean (Spongberg, 1976). Recent investigations by Chester (1966) and Spongberg (1976) indicate that the genus *Halesia* is comprised of five taxa: *H. carolina* L., *H. parviflora* Michaux, *H. diptera* Ellis var. *diptera*, and *H. diptera* Ellis var. *magniflora* Godfrey, all of which are endemic to the southeastern United States. An additional species, *H. Macgregorii* Chun, is native to Southeast Asia.

Halesia carolina, the subject of this study, has been a taxonomic puzzle for more than 50 years. The species undergoes changes in gross morphology from one point in its geographic range to another. On mesic sites in the Piedmont and Coastal Plain of the southeastern United States, *H. carolina* is referred to as Carolina silverbell, and only reaches the size of a shrub or small tree (Gibson, 1913; Coker and Totten, 1945; Peattie, 1950). In the cool, moist coves of the Appalachian highlands of eastern Tennessee and western North

Carolina, however, the tree attains much greater proportions. Stevenson (1967) noted that it is one of the largest forest trees in the Great Smoky Mountains National Park. Locally, the larger form is termed mountain silverbell, peawood, or bell-tree.

Recognizing this disparity of dimension, and coupling it with perceived increases in leaf, petiole, flower, and fruit size in the montane populations, Rehder (1913) first proposed the varietal name monticola. Sargent (1921) elevated this entity to the specific level offering the new name H. monticola (Rehder) Sargent.

The tree's variable habit has led botanists, horticulturists, and nurserymen to apply superfluous names to it over the years. This has resulted in an extremely confused and awkward taxonomy. In an attempt to clarify the situation, Chester (1966) conducted an intensive investigation into the relationships of the H. monticola-carolina complex. He refuted the existing taxonomic status, and concluded that the characters used to define the group were either clincal in nature, or resulted from random variation without correlations worthy of taxonomic recognition:

> The biometrical study conducted in this investigation indicates that . . . the flowers, fruits, and leaves of the montane populations are not significantly greater than those found in H. carolina. The only factor which seems to separate H. carolina and H. monticola is tree size, and there is a gradual increase in size from the piedmont and coastal plain to the mountains; there are no discontinuities even in this characteristic. This lack of genetic barriers between the population

> indicates that only one variable complex is involved and makes it impossible to separate even two well defined varieties.[1]

Although the Chester study attempted to unify the H. monticola-carolina complex under the single name H. carolina, this position remains under debate by taxonomists holding differing viewpoints as to precisely what constitutes a species.

Ecological Distribution and Growth Habit

Mountain silverbell is a dominant, subclimax member of the mixed mesophytic (cove hardwood) forests in the southern Appalachian region of Tennessee and North Carolina (Miller, 1938; Braun, 1950; Shanks, 1954a; Whittaker, 1956) (Figure II-1). Although adaptable to a variety of climatic and edaphic situations, greatest size and frequency are attained on the cool, north and east facing slopes at low and mid-elevations. Here rainfall averages 120 to 200 centimeters (cm.) yearly and the soils are moist, organically rich, and acidic (Shanks, 1954b).

The salubrious upland coves of Blount, Sevier, and Monroe counties in east Tennessee, support stands of the largest known mountain silverbell. In this area, the trees commonly grow to heights of 25 to 30 meters (m.) and attain trunk diameters in excess of 1 m.

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[1]The current investigation of delayed germination in silverbell employs seed collected from the large, mountain form of the tree. It is appropriate, therefore, to treat this entity in the Literature Review. Occasional references will be made to the smaller, piedmont-coastal plain form, but these will be clearly identified as such.

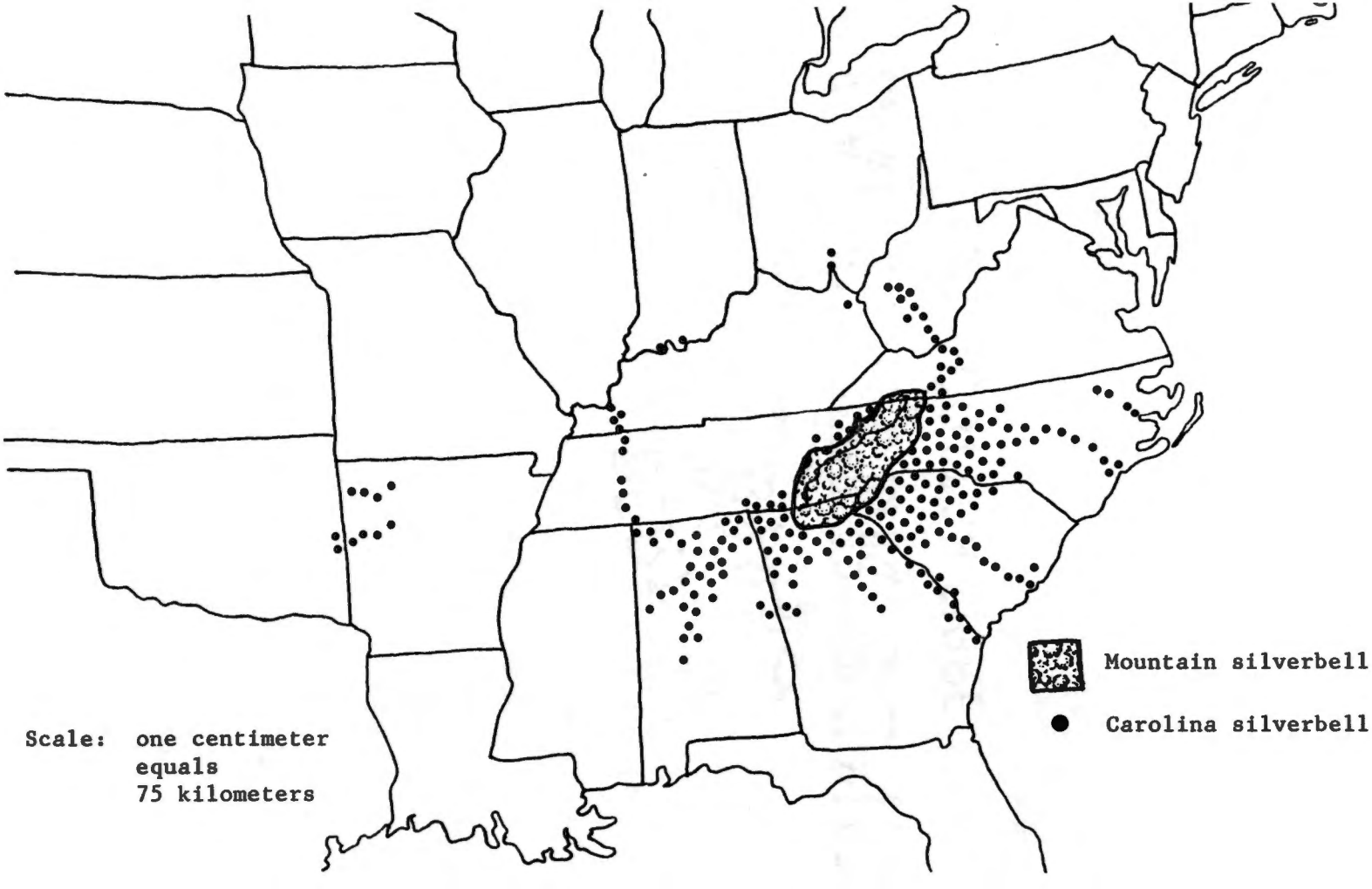

Figure II-1. Geographic Range of Halesia carolina L. Including Carolina and Mountain Forms.

Some individuals may measure 20 m. to the first live limbs (Ayres and Ashe, 1905; Gibson, 1913; Stevenson, 1967).

In 1984, Durr and Hobart reported finding a very large silverbell growing along the upper reaches of Roaring Fork Creek in the Great Smoky Mountains National Park. The specimen was 1.16 m. in diameter (1.37 m. above the ground), 25.6 m. tall, and had an average crown diameter 12.19 m. It was declared national champion by the American Forestry Association (Hunt, 1984).

The boles of thrifty mountain silverbell are generally straight and often exhibit very little taper. They terminate in a crown of erect or spreading branches which gives the tree a pyramidal or irregularly rounded outline. Suppressed individuals, though tolerant of shade, eventually show loss of apical dominance resulting in stunted, flattopped crowns unless overhead light is restored.

Mountain silverbell, when regenerated from seed, is capable of very rapid juvenile growth. Heights of 0.6 to 1.0 m. per year are not unusual if the young trees are grown under good site conditions. When cultivated as an ornamental, Dirr (1977) noted that 2.7 to 3.7 m. growth over 6 to 8 years is not unreasonable, even under average conditions.

Vegetative regeneration from root, and especially basal sprouts, is very common in silverbell. Provided with a readily available source of carbohydrate from the "parent" tree, rapid juvenile growth is even more pronounced than from seed. If given direct sunlight, 5.0 to 6.0 m. of height, and 5.0 to 7.5 cm. of diameter growth can be obtained over a 5 year period.

Following the juvenile stage, the growth of mountain silverbell slows considerably. While little substantive data is available to clearly document the mature tree's performance, Harrar (1967) observed that ring counts made from increment cores frequently reveal 15 to 25 or more layers to the inch. Two hundred or more annual rings have been noted in some of the largest trees.

Increment borings made during the current study consistently demonstrated silverbell's ability to respond favorably to release. Because it is a shade tolerant species, diameter and height growth will continue at low light intensities, albeit very slowly. With the death or removal of competing trees, suppressed silverbell is capable of expanding its crown to capture the increase in light availability. This augmented photosynthetic potential results in increased growth allowing the tree to become productive even after years of suppression.

Economic Importance

Forest Products

During the late nineteenth and early twentieth century, a variety of wood-using industries flourished in the Great Smoky Mountains. Although most of these firms were interested in the vast stands of massive yellow-poplar, chestnut, oak, and spruce, some discovered that the comparatively smaller mountain silverbell also possessed desirable wood qualities.

The diffuse porous wood is characteristically soft, moderately light in weight, and close grained. The sapwood is ivory-white

and shows an abrupt transition to the copper-red heartwood (Record, 1949). Though not particularly strong or decay resistant, attributes such as stability, resistance to splitting, ease of turning and carving, and the ability to take a polish, made silverbell a favored species of several Tennessee and North Carolina companies manufacturing furniture, cabinets, mantels, paneling, and porch columns. These products were not marketed as silverbell, but as birch or cherry (Sudworth, 1897; Ayres and Ashe, 1905; Holmes, 1911; Harrar, 1967).

Perhaps silverbell's chief value is the handsome color and figure which are expressed when the logs are peeled for veneer. Adventitious buds embedded in the wood are responsible for its distorted grain, birdseye figure, and mottled coloration (Barry, 1917; Artman, 1935; Peattie, 1950). Gibson (1913) remarked, "veneers (cut by the rotary method) . . . from logs selected for the figure, possess a rare beauty which no other American wood equals."

Despite these desirable characteristics, very little silverbell found its way into the veneer markets. The scattered occurrence of veneer quality logs and the rugged mountain terrain, in all likelihood, made it difficult for buyers to obtain adequate supplies of raw material. Even more critical was the fact that the best stands of silverbell were removed from commercial exploitation with the founding of the Great Smoky Mountains National Park in the 1930's.

Today, the supply of merchantable-sized silverbell remains rather limited. The United States Forest Service continues to harvest

silverbell from the Pisgah, Nantahala, and Cherokee National Forests, though it is sold as miscellaneous hardwood. It is used locally in the manufacture of lumber, furniture and paper products.[2]

The wood is also a favorite of many Appalachian craftsmen engaged in carving, turning, and fabricating woodenware and wooden novelties found in gift shops catering to the tourist trade (Harrar, 1967).

Ornamental

The silverbells are regarded by many as among the loveliest native flowering trees. Their handsome growth habit, beautiful white or pink bell-shaped flowers, pest resistant foliage, and unusual "gun metal" blue bark, all combine to make them desirable ornamentals (Keeler, 1911; Lemmon, 1955; Wyman, 1971; Wharton and Barbour, 1973; Dirr, 1977).

Both the large mountain silverbell and the more diminutive piedmont-coastal plain form, are tenacious and rather fast growing. The easily transplanted saplings prefer rich, moist, well-drained soils that are somewhat acidic (pH 5.0 to 6.0) and relatively high in organic matter (Bailey, 1935; Grant, 1943; Dirr, 1977). Performance is also good on more basic soils (pH 6.0 to 7.0), although Dirr (1983) reports leaf chlorosis at these higher pH's.

Silverbell is hardy when grown far outside of its natural range. The tree was introduced into England, France, and Germany

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[2]Personal communication with Beecher Colvin, U.S.F.S., Cherokee National Forest, April, 1983.

during the eighteenth and nineteenth century, and continues to be popular there (Chester, 1966; Bean, 1973). In the United States it is hardy to -30° centigrade (C) growing as far north as Massachusetts and New York and as far west as Washington and Oregon (Keeler, 1912; Bailey, 1935; Grant, 1943).

Flowers, Fruit, and Seed

Mountain silverbell is a consistent and prolific flower producer under favorable growing conditions. Although saplings of sprout origin occasionally begin flowering as early as age 5, Harrar (1967) observed that heavy crops are normally not borne until after the twentieth season. In the southern Appalachians, flowering is at its peak from April through mid May. At low elevation flowering can begin as early as late March (Stupka, 1964; Radford et al., 1968).

The flowers themselves (Figure II-2) are actinomorphic, complete, and produced in fascicles or reduced racemes. Each pendulous inflorescence arises from buds of the preceding year, and may contain 2 to 4 (occasionally 5 or more) individual flowers. The calyx tube is obconic with 4 obsolete lobes that are almost or completely adnate to the ovary. The campanulate corolla is epigynous, and consists of 4 lobed petals which are united for at least 2/3 their length. Corolla color varies from pure white to pale pink depending on anthocyanin content (Chester, 1966). The exserted stamens are produced in a single series, vary in number from 8 to 16, and possess

Figure II-2. Floral Characteristics of Mountain Silverbell.

A. Flowering branch (x1.5)

B. Opened flower (x2.5)

C. One stamen (x4)

D. Calyx and pistil (x4)

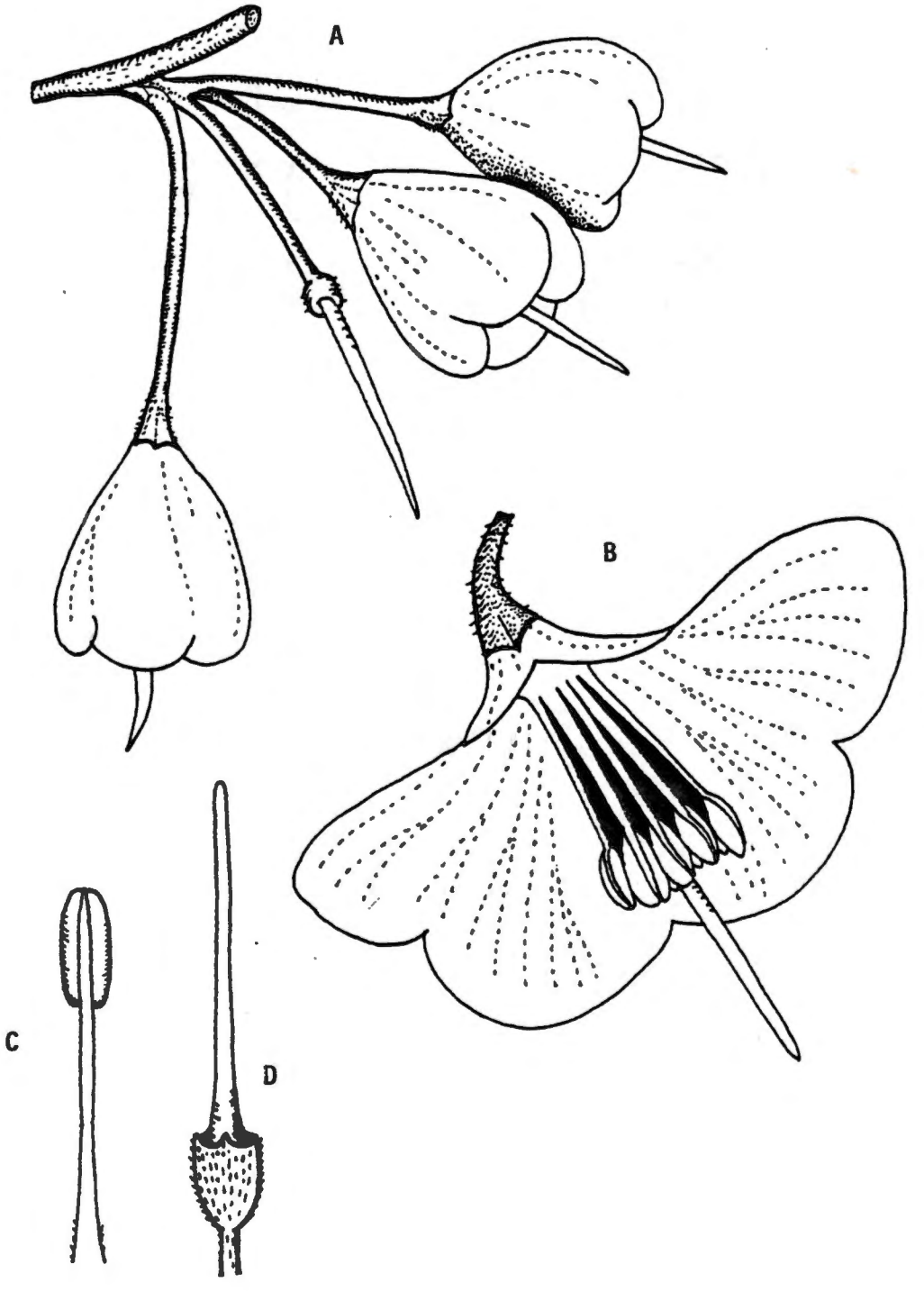

Figure II-2

flattened filaments which are fused to the base of the corolla. Anthers are two-celled, oblong, introrse, and dehisce longitudinally.

The ovary of silverbell is inferior and normally four locular. Anatropous ovules are borne 2 to 8 per placenta but become solitary through abortion (Chester, 1966; Reveal and Seldin, 1976; Spongberg, 1976). Lawrence (1951) noted incomplete septations within the ovary which results in parietal placentation above and axile below.

The drupaceous, indehiscent fruits of mountain silverbell (Figure II-3) reach mature size from mid June to early July. Size and shape vary depending on available moisture and exposure to light (Chester, 1966). Mature, viable fruits commonly range from 30 to 60 millimeters (mm.) in length (including the persistent style). Cleaned fruit range from 3300 to 4100 per kilogram (kg.).

Fruit abscision normally takes place from early October through November, but individual trees often retain a portion of their crop until the following spring. Dispersion is accomplished by wind, water, and various seed predators, most notably red and grey squirrels (Ridley, 1930; Van Dersal, 1938; Miller and Jaques, 1972).

The exocarp is four-winged (rarely five or six-winged), thick and succulent when young, becoming thin, dry, and corky by mid autumn. The hard, bony endocarp is elongated to nearly rounded and variously ribbed externally.

Anatomical studies conducted by Martin (1946) and Chester (1966) indicate the seeds are solitary in each cell and range from 1 to 4 per fruit. They are elongated, somewhat flattened, and broadest

Figure II-3. Fruit Characteristics of Mountain Silverbell.

A. Fruit (x2)

B. Fruit with exocarp removed (x2)

C. Fruit in cross section (x1.5)

D. Fruit in longitudinal section (x3)

   a. exocarp

   b. seed and embryo

   c. distal cavity

   d. cotyledon

   e. endosperm

   f. radicle

   g. endocarp

   h. seed coat

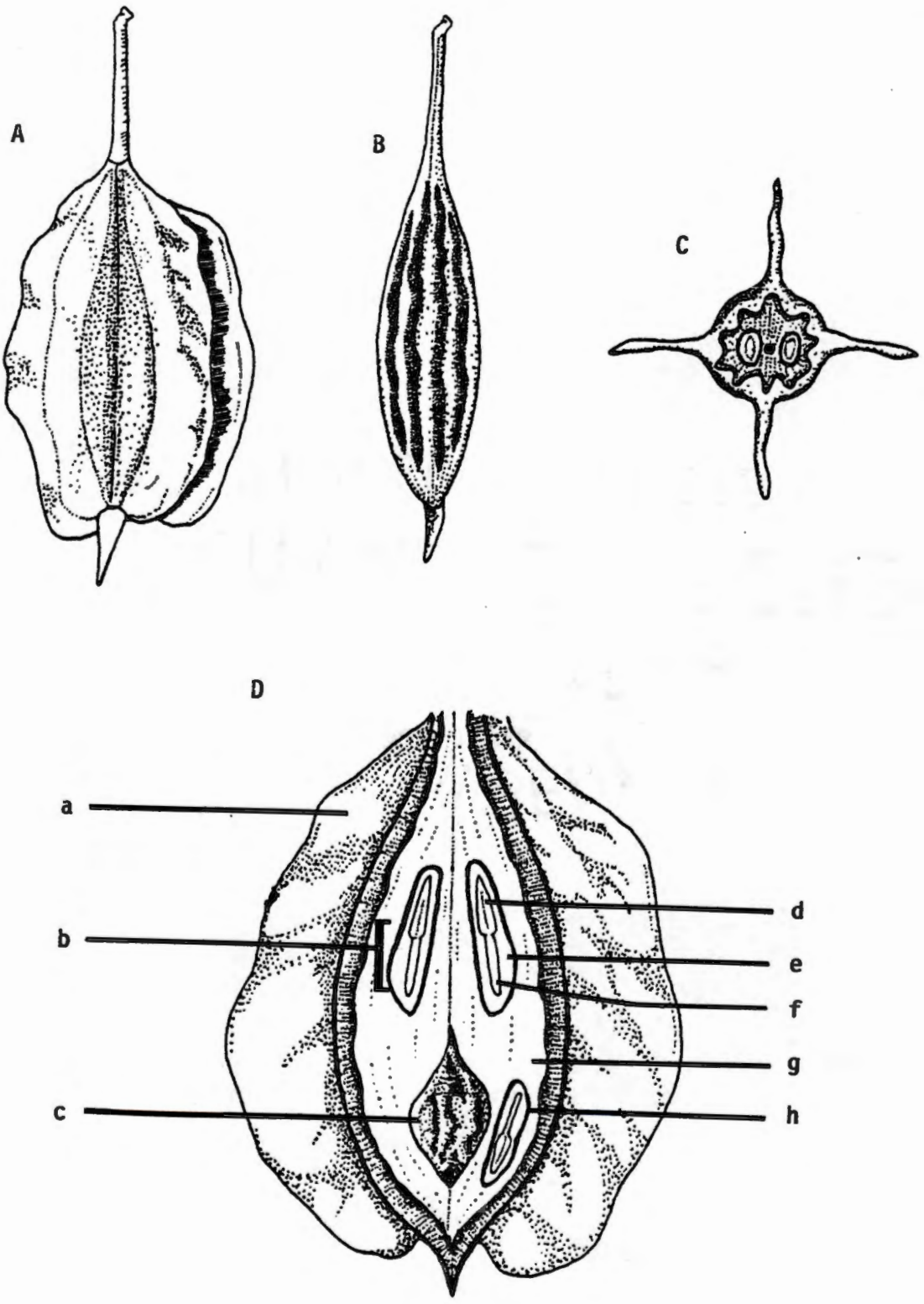

Figure II-3

at the radicle end. The testa is extremely thin, reddish-brown, and encloses the copious endosperm. Embryos are terete, pendulous below, and erect above, and possess two oblong cotyledons (Appendix A).

Dormancy in Seeds

Despite mountain silverbell's potential commercial value, it has not been widely managed for horticultural uses or for forest products. This is because the species is extremely difficult to regenerate from seed and may take up to 2 years to produce a seedling suitable for outplanting. Studies by Jack (1894), Ramaley (1899 in Chester, 1966), and Giersbach and Barton (1932) indicate that this delayed and erratic germination is due primarily to bony seed coverings but the precise nature of the dormancy was never clearly determined.

Seeds of most plants are exposed to seasonal periods of very inclement weather during which they would be damaged or killed if some protective mechanism did not exist. The most common safeguard against freezing cold or extreme dry heat is dormancy. Seed dormancy may be defined as a state of suspended growth or greatly reduced metabolism (Crocker and Barton, 1953; Villiers, 1972; Bidwell, 1979). When dormancy is induced by unfavorable environmental conditions, it is termed imposed dormancy. In temperate zones, dormant seeds often fail to grow even when exposed to ideal conditions. This indicates that dormancy can be caused from within and controlled

by mechanisms in the seeds themselves, or in the fruits enclosing them. Under these circumstances, a condition known as organic dormancy exists (Nikolaeva, 1969).

Though imposed and organic dormancy often work in concert, organic dormancy is the least understood and of most interest to propagators. It is not surprising, therefore, that scientists have concentrated much of their effort on examining and categorizing the biological mechanisms involved in this type of dormancy (Crocker, 1916; Nikolaeva, 1969; Atwater, 1980). Intensive investigations by Nikolaeva (1969) resulted in a classification scheme for organic seed dormancy that has gained wide acceptance (Table II-1). It is this classification scheme, with contributions from various other sources, which will serve as the basis for the following discussion of organic seed dormancy.

Exogenous Organic Dormancy

The first major type of dormancy is termed exogenous organic dormancy and is due to the properties of the outer, non-living fruit and seed wall layers (exocarp, endocarp, outer integument) which inhibit germination of the embryo. The inhibiting effect may be due to a physical barrier which restricts the movement of water and gases to the embryo, or may be mechanical, preventing the expansion and emergence of the embryo. Chemical barriers such as coumarin, certain alkaloids, hydrocyanic acid, etc., located in the external fruit layers can also hinder embryo emergence (Nikolaeva, 1969; Salisbury and Ross, 1979; Hartmann and Kester, 1983).

Table II-1. Types of Organic Dormancy of Seeds (after Nikolaeva, 1969).

Name of Dormancy Type	Factors Causing Dormancy	Conditions Breaking Dormancy	Example
Types of Exogenous Dormancy	Properties of Embryo's Outer Covers (exocarp, endocarp, outer integument)	Various External Treatments	
Physical	Impermeability of External Layer(s) to Water and Gases	Stratification or Other Methods of Injuring the Outer Covers	Black locust (Robinia pseudoacacia); and many other species in the Fabaceae
Chemical	Inhibiting Action of Various Chemical Compounds	Removal of External Covers or Leaching Out Inhibitors	Beet (Beta vulgaris); also several Oriental Ashes (Fraxinus spp.)
Mechanical	Mechanical Resistance of Covers to Embryo's Growth	Various Methods of Destroying the External Covers	Eucalyptus (Eucalyptus spp.), Russian Olive (Elaeagnus angustifolia), and various species in the Rosaceae
Types of Endogenous Dormancy	Properties of the Embryo or of Inner Covers Directly Surrounding It (endosperm, nucellus, inner integument)	Various Physiological Influences	
Morphological	Undifferentiated Embryos or Underdevelopment of the Embryo	Cold Stratification Warm Stratification	African Oilpalm (Elaeis guineensis)
Physiological Shallow	Low permeability of Inner Covers to Gases	Brief Cold Stratification, Injury to Covers, Light, Gibberellin, etc.	Scotch Pine (Pinus sylvestris) and many other members of the Pinaceae
Physiological Deep	Double Mechanism of Inhibition of Germination (Low Permeability of Covers to Gases and the Particular Condition of the Embryo)	Various Durations of Cold Stratification Sometimes in Combination with Warm Stratifications	Apple (Malus spp.) Numerous species from many diverse families.
Types of Combined Dormancy	Combined Causes of Exogenous and Endogenous Dormancy	Application of Physical Treatments in Combination with Various Stratification Regimes	American holly (Ilex opaca) - physical and morphological and physiological American basswood (Tilia americana) - physical and physiological deep

Physical and mechanical forms of exogenous organic dormancy are both directly attributable to extremely hard, non-living fruit and seed wall layers. In the case of physical dormancy, which is characteristic of many legumes, the covers restrict the movement of water and sometimes gases to the embryo and thereby prevent the initiation of the metabolic processes necessary for continued embryo growth and germination (Crocker, 1906; Krugman et al., 1974; Kramer and Kozlowski, 1979). Mechanical dormancy (e.g., Elaeagnus angustifolia), on the other hand, permits water to reach the embryo. Although enzyme activation and the hydrolytic breakdown of reserve substances can occur, the expanding embryo cannot break free of its restrictive enclosures (Nikolaeva, 1969).

In nature, hard coverings are softened by a wide variety of environmental agents including alternate freezing and thawing, attack by soil microorganisms, passage through the digestive tracts of birds or mammals, or even fire (van der Pijl, 1969; Leopold and Kriedemann, 1975; Hartmann and Kester, 1983). Plant propagators, however, employ a process known as scarification to quickly alter these coverings to make them more permeable to water and gases and less mechanically restrictive. This is most commonly accomplished by using abrasives, erosive chemicals, or soaking in water (Isely, 1965; Bonner et al., 1974). Though not as rapid in its action, a procedure termed stratification which involves storing the fruit at warm (15 - 40° C) and cold (0 - 10° C) temperatures under moist conditions has also been shown to effectively overcome physical

and mechanical forms of dormancy in a large number of species (Nikolaeva, 1969; Krugman et al., 1974; Leopold and Kriedemann, 1975; Bidwell, 1979). Harvesting slightly immature fruits and preventing them from drying can also reduce "hardseededness" in some cases (Nikolaeva, 1969; Hartmann and Kester, 1983).

Chemically induced exogenous organic dormancy, due to inhibiting substances present in the external fruit and seed layers, is characteristic of many tropical and desert species (Salisbury and Ross, 1978), but does occur in several temperate climate plants such as tomato, beet, and the oriental ashes. Under natural conditions, these chemicals are leached from the fruits and seed coats during periods of high rainfall, or removed after consumption by birds or mammals. While not normally associated with hard coverings, the presence of these amplify the effects of the chemicals by delaying the leaching out of the inhibiting substances (Nikolaeva, 1969; Bidwell, 1979; Bewley and Black, 1985).

Because chemical inhibitors are, for the most part, water soluble, soaking the fruit for a period of time has been shown to be beneficial to induce germination. When inhibitors are found in association with hard coverings, it is often necessary to either injure or completely remove the external fruit and seed layers in order to initiate embryo germination and growth (Nikolaeva, 1969; Crocker and Barton, 1953; Hartmann and Kester, 1983).

Endogenous Organic Dormancy

The second major form of dormancy identified by Nikolaeva (1969) is endogenous organic dormancy. Endogenous dormancy results

from inhibiting mechanisms located in the inner, living layers of the seed (inner integument, nucellus, endosperm), or may also be related to the physiological state of the embryo itself.

Endogenous morphological barriers exist when the embryos are undeveloped or undifferentiated at the time of fruit maturity. Plants with undifferentiated (rudimentary) embryos produce seeds consisting of little more than a proembryo embedded in a massive endosperm (Atwater, 1980). Embryos of this type occur in various plant families including, the Magnoliaceae (Magnolia spp.), Ranunculaceae (Anemone spp.), Papaveraceae (Papaver spp.) and Araliaceae (Panax spp.) (Nikolaeva, 1969, 1977; Hartmann and Kester, 1983). Though the mechanisms involved in producing undifferentiated embryos remains under debate, the main body of the literature suggests that they are primarily a result of inhibitors which retard normal embryo development. Abscisic acid (ABA) located in the endosperm and embryo proper is most commonly implicated (Evenari, 1949; Villiers and Wareing, 1965; Sondheimer et al., 1968). Since ABA is particularly active at elevated temperatures, exposing seeds to the cold (below 15° C) for a period of time is often recommended to overcome the effects of ABA before the seeds are sown. Treatment with chemicals such as potassium nitrate or gibberellic acid (GA) has also been reported as being beneficial in a few species (Nikolaeva, 1969; Kramer and Kozlowski, 1979; Atwater, 1980; Hartmann and Kester, 1983).

Undeveloped embryos differ from undifferentiated embryos in that they are in a more advanced stage of morphological development,

and may attain a size up to 1/2 of the seed cavity by the time the fruit matures. Plants of this type include members of the Apiaceae (Daucus spp.), Ericaceae (Rhododendron spp.), Primulaceae (Primula spp.), and Gentianaceae (Gentian spp.). Like undifferentiated embryos they are incapable of germination without a preparatory stage during which the development of the embryo is completed. Plant propagators have found that warm, moist stratification (15 - 40° C) aids in the continued growth of the embryo prior to sowing. GA treatments too have been successful in certain instances by greatly accelerating embryo growth (Hartmann and Kester, 1983).

Morphological retardation of embryo development may occur in any species owing to unfavorable environmental conditions such as extremes in temperature or low rainfall at the time of fruit development. Retarded embryo growth in this instance is what Nikolaeva (1969) termed "imposed dormancy." Since it is not organically induced, it cannot therefore be considered as endogenous morphological dormancy in the true sense.

Physiologically shallow dormancy occurs in a wide variety of plant families such as the Pinaceae (Pinus spp.), Poaceae (Triticum spp.), Asteraceae (Xanthium spp.) and Solanaceae (Nicotiana spp.) (Hartmann and Kester, 1983). This form of endogenous organic dormancy is caused by the physiologically active seed layers (inner integument, nucleus, endosperm) which inhibit the exchange of gases between the developing embryo and the outside environment. The causes of low permeability in these layers may be purely physical or, in rare

instances, may also be related to photoactive inhibitors in them which create a chemical barrier to the availability of oxygen to the embryo[3] (Borthwick et al., 1954; Evenari, 1965; Nikolaeva, 1969).

Because of the diversity of mechanisms involved in this type of dormancy, the means by which it is overcome are also diverse. Physiological shallow dormancy due to a physical blocking of oxygen to the embryo is easily broken by injuring or removing the internal seed layers, increasing the partial pressure of oxygen in the surrounding environment, brief cold stratification, or in some instances, dry storage. Photoactive inhibitors, though variable in their mode of action and poorly understood, are most commonly overcome by removing the photochemical barriers which restrict gas permeability. Like physical barriers, this can be accomplished by removing or injuring the internal seed layers. Exposure to light, soaking the seeds in nitrous substances, and treatment with GA have also been found to be effective in some species (Nikolaeva, 1969; Hare, 1981).

A final category of endogenous organic dormancy is physiological deep dormancy. This form of dormancy occurs most commonly in plants of temperate zones and is represented in a wide array of families including the Rosaceae (Prunus spp.) (Heit, 1967), Lauraceae (Lindera benzoin) (Schroeder, 1935), and Hippocastanaceae (Aesculus spp.) (Rudolf, 1969, 1970). While scientists agree that the initiation, maintenance, and breaking of deep physiological dormancy is ultimately

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[3]Some investigators consider cases of impeded germination of photosensitive seeds as a special type of dormancy called photodormancy.

controlled by the balance of growth-inhibiting and growth-promoting substances located in the internal seed layers and embryo, the precise nature and mode of action of these substances remains uncertain and controversial. Equally puzzling are the mediating effects of aeration, temperature, light and moisture on these endogenous growth regulators.

Nikolaeva (1969) indicates that low gas permeability of the internal seed covers is the root cause of deep physiological dormancy, just as it is with shallow physiological dormancy. In the case of shallow physiological dormancy, however, physical and chemical obstruction of oxygen to the embryo is the sole cause of delayed germination. Embryos of such seeds start to grow soon after removal of, or injury to, the covers, and they continue to grow normally.

On the other hand, when seeds are in a state of deep physiological dormancy, the reduction of oxygen below a certain critical level results in the embryo developing its own system of obstructing growth. In combination with the inhibiting action of the seed covers, it creates a powerful double mechanism of germination inhibition. When freed of the covers, embryos of this type start to grow, but their gorwth is to some degree slowed or abnormal. This condition, termed "physiological nanism" or "physiological dwarfing," is most often characterized by reduced hypocotyl and epicotyl growth. Other manifestations may include poor root development, malformed cotyledons and leaves, and callus formation, particularly in the region of the hypocotyl (Crocker and Barton, 1953; Nikolaeva, 1969).

Whereas shallow physiological dormancy can be overcome by a variety of treatments which remove physical and chemical barriers to oxygen availability, Nikolaeva (1969) indicates the only method which effectively breaks deep physiological dormancy is prolonged cold stratification in which seed moisture is above certain "critical levels." Embryo respiration and the enzymatic breakdown of reserve fats, proteins, and carbohydrates located primarily in the endosperm and cotyledons, proceeds at both cold and warm temperatures. However, a reduced respiration rate in the cold allows for the accumulation of still unidentified "growth factors" which overcome the hormonal inhibitors in the embryo and allows germination to take place. The initiation of germination is marked by rapid synthesis of GA which ensures seedling growth.

The inhibitor, which Nikolaeva (1969) identified as indolecetic acid (IAA), occurs in high concentrations in non-stratified dormant seeds. Overcoming the inhibiting effects of IAA under conditions of low temperature is of extreme importance, not only for the successful germination of these seeds, but also during subsequent seedling growth. The influence of IAA persists without cold treatment and is thought to give rise to the abnormal seedling development which has come to be known as physiological nanism.

Contrary to Nikolaeva's (1969) observations, other investigators have implicated abscisic acid (ABA) rather than IAA, as the primary growth-inhibiting substance involved in the initiation and maintenance of deep physiological dormancy. ABA has been isolated from a variety

of deeply dormant seeds including peach (Prunus persica) (Lipe and Crane, 1966), English walnut (Juglans regia) (Martin et al., 1969), apple (Malus spp.) (Rudnicki, 1969), plum (Prunus spp.) (Lin and Boe, 1972), and European hazel (Corylus avellana) (Williams et al., 1973). Although the growth-inhibiting effect of this substance on the seed and embryo has not been proven conclusively, it is believed to interfere in protein synthesis (Salisbury and Ross, 1978).

The gibberellins (GA) comprise the most common class of growth promoting hormones in seed. Working in opposition to ABA, they are thought to promote the germination of deeply dormant seeds by stimulating the secretion of hydrolytic enzymes which then mobilize stored food reserves in the endosperm. GA has also been shown to promote cell elongation, particularly in the radicle (Salisbury and Ross, 1978; Hartmann and Kester, 1983; Bewley and Black, 1985).

Exposing deeply dormant seeds to cold temperature effectively overcomes dormancy by improving embryo aeration and by altering the concentration of growth-inhibiting and growth-promoting hormones. Recent studies indicate that the effect of cold stratification on these hormones is complex and varies from one species to the next. In some seeds such as plum, cold stratification is believed to promote germination by both reducing the concentration of ABA, and at the same time increasing the level of GA (Lin and Boe, 1972). In other species like European ash (Fraxinus excelsior), ABA is not removed by chilling but its inhibitory effect is offset entirely by an increase in the growth-promoter GA (Villiers and Wareing, 1965).

In still other species such as peach, ABA levels decrease gradually in the cold. Though GA synthesis is presumably activated by low temperature, it does not reach detectable levels until the seeds are exposed to warmth (Lipe and Crane, 1966). This provides an effective mechanism to prevent premature germination. ABA present in the seed ensures that the seed is initially dormant. Exposure to cold winter temperatures slowly overcomes the effect of the inhibitor and at the same time activates growth-promoting GA. With the onset of warm spring temperatures, GA concentrations rise to such levels, that dormancy is broken and germination can then take place (Bidwell, 1979).

Combined Dormancy

The types of dormancy described previously are by no means mutually exclusive and more than one mechanism for the imposition of dormancy may be possessed by fruits. Often exogenous and endogenous dormancy factors act in a combined way to delay the germination of the embryo. This has given rise to the term "combined dormancy" (Nikolaeva, 1969; Villiers, 1972).

Although combined dormancy can result from a wide variety of exogenous and endogenous mechanisms, in temperate zones it is most commonly equated with fruits having a physical barrier to germination plus a physiologically dormant embryo. After fruit abscision, various agents in the environment soften the external covers and allow water and oxygen to reach the embryo. The physiological

component of dormancy is then relieved by low temperature as the fruit overwinters on the ground.

To bring about germination in the nursery, plant propagators mimic conditions in the environment. Warm stratification in a moist medium softens the external fruit layers and promotes proper aeration and hydration of the embryo. Outdoor cold stratification during the winter then serves to bring about a favorable balance of hormonal growth-promoters and allows germination to occur after the fruits are planted in the spring. This process may be artificially hastened by employing mechanical or chemical scarifiers to erode the external coverings. Cold storage in a refrigerator may also speed the presowing treatment because the embryo is exposed to constant and controlled low temperature rather than widely fluctuating temperatures that occur outdoors (Hartmann and Kester, 1983).

Advances in _in vitro_ technology have made possible a procedure known as embryo culture. Embryo culture involves excising the embryo from the fruit and seed and germinating it on a nutrient solution under aseptic conditions. Plants currently being commercially propagated by this method include _Iris_ spp., _Maranta_ spp., _Prunus_ spp., _Rosa_ spp., and _Olea_ spp. (Lammerts, 1942; Hartmann and Kester, 1983).

Embryo culture has been particularly useful in inducing prompt germination of embryos that would normally remain dormant for long periods by removing the physical, mechanical, and chemical restraints of the fruit coverings. Certain forms of morphological and physiological endogenous dormancy are also overcome by the procedure mainly

by removing perturbing chemicals located in the endosperm or by ameliorating the conditions of the embryo aeration.

The in vitro culture and germination of immature embryos has been attempted by several investigators (Hesse and Kester, 1955; Norstog, 1977). Seedling production from grossly underdeveloped embryos has been largely unsuccessful due to incomplete embryogenesis and because such embryos appear unable to adequately mobilize and utilize reserve material from the endosperm and cotyledons (Hesse and Kester, 1955). Once the embryos reach 1/3 to 1/2 mature size, however, viability percentages increase significantly. This is particularly true if the embryos are provided with high sucrose concentration (8 to 18 percent) and mineral solutions in the culture medium (Hartmann and Kester, 1983).

Although the use of more developmentally advanced embryos promotes a greater degree of success in vitro, this is not true with embryos under the control of deep physiological dormancy. While fully developed embryos of this type are usually capable of growth (and therefore cannot be called truly dormant), their growth is delayed and abnormal, indicating an imbalance of hormonal growth promoters and inhibitors. Because of the persistent effects of physiological nanism, successful embryo culture can be attained only after the seeds have been exposed to an initial cold stratification treatment (Crocker and Barton, 1953; Nikolaeva, 1969).

Propagation of Silverbell

Seed Propagation and the Problem of Dormancy

Sound and uninjured seeds of approximately 2/3 of North American tree species fail to germinate even when placed under conditions adequate for their germination, indicating a state of organic dormancy (Krugman et al., 1974). Such is the case with both the mountain and smaller Carolina silverbell.

The earliest references to delayed germination in silverbell were purely speculative. Conclusions were drawn not from controlled experimentation, but from what was casually observed in nature or in the nursery. Jack (1894) attributed dormancy to a hard, bony seed covering and categorized Halesia as among those species that "may commonly be expected to grow in the second year rather than the first." In 1899, Ramaley (in Chester, 1966) noted that "seeds of this plant, the snow-drop tree, planted in the spring of the year following their ripening, lie dormant an entire year before germinating."

Giersbach and Barton (1932) also recognized the physical barrier of the bony encarp as a major obstruction, but were the first to stress the importance of varying temperature and adequate moisture in bringing about successful germination. Elaborate studies involving cold and warm-cold stratification were conducted in an attempt to optimize key conditions in the environment in order to hasten seedling emergence. Seedlots, however, were found to vary

widely in their stratification requirements, thereby yielding erratic and often contradictory results.

While cold stratification alone was successful in some seedlots, the most consistent germination resulted from a 90 day warm stratification at 18° C followed by a similar interval outdoors in a cold frame. Germination was found to range from 33 to 44 percent, 3 months after sowing.

Recent investigations have been attempted to either replicate or improve upon the methodology of Giersbach and Barton (1932). Wide variations in stratification requirements among different seedlots have, however, continued to confound seed scientists and commercial growers.

Bonner and Mignery (1974) moist stratified silverbell fruits at 13 to 30° C for 60 to 120 days, followed by 60 to 90 days at 1 to 5° C. Greenhouse germination tests were conducted in flats of sand or sand-peat mixtures with 30° C day and 20° C night temperatures. Based on seven samples, germination averaged 53 percent over a 90 day period.

Dirr (1983) employing the same stratification techniques as Bonner and Mignery (1974) reported that germinating silverbell seed "was no easy task." Although the sample size was unknown, this test yielded no germination whatsoever until an additional 60 days of cold treatment was provided.

While all previous tests presumably used the fruits of the smaller piedmont-coastal plain form of silverbell, Chester (1966)

worked with fruits collected from the larger mountain silverbell. Trials were conducted following the stratification recommendations of Giersbach and Barton (1932). Eighty days after sowing, the 200 stratified fruits yielded only 10 percent germinative capacity.

Current nursery practices do not improve upon the seed propagation techniques cited above. Methods used by growers vary depending upon available greenhouse facilities, workforce, and most importantly, their past experience working with the species.

The simplest procedure is to plant the freshly collected fruits at shallow depth in mulched, outdoor beds in the fall without pretreatment. This allows warm and cold stratification to occur under "natural" conditions. The inherent ease of direct planting makes this method attractive. Variable environmental conditions, coupled with different stratification requirements among seedlots though, result in extremely variable germination rates which may be prolonged for up to 3 years especially if the fruits are allowed to dry (Bailey, 1935; Wright, 1973).

Another reported method entails stratifying fruits collected in September in polyethylene bags containing moist sand or peat at normal greenhouse temperatures, or burying them in flats containing soil. In January, the fruits are transferred to outdoor board-covered cold frames for the cold part of the conditioning. In the spring, the stratified fruits are sown outdoors (Wyman, 1953; Bonner and Mignery, 1974).

Beginning with the earliest attempts to propagate silverbell from seed, most investigators have identified the lignified endocarp

as the primary impediment responsible for the extremely delayed and erratic germination of the species. Confusion arises, however, because the propagation techniques recommended are not the most helpful for overcoming this form of dormancy. Scarification, which quickly erodes hard seed coverings, is much more efficient. Warm and cold moist stratification, on the other hand, is most commonly reserved for those fruits which possess a combined form of dormancy involving both hardseededness and an accompanying chemical, morphological, or physiological barrier.

Since researchers have failed to provide any specific information, the workings, or even the existence of an additional dormancy mechanism must remain a matter of speculation. Combined mechanisms of this kind though could easily contribute to the poor germinative performance so frequently observed.

Vegetative Propagation

Past research has shown that silverbell can be successfully propagated using various vegetative methods including softwood and semihardwood cuttings, root cuttings, and layering (Vines, 1960; Bonner and Mignery, 1974; Hora, 1981).

Laurie (1930) collected semihardwood cuttings from Halesia during the summer and attempted to root these in three different types of media. No exogenous hormones were applied. Ninety percent rooting was achieved using sphagnum peat, while 70 percent was obtained with pure sand. Roots failed to develop from cuttings placed in a half-sand, half-peat mixture.

Doran (1957) employed semihardwood cuttings from Carolina silverbell and obtained 40 percent rooting in sandy soil. When the cuttings were treated for 20 hours with 25 parts per million (ppm) indolebutyric acid (IBA), rooting increased to 80 percent after a 6 week period. Successful rooting was also accomplished using a sand-peat mixture.

Seventy percent rooting of mountain silverbell was reported by Halward (1965) when the semihardwood cuttings were treated with "Seradix No. 1." The rooting media was four parts sand and one part peat supplied with intermittent mist and 21° C bottom heat.

Burd (1976) tested the reaction of greenwood and semihardwood cuttings of Carolina silverbell to various concentrations and combinations of IBA and napthaleneacetic acid (NAA). Best rooting responses were elicited by IBA alone at 2500 ppm. Rooting ranged from 70 to 100 percent after 7 weeks in a peat-perlite mixture under intermittent mist.

Although silverbell cuttings readily produce roots and are easily transplanted, there are indications that they are difficult to harden-off. Several commercial growers in the Southeast have observed that many of the rooted cuttings, when moved to outdoor cold frames, fail to cease growth even with the onset of freezing temperatures. As a result, stems often split, badly degrading or killing the stock. Crown[4] reported the loss of 30 percent of his

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[4]Personal communication with Richard Crown, Cedar Lane Farms, Madison, Georgia, March, 1983.

rooted Carolina silverbells when they failed to enter deep bud rest. Shadow[5] recommended the use of microfoam to insulate the plants during the winter months but found this to be costly. Because of this, he now relies on seed propagation.

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[5]Personal communication with Donald Shadow, Shadow Nursery, Winchester, Tennessee, June, 1982.

CHAPTER III

OBJECTIVES

Delayed and erratic germination in Carolina and mountain silverbell is a potentially complicated situation perhaps involving both endogenous and exogenous forms of dormancy. Germination problems such as these can be a nuisance to nursery operators, since its occurrence in the nursery bed results in irregular stocking of different ages and sizes. Furthermore, bed space may be tied up for several years greatly increasing production costs. Delayed germination can also hamper direct-seeding operations by increasing the time sown seed is exposed to predators and adverse weather conditions (Bonner et al., 1974).

It is clear that delayed germination in silverbell, whatever the underlying cause, is largely responsible for the limited use of this potentially valuable species by both the ornamental and forest products industries. Thus discovering a means by which to overcome this problem is of practical and economic importance.

The primary objective of the current study was, therefore to investigate previously unexplored means of overcoming delayed germination in mountain silverbell in order to obtain merchantable seedlings from seed within one year. To accomplish this, attempts were made first to negate the most commonly cited source of delayed germination, the lignified endocarp, by employing chemical, water,

and mechanical scarification. The feasibility of *in vitro* propagation using embryo culture was also explored.

CHAPTER IV

EXPERIMENTAL METHODS FOR STIMULATING EARLY GERMINATION OF MOUNTAIN SILVERBELL

Although dormancy in silverbell may be related to more than one factor, the most apparent cause is the hardened endocarp layer. This layer may delay germination by physically preventing the access of water and oxygen to the embryo or by mechanically restricting embryo emergence once seed hydration has occurred. Propagators have found that various scarification treatments which quickly break down these bony seed coverings are often very effective in bringing about germination at a much faster rate than would be otherwise possible under natural conditions. Such conventional techniques, however, have never been used in attempt to hasten germination of silverbell.

The following chronology describes a series of experiments designed to promote the rapid germination of mountain silverbell by overcoming the problem of hardseededness. The rationale was to begin with the simplest and least expensive scarification methods, undertaking more complicated procedures only as was necessary to meet the study objective.

All fruits used in the subsequent tests were gathered from the ground at random so the "parent" tree was unknown. Three separate collections were made during the month of January, 1982, and consisted

of about 2000 fruits each. Pertinent information concerning the location of the collection sites is provided in Figure IV-1.

The fruits of the respective seedlots were kept segregated in perforated polyethylene bags and stored in the refrigerator at 2° C until needed.

Chemical Scarification

Sulfuric Acid Scarification

Commercial grade sulfuric acid (95 percent pure, specific gravity 1.84) is by far the most commonly recommended chemical agent used to destroy lignified seed coverings. To evaluate its effectiveness in mountain silverbell, 50 fruits from each of the three seedlots were first allowed to come to laboratory temperature in preparation for their immersion in the acid. Once ambient temperature was reached, the fruits from the three collections were dewinged by hand and placed in separate glass containers.

Following the general recommendations of Bonner et al. (1974), enough sulfuric acid was added to assure that the fruits were completely covered. Each mixture was slowly stirred every 15 minutes to promote uniform etching of the endocarp and to loosen the accumulation of dark residues which had formed on the surface of the endocarp due to the action of the acid.

At 30 minute intervals (30, 60, 90, 120, and 150 minutes), 10 fruits from each seedlot were removed from the acid and rinsed in constantly circulating water in a pipette washer. Because of

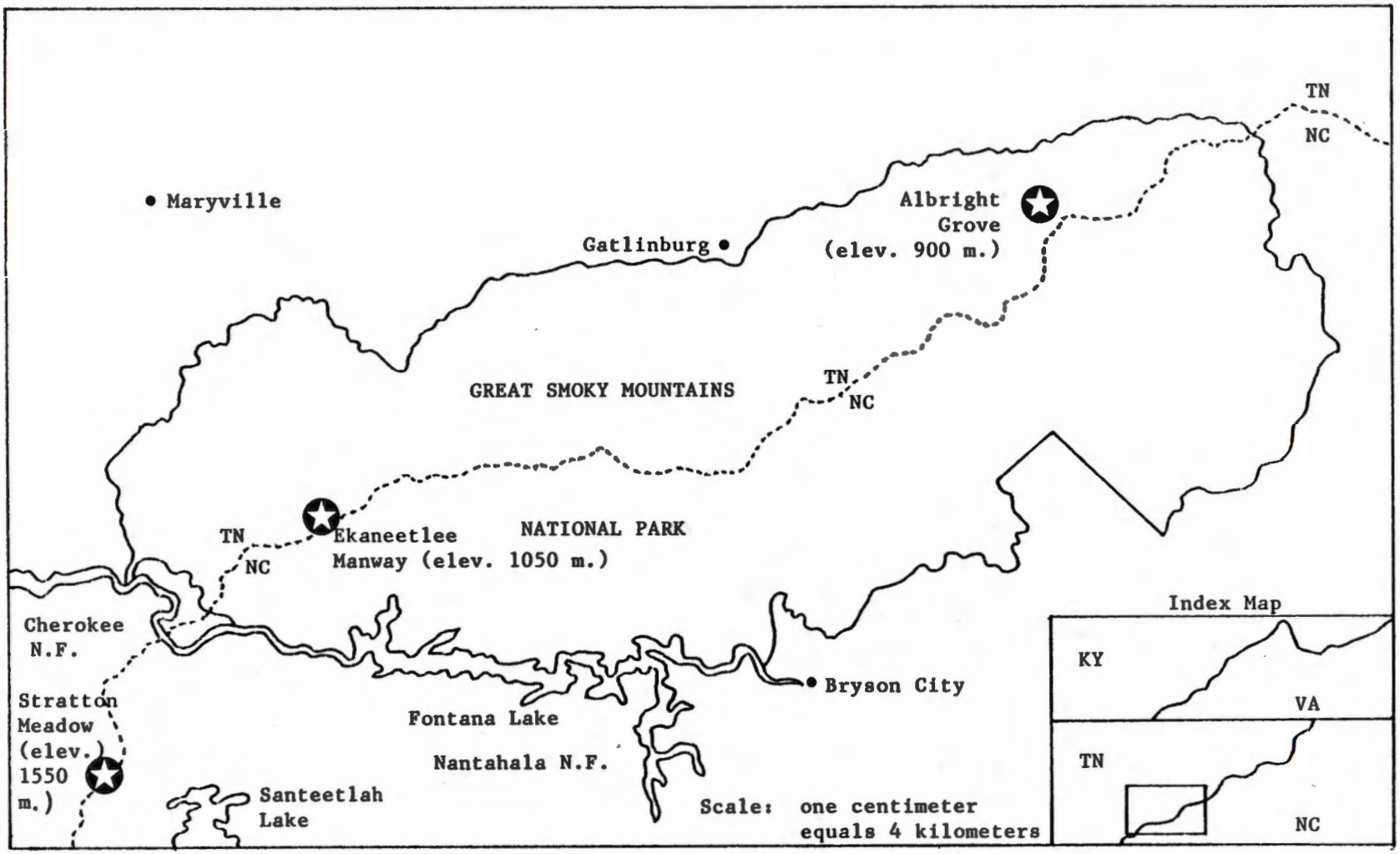

Figure IV-1. Mountain Silverbell Fruit Collection Areas: Great Smoky Mountains National Park and Vicinity.

the strongly plicate and pitted nature of the outer endocarp, the treated fruits were allowed to rinse for a 72 hour period to assure complete removal of the acid.

After rinsing, the fruits were taken from the water bath and planted in flats of "Redi-Earth" (sphagnum peat-vermiculite mixture) at a depth of approximately 1 cm. The flats were then placed in the greenhouse under normal greenhouse temperatures and natural light. The growing medium was kept moist by hand watering. Fruits were monitored for 3 months at the time of watering for indications of sprouting. Periodically, fruits were removed at random from the medium, checked for signs of endocarp rupturing and radicle emergence, then replanted.

Although prolonged immersion of silverbell fruits in concentrated sulfuric acid appeared effective in eroding the external endocarp layer, no germination resulted from this procedure. Furthermore, no indications of endocarp swelling or radicle emergence were in evidence even after 3 months in the moist growth medium.

Hydrogen Peroxide Scarification

Chemical scarification of the endocarp using 30 percent hydrogen peroxide was tested concurrently with sulfuric acid to determine if a strong base was effective in reducing the barriers of hardseededness. The same procedures employed in the previous section were used here.

Again, the external endocarp tissue became strongly pitted and discolored from prolonged exposure to the caustic scarifier.

Slight softening of the outer endocarp was observed after 2 weeks in the moist planting medium, but no germination, swelling, or endocarp splitting had occurred after 3 months in the greenhouse.

The inability of conventional chemical scarifiers to initiate germination was enigmatic since the endocarp appeared to be severely eroded, particularly after prolonged immersion. The lack of endocarp swelling after several months in the moist planting medium, however, seemed to indicate that these treatments failed to completely overcome the physical barriers of hardseededness and that water and gases were not reaching the embryos. It was possible that only the outer layers of the endocarp were destroyed or softened, and the hardened inner layers remained unaffected.

In contrast, infiltration of sulfuric acid and hydrogen peroxide too deeply into the seed cavity could have prevented germination by causing the premature death of the embryos. Though access of the chemicals probably did not occur through the endocarp itself, entry to the internal region of the fruit may have been gained as the chemicals moved inward by capillary action along the fruit stalk.

To test these possibilities, the sulfuric acid and hydrogen peroxide scarification procedures were repeated in a similar fashion to monitor their effectiveness in penetrating the restrictive endocarp tissue. The condition of the embryo and endosperm layers were also observed after each treatment.

Sulfuric Acid/Hydrogen Peroxide Penetration of the Endocarp Wall

The dewinged fruits collected in January, 1982 were again treated for varying time intervals. Unlike the initial chemical scarification tests, the fruits were not agitated during the scarification so that the amount of burned tissue dislodging from the endocarp would be minimized. In this way, a more accurate appraisal of infiltration could be made.

One hundred and twenty dewinged fruits from each of the three seedlots were removed from storage in preparation for their immersion in the chemical scarifiers. Half of the fruits (60 per seedlot) were then placed in concentrated sulfuric acid and the other half in 30 percent hydrogen peroxide. At 30 minute intervals (30, 60, 90, 120, and 150 minutes), 10 fruits were removed from the sulfuric acid and 10 were removed from the hydrogen peroxide. The final group of fruits was allowed to remain an extra 90 minutes in the chemical baths for a total scarification time of 240 minutes.

Following the chemical scarification treatment, each fruit was rinsed with water to remove the chemicals while at the same time taking care not to disturb the endocarp remnants. The fruits were then sectioned longitudinally using a high speed electric grinding wheel (1/4 horse power; 3450 rpm; aluminum oxide wheel; grit 36; grade N). The average depth of penetration of the chemicals into each individual fruit was calculated by measuring the thickness of the chemically burned tissue in four places (two proximal and two distal) along the endocarp wall. These measurements were then

used to calculate the average depth of chemical penetration for each seedlot after each exposure time (Tables IV-1 and IV-2). Possible damage to the endosperm tissue and embryo proper due to the chemical scarifiers was also evaluated by observing changes in their color and texture.

Although slow in their action, concentrated sulfuric acid and 30 percent hydrogen peroxide produced approximately equal penetration rates. Generally, longer exposure resulted in deeper penetration. In several instances, however, the reverse was true. This occurred because of the wide variation in endocarp density among individual fruits within the same seedlot. In some of the more dense fruits, for example, penetration of concentrated sulfuric acid proceeded as slowly as 1 mm. in 4 hours, whereas in more porous fruits, the same depth of penetration was achieved in only 30 minutes.

Differences in endocarp density were detected not only in individual fruits within the same seedlot but also among the seedlots themselves. Both concentrated sulfuric acid and 30 percent hydrogen peroxide entered the denser endocarps of the Stratton Meadow collection more slowly than in either the Albright Grove or Ekaneetlee Manway collections.

Wide variation in the positioning of seeds within the fruit was also observed in mountain silverbell. Some seeds were located within 1 mm. of the outer surface of the fruit, while others, deeply embedded within the endocarp tissue, were found adjacent to the medial axis.

Table IV-1. Depth of Sulfuric Acid Penetration into the Endocarp of Cold Stratified Mountain Silverbell Fruits.

Exposure Time (Minutes)	Average Depth of Penetration Per 10 Fruits (mm.)[a] Seed Source		
	Albright Grove	Stratton Meadow	Ekaneetlee Manway
30	0.48	0.55	0.48
60	0.65	0.43	0.63
90	0.78	0.75	0.78
120	1.15	0.95	1.13
150	1.10	1.00	1.23
240	1.43	1.33	1.45

[a]Measurements estimated to nearest 0.5 mm., values less than 0.5 mm. tallied as 0.0 mm.

Table IV-2. Depth of 30 Percent Hydrogen Peroxide Penetration into the Endocarp of Cold Stratified Mountain Silverbell Fruits.

Exposure Time (Minutes)	Average Depth of Penetration Per 10 Fruits (mm.) Seed Source		
	Albright Grove	Stratton Meadow	Ekaneetlee Manway
30	0.38	0.28	0.30
60	0.73	0.45	0.45
90	0.53	0.68	0.98
120	0.98	0.93	0.78
150	1.08	0.98	1.13
240	1.28	1.18	1.35

The heterogeneous nature of mountain silverbell fruits helped to explain, at least in part, why the original chemical scarification experiments failed to bring about germination. Seeds located close to the outer surface of porous fruits were often destroyed 30 minutes after immersion. The once firm, starchy endosperm tissues were partially dissolved and the pure white embryos became purple (sulfuric acid) or gray (hydrogen peroxide) and withered. Those seeds positioned deep within the endocarp of very dense fruits, on the other hand, were totally unmolested even after 4 hours of exposure. They remained surrounded by several millimeters of dry, hardened endocarp, which, in all likelihood, continued to be a barrier to embryo hydration and emergence. No entry of the chemicals into the inner seed cavity along the fruit stalk was noted.

The combination of variable endocarp density and variable positioning of seeds within the endocarp makes the use of chemical scarifiers highly impractical for overcoming potential physical or mechanical barriers to germination. These same factors make it extremely difficult to predetermine the proper duration of exposure necessary to be effective. It is also possible that even though the treated fruits used in the original experiments received a 72 hour soak in water after their scarification treatment, enough sulfuric acid residue remained in the endocarp tissue to constitute an artificial chemical barrier to germination.

Water Scarification

Cold Water Scarification

Soaking fruits in water at room temperature for several days has successfully hastened germination in some species, particularly conifers (Rudolf, 1950). Though such treatments are not usually applied where extreme physical and mechanical barriers to germination exist, prolonged soaking for several weeks may induce germination by softening the bony endocarp, by completing the water imbibition requirement of the embryo, or perhaps by leaching germination inhibitors from the fruit and seed.

To test the possibility, an experiment was begun involving 40 fruits from each of the three seedlots. The fruits were subdivided into individual lots of 10 fruits each and placed in cotton bags to keep them segregated. These were then put in a pipette washer and continually flushed with tap water. Ten fruits from each seedlot were removed from the water bath after 1, 2, 4, and 8 weeks of soaking and planted in the greenhouse. Germination was then monitored over a 3 month period.

After 2 weeks of constant soaking, the fruits appeared essentially unaltered. By 4 weeks, however, the outermost endocarp became slightly swollen and pliant. Inspection of the fruits after 8 weeks showed continued softening of the external endocarp tissue along with a change in coloration from the original brown to a light beige.

Despite these changes, no germination occurred in any of the fruits regardless of the duration of the cold water treatments.

Cold Water Penetration of the Endocarp Wall

In an attempt to understand the reasons for the failure of water scarification to bring about germination and in order to test the effectiveness of water in reducing the physical and mechanical barriers to germination, 40 cold stratified fruits from each seedlot were again put into constantly circulating water in a pipette washer. The fruits from each seedlot (kept segregated in cotton bags) were then removed from the water bath after 1, 2, 4, and 8 weeks and examined for endocarp swelling. After being dissected longitudinally, water penetration was ascertained by measuring the darkened, wetted endocarp tissue. As in the chemical scarification experiment described previously, the average depth of infiltration was calculated on an individual fruit basis and then these measurements were used to derive average penetration depths per seedlot at each time period (Table IV-3). The endosperm tissues and embryos were also examined for hydration and overt signs of damage.

Sectioning the fruits revealed that infiltration of water into the endocarp proceeded at a very slow rate throughout the course of the experiment. The variation in the porosity of individual fruits that was so apparent during the previous chemical scarification treatments was not clearly expressed when water was used. Differences in the porosity among fruits of the same seedlot, however, remained noticeable, with water penetrating into the Stratton Meadow collection more slowly.

Observation of the wetted tissues showed that water had reached several of the outermost seeds (at the distal end of the fruits),

Table IV-3. Depth of Water Penetration into the Endocarp of Cold Stratified Mountain Silverbell Fruits.

Exposure Time (Weeks)	Average Depth of Penetration Per 10 Fruits (mm.)[a] Seed Source		
	Albright Grove	Stratton Meadow	Ekaneetlee Manway
1	0.23	0.20	0.20
2	0.25	0.20	0.30
4	0.60	0.48	0.65
8	0.78	0.68	0.75

[a]Estimated to the nearest 0.5 mm., values less than 0.5 mm. tallied as 0.0.

but this only occurred after 8 weeks of continued immersion. No seed swelling, however, was apparent. Again, because of the variable positioning of the seeds within the endocarp, most seeds remained well out of reach of the water scarifier. Those few seeds which were in contact with the water had begun to die, as evidenced by their grey coloration and fetid odor. The cause of death, although not clear, most likely resulted from anaerobisis, waterborne pathogens, or from endogenous seed contaminants which were activated or exacerbated in the presence of water.

The results of these experiments clearly showed that water scarification was unsuccessful in bringing about germination in silverbell since the vast majority of seeds positioned deep inside the fruit were unaffected and remained tightly bound within the dry, hardened endocarp even after 8 weeks of water exposure. Although the extreme outer tissues of the endocarp became somewhat swollen and pliant, and did permit water access to the most peripherally located seeds, the death of those seeds prior to germination pointed to the necessity of examining other alternative methods of breaking exogenous barriers to germination.

Hot Water/Boiling Water Scarification

Unlike cold water scarification, soaking fruits in hot water has been found to rapidly increase the permeability of hardseeded species such as legumes, by profoundly altering the physical integrity of the seed coat (Hartmann and Kester, 1983). After being planted,

the fissured seed coat allows the unencumbered movement of water to the inner living tissues of the seed. Once hydration occurs, enough pressure is exerted by the swelling of the embryo and endosperm to cause the seed coat to break open along naturally occurring suture lines.

Hot water scarification has been found to be extremely difficult to standardize and often yields erratic results. Each species (or even seedlot) requires a different exposure time to achieve success. Treatment for too long a time causes the embryos to succumb because of high temperature, while undertreatment does not adequately erode the physically inhibiting layers (Bonner et al., 1974).

Although the fruits of mountain silverbell are very different from the legumes in that they are sutureless, the fracturing of the hardened fruit layers produced by high temperature exposure was a desirable aim. To investigate the effectiveness of this procedure, 40 fruits from each seedlot were segregated in cotton bags and immersed in a 50° C hot water bath. After each time period (30, 60, 90, and 240 minutes), 10 fruits from each seedlot were removed, shallowly planted in the greenhouse, and checked over a 3 month period for germination.

Bonner et al. (1974) reported that placing fruits in boiling water is sometimes beneficial in overcoming delayed germination due to hardseededness. In accordance with this observation, 10 fruits from each of the seedlots were placed in water preheated to 100° C and allowed to remain in the gradually cooling water for

a period of 24 hours. These were also planted in the greenhouse and observed for indications of germination.

Both exposure to hot water and boiling water failed to markedly change the texture or outward appearance of the bony endocarp tissue. These procedures were also unsuccessful in bringing about germination even after 3 months in the planting medium.

Hot Water/Boiling Water Penetration of the Endocarp Wall

After a repeat of the experimental procedures, cutting tests showed that soaking mountain silverbell fruits in 50° C water was ineffective in overcoming the barriers of hardseededness regardless of the duration of the treatment (Table IV-4)

Although more rapid in its action than cold water, infiltration of hot water proceeded at such a slow rate that all but two of the outermost seeds were well out of reach of the water even after 4 hours of exposure. Those seeds which did come in contact with the hot water did not appear outwardly affected except that the endosperm had become softened due to hydration. Their viability, however, must be questioned since exposure to such high temperatures normally causes the breakdown of proteins in living tissues.

Inspection of the treated fruits under the microscope showed that hot water exposure produced none of the desired fissuring of the endocarp. Movement of water into the peripheral cell layers of the endocarp, therefore, must have resulted purely from capillary action just as it had when cold water treatments were employed.

Table IV-4. Depth of 50° C Hot Water Penetration into the Endocarp of Cold Stratified Mountain Silverbell Fruits.

Exposure Time (Minutes)	Average Depth of Penetration Per 10 Fruits (mm.)[a] Seed Source		
	Albright Grove	Stratton Meadow	Ekaneetlee Manway
30	0.00	0.00	0.00
60	0.00	0.00	0.00
90	0.25	0.20	0.25
240	0.33	0.28	0.30

[a]Estimated to the nearest 0.5 mm.; measurement less than 0.5 tallied as 0.0.

Boiling water scarification yielded penetration rates similar to those recorded for fruits immersed in 50° C water for 240 minutes (Albright Grove 0.35 mm., Stratton Meadow 0.30 mm., and Ekaneetlee Manway 0.30 mm.). As before, only a very few seeds located near the outermost layer of endocarp came in contact with the water and again it must be summarized that these were killed by high temperature though no overt sign of damage was observed. Finally, no physical alteration of the endocarp was noted even when magnified 100 times.

It is clear that hot and boiling water scarification was an ineffective and impractical method for bringing about the early germination of mountain silverbell seeds. The only advantage gained from these procedures was that water infiltration into the endocarp took place at a slightly faster rate than when cold water was used. This, though, was offset by the fact that the outer, hydrated seeds were probably killed by high temperature exposure. Also significant was the inability of the high temperature treatments to produce any noticeable fissuring of the endocarp layers. Without this, the endocarp remained a formidable barrier to the inward movement of water to the majority of seeds located deep within the fruit. Also, without such alterations the expansion of germinating embryos, if it were to occur, would probably be mechanically inhibited by the sutureless endocarp.

Mechanical Scarification

Another procedure which is often successful in overcoming the effects of exogenous dormancy is mechanical scarification (Isely,

1967; Bonner et al., 1974; Salisbury and Ross, 1978; Hartmann and Kester, 1983). Like chemical and water scarification, its aim is to erode or destroy the impervious layers so that water and gases can reach internal living tissues and permit germination to occur. Mechanical scarification though, appears more suited to use with the drupaceous fruits of mountain silverbell than any of the other conventional treatments since the propagator can predetermine the amount of scarification required for each individual fruit. This is particularly noteworthy considering that each fruit can vary considerably not only in seed number and endocarp density but also in seed positioning.

A two part experiment was undertaken to test the efficacy of mechanical scarification treatments on cold stratified fruits of mountain silverbell. Part one involved carefully exposing the seeds by wearing away a portion of the endocarp using the high speed electric grinding wheel. Twenty fruits from each seedlot were ground parallel to the longitudinal axis exposing a single seed.[6] The endosperm tissue was left intact to provide nutrition and protection for the embryos. Half of the exposed seeds received a 10 second dip in "Benlate" fungicide which had been mixed at the rate of 3/4 tablespoon per gallon of distilled water. The remaining half were not treated with "Benlate." Both were then planted at a depth of approximately 1 cm. in sterilized "Redi-Earth" and placed in the

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[6]Over the course of this study 1,208 fruits were dissected and found to contain an average of 1.76 seeds each.

greenhouse where they were hand watered and kept under natural light. During watering, the fruits were randomly uncovered and examined for indications of germination.

Part two of this experiment was designed to determine whether seeds, completely excised from the fruit, would germinate when planted. Twenty seeds from each seedlot were carefully freed of their bony endocarps using the grinding wheel. As before, half of the seeds received a 10 second dip in "Benlate" fungicide while the other half was untreated. Again, these were shallowly planted in sterilized "Redi-Earth," placed in the greenhouse, and monitored for signs of germination.

Moderate swelling of both the exposed and completely excised seeds during the first several days in the moist planting medium indicated that the endosperm tissue did not constitute a physical barrier to the ingress of water to the embryo. Hydration of viable seeds normally leads to enzyme activation and embryo germination provided that the seeds are exposed to appropriate environmental conditions and that there are no intervening chemical, physiological, or morphological restraints. An accurate appraisal of the germinative capacity of the seeds could not be made, however, due to heavy contamination by fungi and bacteria. Regardless of whether the seeds were completely excised from the external coverings or simply exposed, those not treated with fungicide became noticeably contaminated 7 days after planting. By day 20 all untreated seeds had apparently died as indicated by their watery texture, discoloration, and foul

odor. Even the seeds treated with "Benlate" succumbed within 20 days in the greenhouse although the onset of contamination, in this case, did not occur until day 9.

From the experimental results presented thus far, it can be seen that all attempts to induce the rapid germination of mountain silverbell seeds met with total failure when conventional scarification treatments were employed. The primary reasons for these failures seemed to be directly linked to either the inability of the various scarifiers to adequately break down the hardened endocarp tissues, or in some cases, the inability of the treatments to effectively overcome the persistent problem of endogenous fungal and bacterial contamination. The question then arose, what other procedures could be used to circumvent both of these problems so that germination would take place?

In Vitro Culture of Excised Embryos

Embryo culture has been found to be particularly helpful in bringing about the prompt germination of embryos that would otherwise be dormant due to the physical and mechanical restraints imposed by the hardened fruit coverings. It also provides the propagator with the unique opportunity to rid the embryo of contamination through the application of chemical sterilizers or antibiotics. Olive (Olea spp.), for example, a species which bears indehiscent drupaceous fruits with a bony endocarp similar to those found in silverbell, are easily propagated when embryo culture is used. Once surface

sterilized, the "naked" embryos are transferred to a culture medium from which they receive a readily available supply of water, essential minerals, and a source of carbohydrate. The latter two must be provided since the embryo's normal source of these materials, the endosperm, is also removed prior to culturing (Crocker and Barton, 1953; Norstog, 1977).

Unlike conventional scarification techniques, embryo culture is costly, requiring specialized equipment and skilled labor. However, because of its proven ability to eliminate the problems of hardseededness and endogenous contamination, the use of embryo culture in attempting to meet the primary objective of the study was justified.

Developing a methodology for the effective in vitro culture of mountain silverbell embryos proved to be a difficult and time consuming task. Numerous test trials were conducted in order to refine the manual skills necessary for the removal of the seed from the fruit and to perfect the delicate process of excising the small embryos from the seed. Of particular difficulty was determining which chemical sterilizing agents to use and in what concentrations. Too harsh a treatment caused the death of the fragile embryos, but incomplete sterilization proved to be equally harmful. The rapid growth of pathogens in the nutrient rich culture medium quickly overcame the embryos and prevented their germination.

In time, a workable sterilization and culture regimen was achieved. The following is an account of the first successful trial

and includes a general description of the procedures used as well as an account of how the embryos reacted to those procedures.

Ten seeds from each of the three seedlots were carefully extracted from the bony endocarp using the high speed electric grinding wheel. Within the aseptic environment of a laminar flow hood, the extracted seeds were surface sterilized by soaking them for 3 minutes in a 5 percent chlorox solution. Next, in order to remove the chlorox residues, the seeds were dipped in sterile double distilled water, immersed for 15 seconds in a 0.01 normal solution of hydrochloric acid, and then rinsed a second time in sterile water. Finally, the seeds were allowed to hydrate in a third sterile, double distilled water bath at 4° C for 48 hours to soften the moderately cornified endosperm tissue in preparation for embryo excision.

The embryos were excised from the sterile, hydrated endosperm, again within the laminar flow hood utilizing scalpel and forceps. A 10 power dissecting microscope provided the needed magnification. Once removed, the naked embryos were subjected to the same sterilization procedure as the seeds, but here the chlorox solution was diluted to 1 percent to minimize the chemical "burning" of the delicate tissues.

The surface sterilized embryos were then aseptically transferred to the test tubes (25 x 125 mm.) containing agar-based Knop's medium (Appendix B) and placed in a germinator at 25° C with a 16 hour light (2 E/m sec.), 8 hour dark photoperiod.[7]

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[7]A detailed description of the seed and embryo sterilization and culturing procedures employed in all *in vitro* experiments is located in Appendix B.

Earliest indications of germination occurred in two embryos of the Ekaneetlee Manway collection after only 2 days *in vitro*. By day 7, germination had occurred in 100 percent of the cultured embryos (Figure IV-2). Initiation of the germination process was marked first by divergence of the cotyledons and followed, normally within 24 hours, by greening of the apical meristem. Further indications of the awakening of metabolic activity took place shortly thereafter and included the acropetalous greening of the cotyledons, swelling and greening of the hypocotyl, radicle elongation, and finally the appearance of the plumule. Observations made over a 6 week period revealed a rather wide variation in the cotyledon and radicle lengths of individual seedlings regardless of seed source. While this variability may have been due to, among other things, seed quality and genetic factors associated with open pollination, the most likely cause appeared to be the differential reaction to the seed and embryo sterilization procedures. Tip burning of the cotyledons, for example, was readily observable in 40 percent (12 of 30) of the seedlings. Similarly, the chemical degradation of the mucilage layer, rootcap, and meristem may have been related to the production of stunted or malformed radicles in 23 percent (7 of 30) of the seedlings.

Despite these malformations, the overall development of the seedlings above the level of the root collar did not appear to be adversely affected. Although those seedlings with damaged radicles could have originally been limited in their capacity to receive

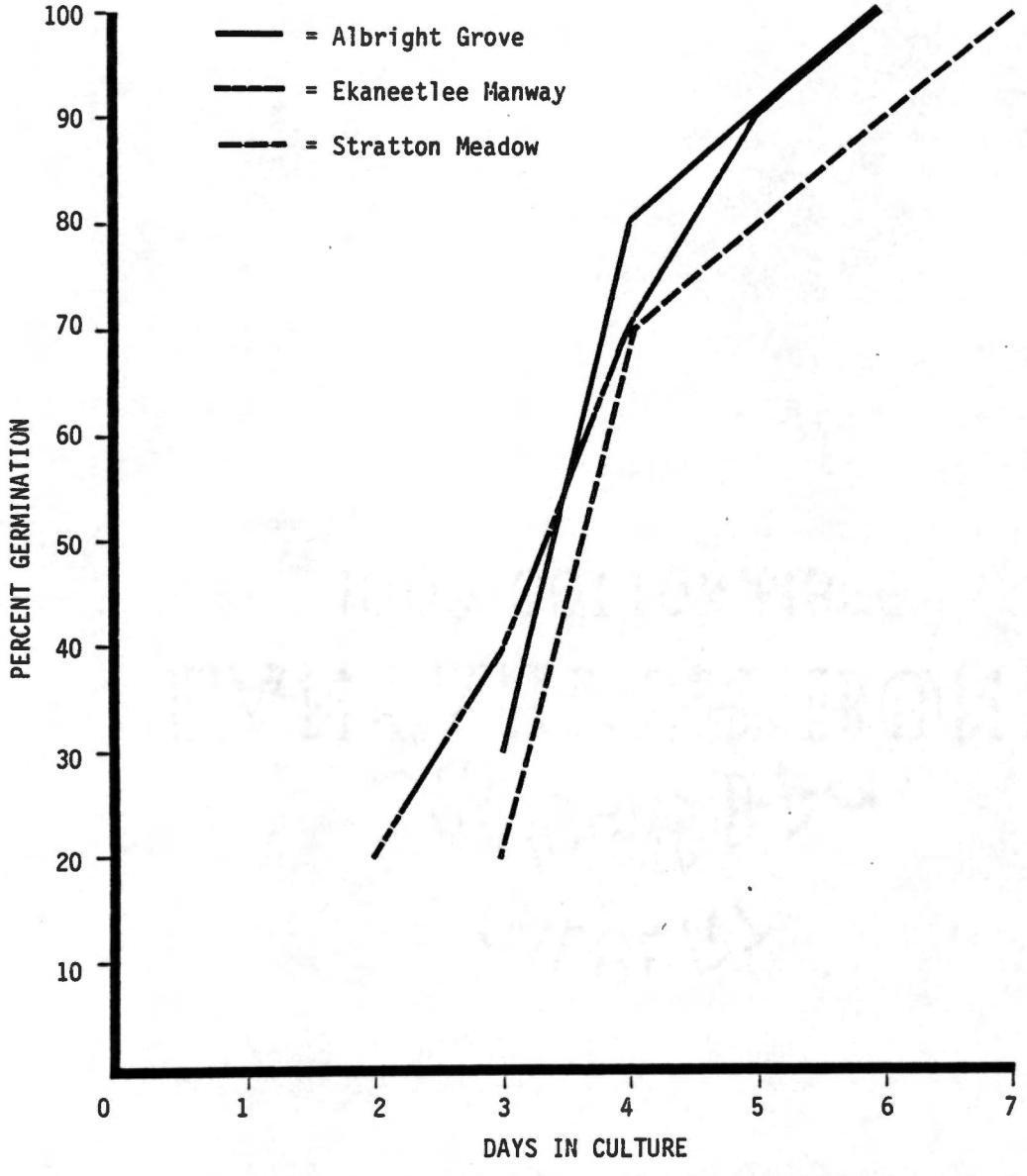

Figure IV-2. Germination Rate of 30 Cold Stratified Mountain Silverbell Embryos Cultured *in vitro*. Each Collection Consisted of 10 Embryos Each.

nutrients from the Knop's medium, this did not appear to be the case after the fourth week in culture. Each responded by producing a network of lateral roots which emanated from the lower portion of the hypocotyl.

Transfer of Cultured Seedlings to Soil

At the end of the sixth week in culture, five seedlings from each seedlot were removed from the Knop's medium using forceps. Upon removal, the root systems were thoroughly rinsed with distilled water to remove the excess agar. These seedlings were then planted in separate clay pots containing standard nursery potting soil and observed closely. Within 60 minutes, 11 of the 15 seedlings had begun to wilt and after 90 minutes all 15 showed signs of severe desiccation.

The desiccation and subsequent death of the seedlings was attributed to their having been germinated in closed test tubes. In this protected environment it is likely that they failed to produce well developed stomatal control or sufficient epidermal waxes.

A second attempt was initiated to determine if the viability of planted seedlings could be maintained by providing them temporarily with a protected atmosphere. Two seedlings from each seedlot were removed from the culture medium and their total length measured. Special care was taken to be sure that half of the sample included seedlings whose radicles appeared abnormal. This was done because their performance compared to "normal" seedlings was also of interest.

Once transplanted into the potting soil, all seedlings were covered with 100 ml. glass beakers. At 2 day intervals the beakers were progressively raised to increase the seedlings' exposure to the outside environment. Each plant responded by producing thicker leaves and more hardened stems. By the tenth day of the experiment the beakers were totally removed and the well acclimated seedlings appeared green with no indications of wilting. They were then placed in a south facing window in the laboratory and monitored for a period of 1 year (Table IV-5). During that time they received four fertilizer applications ("Miracle-Gro," 15-30-15) and were twice transplanted into larger pots.

Based on this small sample, the seedlings appeared to respond favorably to this method of transfer to the soil medium. After an initial lag period of approximately 2 weeks, all seedlings began to increase in height and to produce well formed leaves. By the sixth month the striped bark pattern characteristic of juvenile silverbell seedlings became apparent on the lower portion of the stems. Several small lateral branches were also in evidence at that time. Height growth continued at an increasing rate and at the end of the twelfth month all plants were green and vigorous.

The measurements taken throughout the course of this trial revealed wide variation in height growth among seedlings regardless of seed source. Because seedlings with well developed root systems normally have a superior potential for the uptake of nutrients from the soil, it was hypothesized that those which originally had the

Table IV-5. Stem Height (cm.) of 6 Mountain Silverbell Seedlings Derived from Cultured Embryos and Transplanted into Soil.

Months Since Transference to Soil	Embryo Source					
	Albright (1)[a]	Grove (2)	Stratton (3)[a]	Meadow (4)	Ekaneetlee (5)[a]	Manway (6)
2	10.2	18.3	10.9	8.8	12.7	15.4
4	19.0	33.2	17.7	13.9	25.4	27.9
6	35.6	43.2	31.5	27.4	34.5	41.9
8	66.4	73.6	55.8	38.1	48.2	68.5
10	78.7	111.6	83.8	50.8	75.8	99.6
12	104.0	175.2	110.9	76.2	96.8	127.5

[a]Odd numbered seedlings exhibited damaged radicles while in culture.

strongest root systems would ultimately yield the tallest and most vigorous individuals. This, however, was not consistently demonstrated. Although two of the largest plants did originate from seedlings with undamaged radicles, the smallest was also derived from an undamaged seedling.

Those whose radicles had been injured, presumably during the sterilization procedures, recovered well when transplanted and yielded healthy well-formed seedlings. Their height growth too was more consistent than in undamaged individuals, ranging from 96.8 to 110.9 cm. after 12 months.

Outplanting the Seedlings

During the month of June, 1983, approximately 1 year after their culture, 5 of the 6 seedlings were outplanted at 4 different locations within 20 miles of Knoxville, Tennessee.[8] Despite the rather high clay content of the soils, which is considered less than ideal for the growth of the species, and without any special care such as mulching or fertilization, each plant adapted well to the transfer. Height growth and lateral branch development continued until the onset of winter dormancy in late September.

In spite of -20° C temperatures which occurred in late December, 1983, all of the planted seedlings survived the winter in good condition. Even the potted seedling, whose root system remained above ground uninsulated from the extreme cold, endured without sign of

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[8]One seedling was kept in a 1 gallon container outdoors under shade.

injury. Normal bud swelling, leaf production, and stem elongation began the following April and good growth continued throughout the 1984 growing season.

Though caution must be exercised when making inferences from such a small sample, it appeared noteworthy that all of the mountain silverbells grown from cultured embryos successfully hardened off and survived the bitter winter, while Carolina silverbells, started from soft or semihardwood cuttings, commonly do not. Such differences seem to indicate that the physiological processes involved in preparing the plant for the onset of winter may function more efficiently when the plant originates from seed. Not to be overlooked too is the possibility that the mountain silverbell, which is adapted to surviving in a harsh montane climate, may be more cold hardy than the Carolina entity. Answers to both of these questions would be of great interest to plant propagators and therefore warrant further study.

CHAPTER V

INVESTIGATION OF SEED DORMANCY MECHANISMS IN MOUNTAIN SILVERBELL

The successful *in vitro* germination of excised mountain silverbell embryos, and their development into merchantable-sized seedlings within a year after transference to soil met the primary objective of the study. Though labor intensive, it demonstrated that embryo culture can be used to grow seedlings to merchantable proportions in a far shorter period of time and with much greater certainty than is possible under forest conditions or with any of the other seed propagation methods currently detailed in the literature. Such a stark contrast in germinative performance suggests that the effectiveness of *in vitro* culturing is tied to its unique ability to bypass natural germination inhibiting mechanisms while at the same time providing the proper environmental conditions necessary for rapid and complete embryo activation. These positive results, nevertheless, do not provide a clearer understanding of the germination inhibiting mechanisms that are most likely involved in the natural environment.

Accordingly, a second set of study objectives were established in an attempt to provide propagators and seed scientists with a better insight into these mechanisms. The additional objectives endeavored to:

1. determine the possible existence of exogenous chemical barriers to early germination
2. examine the potential role of morphological dormancy
3. test for the possible occurrence of physiological dormancy
4. assess the beneficial role which natural stratification plays in promoting germination

Chemical Dormancy

Previous tests in this study have been based on observations made by Jack (1894), Ramaley (1899), and Giersbach and Barton (1932) which suggest that germination inhibition in silverbell is due to the barriers imposed by the bony, indehiscent endocarp. The possibility exists, however, that chemicals may also be responsible for inhibiting germination. The complete excision of the embryo during the original embryo culture experiments may have contributed significantly to the efficacy of this procedure since it not only allowed the unencumbered hydration, aeration, and expansion of the embryo to take place, but it also removed the fruit and seed layers which could have contained chemicals that inhibit or delay germination. In contrast, the failure of some of the conventional scarification procedures to alleviate the barriers of hardseededness, and also their failure to remove chemical inhibitors, could explain why those methods were ineffective.

The inhibitory influence produced by naturally occurring chemicals is the least understood form of dormancy. As recently as 1983, Hartmann and Kester noted the difficulty in "establishing a direct relationship between specific chemicals and germination (inhibition)." Confusion arises because of the wide variety of inhibiting agents involved, and because of their dynamism in the natural environment. The concentration of these substances, and hence their degree of inhibition, may change depending on the developmental stage of the fruit or under differing conditions of temperature, aeration, light, and moisture.

Further uncertainty occurs because modern researchers have had to rely primarily on external applications of extracts from fruit and seed layers to judge their inhibitory effects on the germination of excised embryos. This type of bioassay does not always accurately portray conditions as they exist in vivo. Particularly troublesome are fruits which contain more than one inhibitor or possess hardened covers which restrict aeration.

Despite the obvious drawbacks associated with this form of experimentation, it represents a valuable first step in the understanding of chemical dormancy. However, until a more precise procedure is developed, conclusions about the workings of chemical inhibition as it occurs in nature will remain uncertain and open to debate.

Test for Chemical Inhibitors in Stratified Fruits

The objective of this experiment was to determine if the exocarp, endocarp, seed coat, and endosperm of mountain silverbell

fruits contain soluble chemical substances that inhibit embryo germination even after having been exposed to natural overwintering. No attempt was made to specifically identify these substances. Fruits were collected from the Stratton Meadow collection area in March, 1983. Separate extracts from each fruit and seed layer were prepared and incorporated directly into the Knop's culture medium. Embryos, also obtained from these fruits, were excised and cultured in the mixture. Germination rates and percentages were then monitored daily. For these trials, germination was considered to include both the greening of the apical meristem and cotyledons, and elongation of the radicle and plumule (Esau, 1977; Kramer and Kozlowski, 1979).

There were no guidelines for determining the amount of material to use for the derivation of the extracts. Ideally, the concentration of extract material surrounding the cultured embryos should equal those found *in vivo*. Like so many experiments dealing with chemical inhibitors, precise duplication of such concentrations was impossible to attain. For experimental purposes, 1.5 g. each of exocarp, endocarp, and endosperm-seed coat[9] was used as a base-line quantity for the extractions. The prescribed amount of each tissue was dissolved in 70 percent acetone (70 ml. pure acetone plus 30 ml. double distilled water). A control containing only the 70 percent acetone solvent was also included. After agitating the tissues and solvent

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[9]Difficulty in separating the membranous seed coat from the endosperm made it necessary to combine these tissues into a single extract.

on an electric shaker at laboratory temperature for 48 hours, the contents were filtered leaving behind the tissue remnants.

Volatilizing the acetone from each solution was accomplished by using a flash evaporator. The remaining solution was again filtered and brought to standard volume (30 ml.) with double distilled water. Each extract was next centrifuged for 10 minutes at 10,000 revolutions per minute (12,000 g-forces) to further remove insoluble components.

Contamination of the fruits and seed tissues by fungi and bacteria further complicated the extract synthesis. While autoclaving the material would ensure elimination of the contaminants, it also involved the risk of destroying possible chemical inhibitors. To circumvent these difficulties, the extracts were drawn through a 0.2 micron bacteriological suction filter to assure complete sterilization.

In the laminar flow hood, each extract was transferred to a separate sterile beaker. Knop's medium (approximately 40° C) autoclaved earlier, was then combined with the extracts. The contents (280 ml. per treatment) were thoroughly mixed with a magnetic stirrer, decanted into test tubes (20 tubes per treatment), and allowed to solidify.

Finally, a total of 80 excised embryos were cultured in the media, placed in the germinator, and monitored for germination.

Two weeks later the experiment was repeated and the same parameters recorded.

Germination rate and percentage. Neither embryo germination rates nor germination percentages were found to be noticeably reduced by extract culturing. As with the controls, several extract treated embryos began to exhibit meristematic and cotyledonary greening in as little as 2 days. By the end of the first week, all embryos, regardless of treatment type had initiated the greening process and showed some degree of plumule and radicle elongation. Such rapid and complete germination would therefore seem to suggest either the complete lack of soluble germination inhibiting substances in the media or insufficient levels of such substances necessary to prevent germination.

Although extracts derived from the various fruit and seed layers of mountain silverbell failed to prevent germination from occurring, researchers have observed that endogenous chemicals sometimes adversely affect subsequent seedling growth and morphological development. Temporary growth inhibition, for example, has been reported in wheat (Triticum spp.) and mustard (Brassica spp.) even after the initiation of the germination process due to phenols and coumarin. While these substances are initially present in concentrations sufficient to totally arrest germination, they are eventually degraded to the point where germination can occur. Their persistent low-level activity, however, interferes with normal seedling development especially when the outer fruit coverings remain in contact with the young plant (Evanari, 1949; Hartmann and Kester, 1983).

Aware that this might take place in mountain silverbell, the extract experiment was designed with enough flexibility to allow

for the monitoring of seedlings should germination occur. At 1, 2, 4, and 6 weeks, 5 seedlings were removed from each extract type and their morphological development documented by measuring length of longest rootlet, cotyledon, and leaf, as well as total axial length. In addition to these, oven dry weights were recorded since they are conventionally considered to be the best indicators of individual seedling growth in terms of biomass production. (Data detailing seedling growth is documented in Appendix C).

Dry weight growth. Dry weights of all seedlings (oven dried at 105° C for 24 hours), regardless of treatment, continued to increase throughout the 6 weeks of the trial. Controls, however, appeared to outperform any of the extract treated seedlings (Figure V-1).

To better understand the growth performance of the extract treated seedlings in relation to one another, and in relation to the controls, mean separation techniques were used. Data were analyzed on the University of Tennessee IBM 3031 computer using the general linear models (GLM) procedures of the Statistical Analysis System (SAS) (Barr, et al., 1979). Mean separations were accomplished by employing linear contrasts. Test results showing differences at the 95 percent confidence level were considered significant.

The data evaluated were seedling dry weight measurements at 6 weeks. It was reasoned that values obtained at the end point of the experiment would best reflect comparative growth in terms of biomass accumulation since those seedlings were exposed to the extracts for the longest period of time.

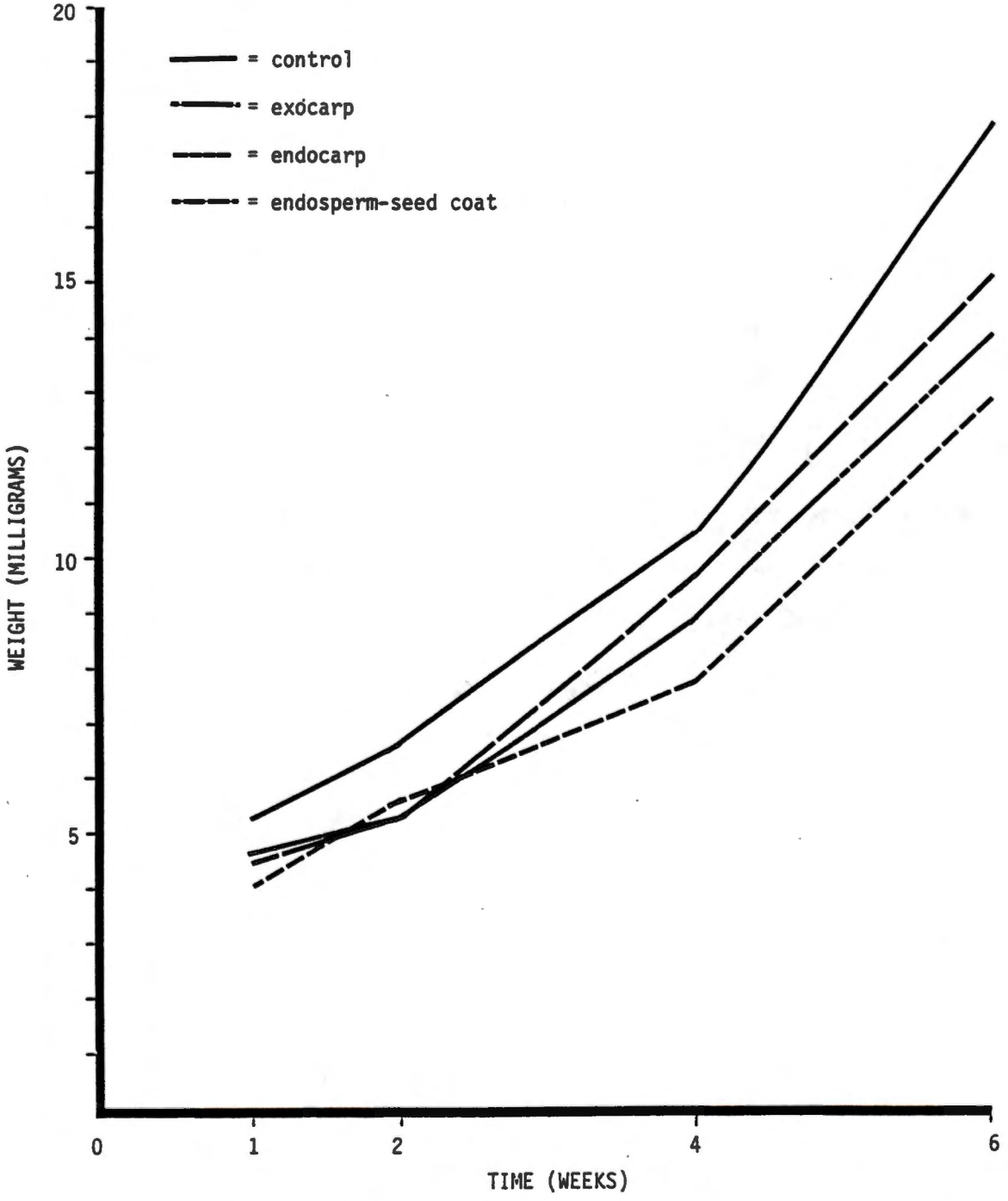

Figure V-1. Oven Dry Weight of Control and Stratified Extract Treated Seedlings.

The dry weights of control seedlings at 6 weeks were found to be significantly greater than those cultured in either exocarp, endocarp, or endosperm-seed coat extract ($pr>f = 0.0003$) indicating a strong treatment effect. Differences in dry weights among the extract treated seedlings themselves, though, were not significant despite a noticeable downward trend in dry weight accumulation corresponding with increasing depth of origin of the materials from which the extracts were made (Appendix C).

While dry weight measurements showed that extract applications strongly reduced seedling growth, they revealed nothing about the morphological development of the treated seedlings or how the growth was distributed on each plant. In order to make that determination, the linear dimensions of major plant structures were recorded.

Previous *in vitro* experimentation by the author has shown that mountain silverbell seedlings vary in their morphological development despite uniform growing conditions. This variation may be attributed, in part, to individual genotypic differences associated with open pollination. However, in the case of root and cotyledon development, it appeared that at least some of the observed variation was a direct result of the differential reaction of the embryos to the sterilization procedures. In the first successful embryo culture test it was noted that 40 percent of the embryos exhibited obvious cotyledonary bleaching and 23 percent possessed what were assumed to be chemically deformed radicles.

Because only a portion of the embryos react in this manner, and to varying degrees, it is difficult to assess damage to these structures in a quantitative sense. These confounding influences, therefore, further complicate an evaluation of the effect of chemical inhibitors on seedling ontogeny.

Individual variation in seedling morphology due to genetic diversity and variable response to embryo sterilization both must be considered unavoidable components of these experiments. Mountain silverbell fruits could be obtained only from wild populations and embryo sterilization prior to in vitro culturing was necessary to assure contaminant free growth. By employing embryos collected from the same locale, and assuring that each received identical sterilization treatments, it was felt that both forms of innate variation would be equalized among treatments and that a generalized picture of extract effect could be established.

Root development. Extract effect on root development was initially tested by measuring the linear distance from the tip of the radicle to the root collar. As the seedlings matured and began to produce secondary rootlets which often exceeded the length of the radicle, it became necessary to measure from the tip of the longest rootlet to the root collar. Also to more accurately reflect the influence of each extract on the expansion of the root system, the number of secondary and tertiary rootlets formed was recorded.

Control seedlings cultured in Knop's medium alone gave rise to longer root systems than any of the seedlings treated with extract

at each measurement interval (Figure V-2). Although the effects of the extracts varied over time, endosperm seed coat was generally the most inhibiting, followed by endocarp and finally exocarp.

This same pattern was reflected in the number of secondary and tertiary roots produced (Table V-1), which seems to suggest that those seedlings capable of producing the longest roots will ultimately give rise to a higher number of lateral roots.

Clearly, extract applications produced a negative effect on overall root development. Because the identity of the inhibiting substance or substances was beyond the scope of this study, the precise site and mode of action could not be determined. However, since secondary and tertiary roots typically arise from the pericycle, reduced lateral root frequency in extract treated seedlings was most likely the result of a moderate perturbation in this region. This may have been due to either an internal physiological reaction or to an external irritation.

Cotyledon development. Like roots, the cotyledons of silverbell embryos seemed particularly susceptible to the pre-culturing sterilization process. Because of this, it was possible that the resulting "tip burn" would severely retard their growth and mask the inhibiting influence of extract applications. Since the number of embryos affected in each treatment type was similar (20 to 30 percent), and because the "burning" did not appear extensive, it was felt that valid comparisons could be made.

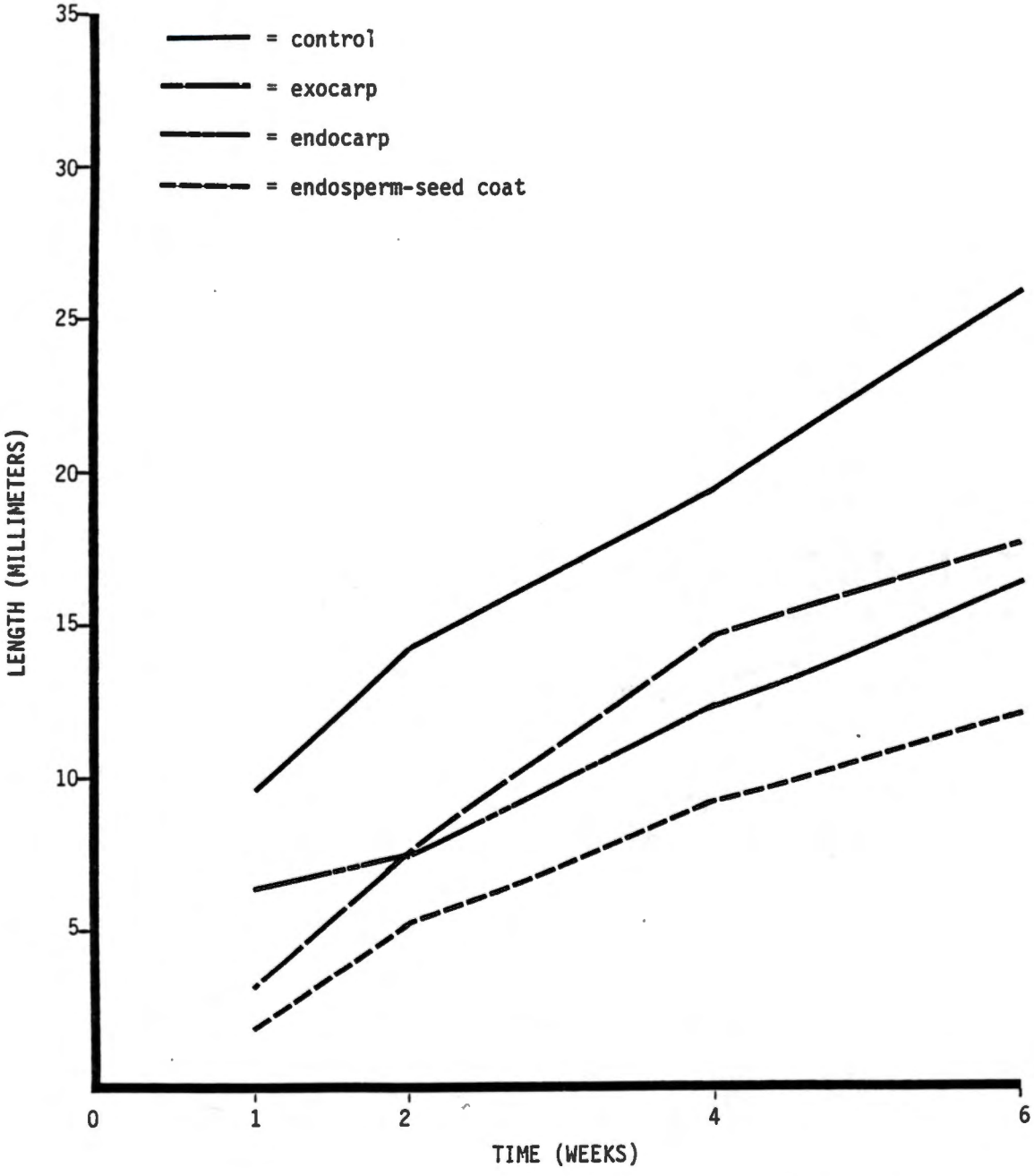

Figure V-2. Length of Longest Root Produced Control and Stratified Extract Treated Seedlings.

Table V-1. Number of Secondary and Tertiary Roots Produced on Control and Stratified Extract Treated Seedlings.[a]

Weeks in Culture	Control		Stratified Extract Treatment					
			Exocarp		Endocarp		Endosperm - Seed Coat	
	Secondary	Tertiary	Secondary	Tertiary	Secondary	Tertiary	Secondary	Tertiary
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.70	0.00	0.00	0.00	0.30	0.00	0.00	0.00
4	3.40	0.50	2.40	0.20	2.10	0.00	1.10	0.00
6	6.10	1.20	5.40	0.50	3.70	0.80	2.80	0.50

Cotyledons (measured from tip of longest cotyledon to its junction with the stem), regardless of treatment type elongated at a slow and rather steady rate throughout the course of the trial. Control embryos continually produced longer cotyledons than those treated with the extracts (Figure V-3). The inhibitory effect of each extract also appeared to increase with their increasing depth of origin within the fruit and seed. Those derived from tissues nearest the embryo were the most debilitating.

Leaf development. Observation of control seedlings germinated in Knop's medium alone revealed that the production of the first true leaf occurs approximately 1 week after culturing, and the appearance of this first leaf is always preceded by cotyledon and hypocotyl greening and some degree of radicle elongation. Although treatment of seedlings with various fruit and seed extracts did not slow the timing of the greening process, they did seem to greatly retard the onset of leaf production.

The delayed appearance of the first leaf in extract treated seedlings is shown in Table V-2. At the end of 1 week in culture the endosperm-seed coat treated seedlings had produced on average only 0.10 leaves (1 of 10 seedlings exhibited organized leaf structure) compared to 0.90 leaves in the controls. Those seedlings cultured in exocarp and endocarp extract developed somewhat better setting an average of 0.20 and 0.30 leaves over the same period.

As the experiment progressed into the second week and beyond, the inhibiting influence of the extracts abated, at least in terms

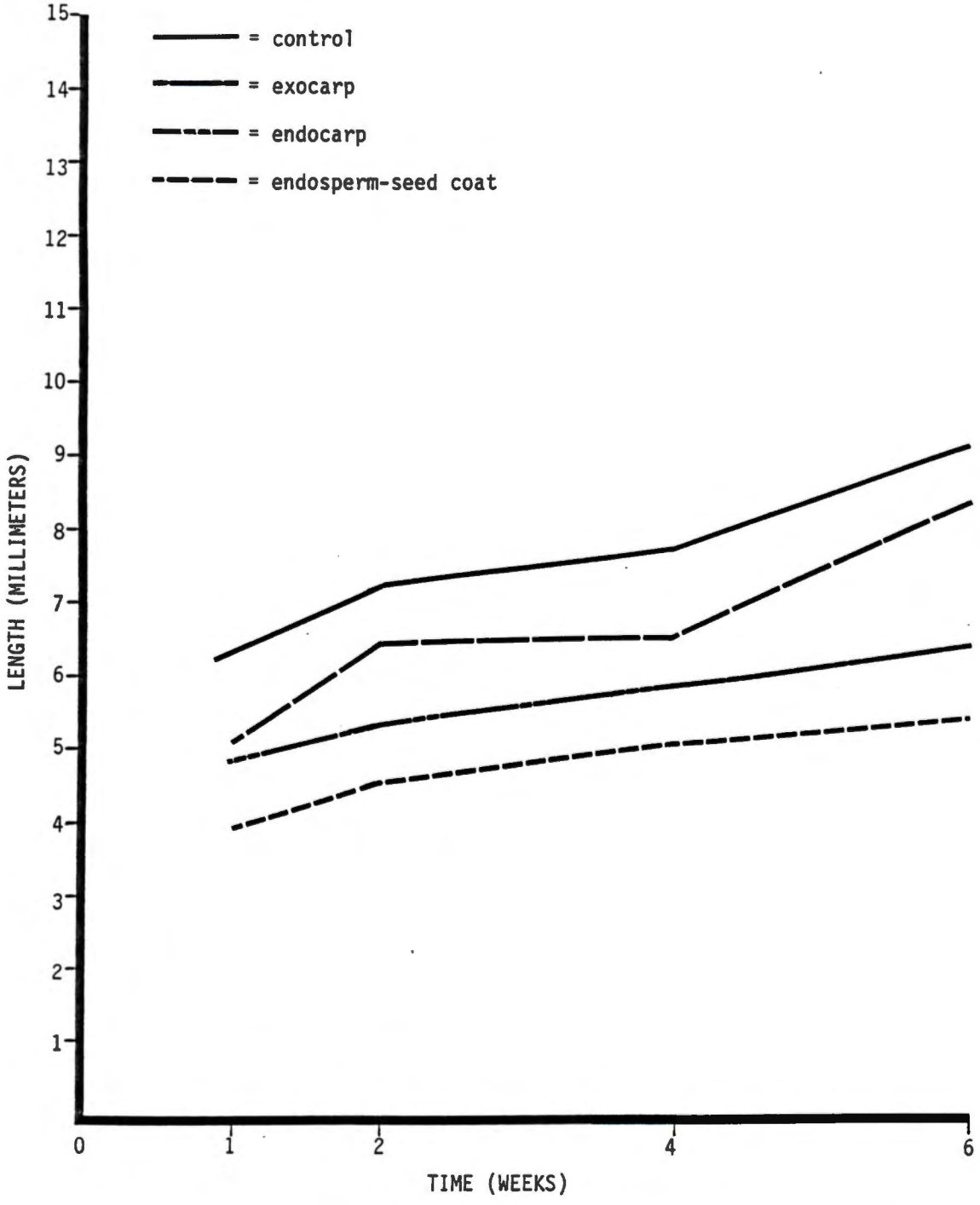

Figure V-3. Length of Longest Cotyledon Produced on Control and Stratified Extract Treated Seedlings.

Table V-2. Number of Leaves Produced on Control and Stratified Extract Treated Seedlings.

Weeks in Culture	Control	Stratified Extract Treatment		
		Exocarp	Endocarp	Endosperm - Seed Coat
1	0.90	0.20	0.30	0.10
2	2.40	2.00	2.20	1.80
4	3.80	4.30	4.00	4.10
6	5.40	5.20	5.30	4.80

of the number of leaves produced. In some instances, extract treated seedlings actually gave rise to more leaves than the controls even though the length of those leaves (measured from the leaf apex to node) continued to lag noticeably behind the leaf length of the controls (Figure V-4).

Since leaf initiation takes place in the peripheral region of the shoot apex, an area distinctly removed from direct and possibly irritating contact with the culture media, the results of this test seemed to indicate that the initial lag in leaf production and subsequent reduction in leaf size was probably due to an agent within the extracts that was translocated upward through the stem.

Development of seedling axis. Total axial length, or the linear distance from the tip of the plumule to the tip of the longest rootlet, was recorded for each individual seedling. These measurements were then averaged to detect differences in elongation between extract treated plants and those of the control.

The results, depicted in Figure V-5, show that the controls outperformed all extract treatments over the entire 6 weeks of the test, indicating a negative reaction to the extracts. Exocarp extract tended to produce the least inhibitory effect while those cultured in extract endosperm-seed coat (derived from the most deeply located tissues immediately surrounding the embryo) were consistently the most effective at inhibiting elongation.

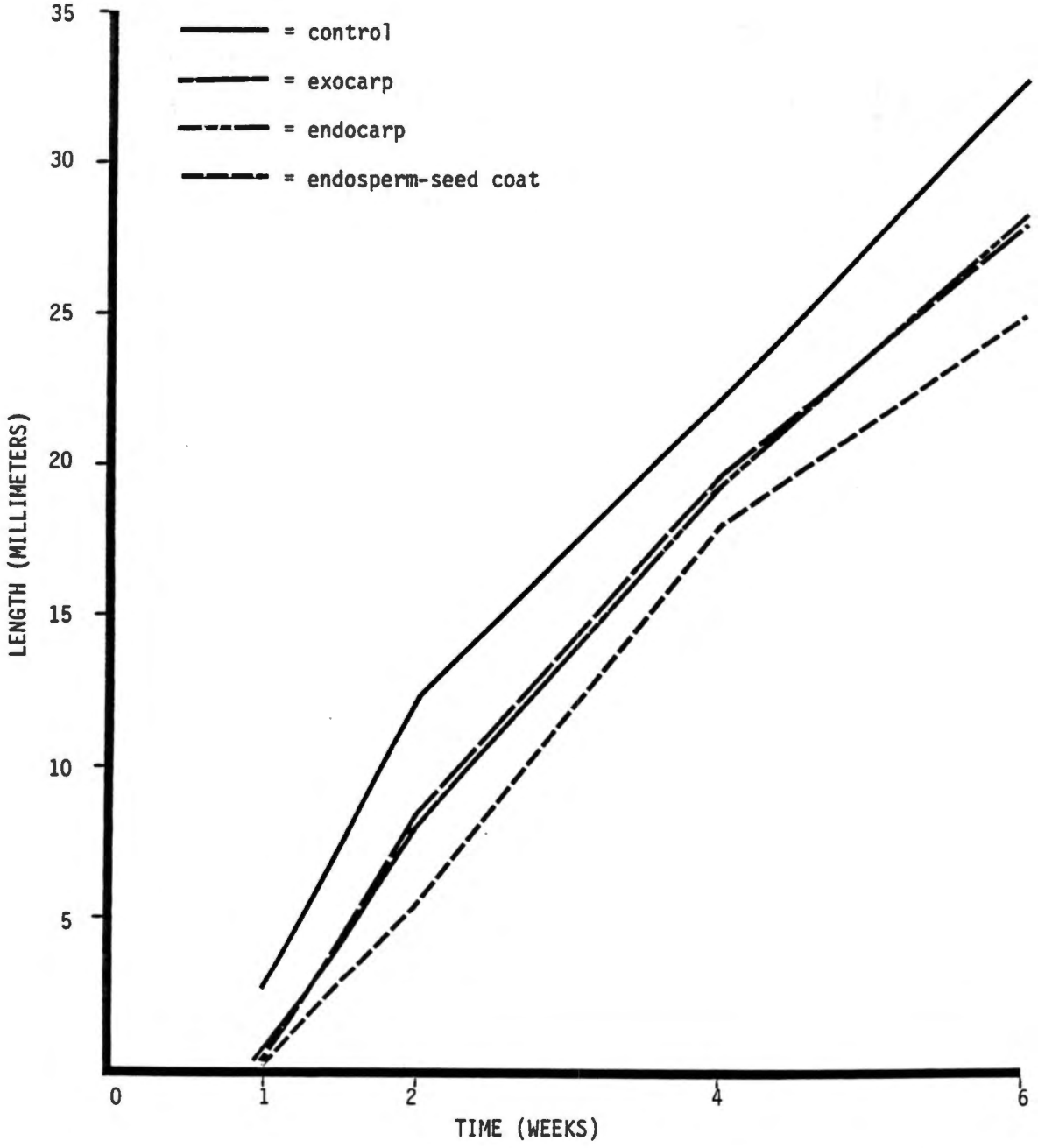

Figure V-4. Length of Longest Leaf Produced on Control and Stratified Extract Treated Seedlings.

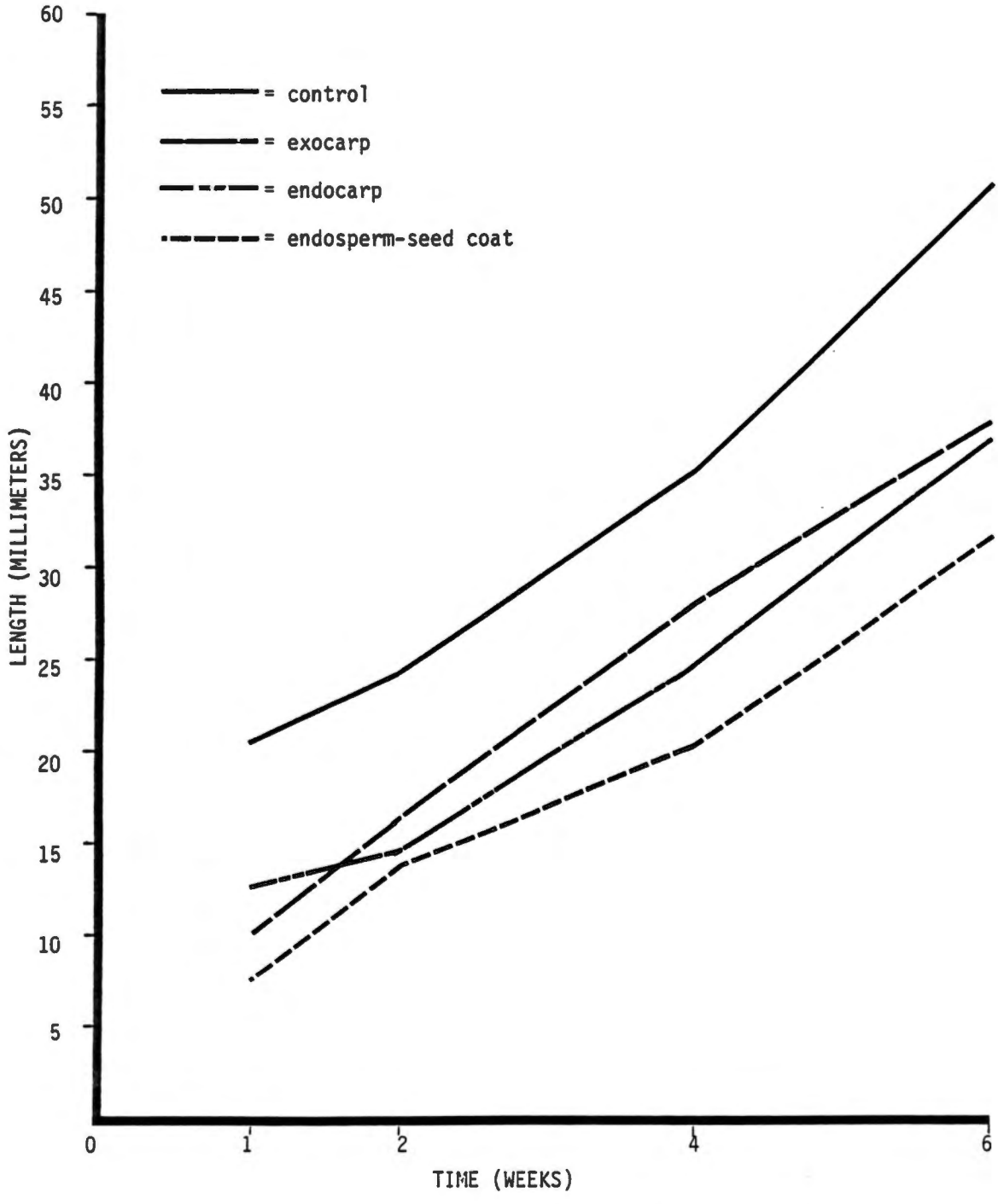

Figure V-5. Major Axial Length of Control and Stratified Extract Treated Seedlings.

Test for Chemical Inhibitors in Non-Stratified, Green Fruits

Because fruits used in the previous chemical inhibitor experiments were collected from the ground in March, the once succulent exocarps had become withered and papery. By lying on the moist forest floor for several months, inhibiting substances that may have been present in the external layers could have been leached away. Prolonged exposure to the cold could have also contributed to the degradation of such substances.

Considering these possibilities, an experiment was undertaken to test for chemical inhibitors in fresh, non-stratified fruits. In early September, 1983, approximately 200 fruits were harvested from the Stratton Meadow collection area. These had not yet abscised from the parent trees and were green and succulent.

The preparation of extracts from the fruit and seed layers for this test were procedurally the same as for stratified fruits. There was, however, one exception. Non-stratified green fruits were found to contain a higher percentage of liquids than stratified fruits. To compensate for the extra weight of these liquids, it was necessary to use more than 1.5 g. of green material to prepare each extract. In this way the same relative amounts of solid material were used in the derivation of extracts for both the stratified and non-stratified tests. Table V-3 shows the amount of green tissues used in the extract synthesis corrected for the extra weight of liquids.

Embryos used for this test were excised from naturally stratified fruits collected the previous March. These fruits had been

Table V-3. Moisture Content of Stratified and Green Tissues and Weight of Green Tissues Corrected for Extra Weight of Liquids.[a]

Tissue	Moisture Content of Stratified Tissue	Moisture Content of Green Tissue	Weight of Stratified Tissue Used to Derive Extract	Weight of Green Tissue Used to Derive Extract (Corrected for Extra Weight of Liquids)
	Percent		Grams	
Exocarp	31.6[b]	87.2[b]	1.5	4.1
Endocarp	4.2[b]	7.6[b]	1.5	2.7
Endosperm-Seed Coat	24.9[c]	26.4[c]	1.5	1.6

[a]All values calculated on a green weight basis.

[b]Approximate values based on a sample of 5.0 g. of tissue.

[c]Approximate values based on a sample of 1.0 g. of tissue.

stored in sealed, polyethylene bags in the refrigerator and held for 6 months at 2° C. Stratified rather than non-stratified embryos were selected because their performance in culture was already known from the earlier stratified extract trials. Using embryos from the September seedlot risked the possibility of a total germination failure, not necessarily due to green extract applications, but as a consequence of their immaturity.[9]

Germination rate and percentage. As with the previous stratified trials, extracts derived from non-stratified fruits failed to prevent the germination of the embryos or slow their rate of germination. Apical meristem and cotyledonary greening occurred in at least one embryo of each treatment type within 2 days of culturing. After 1 week, all had begun the greening process along with plumule elongation. (Data detailing seedling growth is given in Appendix C.).

Dry weight growth. Control seedlings continually outperformed any of those cultured in extracts (Figure V-6). Despite a steady weight increase in all extract treated plants, the relative effect of each appeared quite variable.

Mean separation techniques indicated that dry weight growth of control seedlings was significantly greater than any of the seedlings grown in media containing green extracts ($pr>f = 0.0097$) suggesting

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[9]Statistical tests revealed no significant differences among controls used for stratified extract and non-stratified extract trials ($pr>f = 0.4978$) indicating a valid comparison.

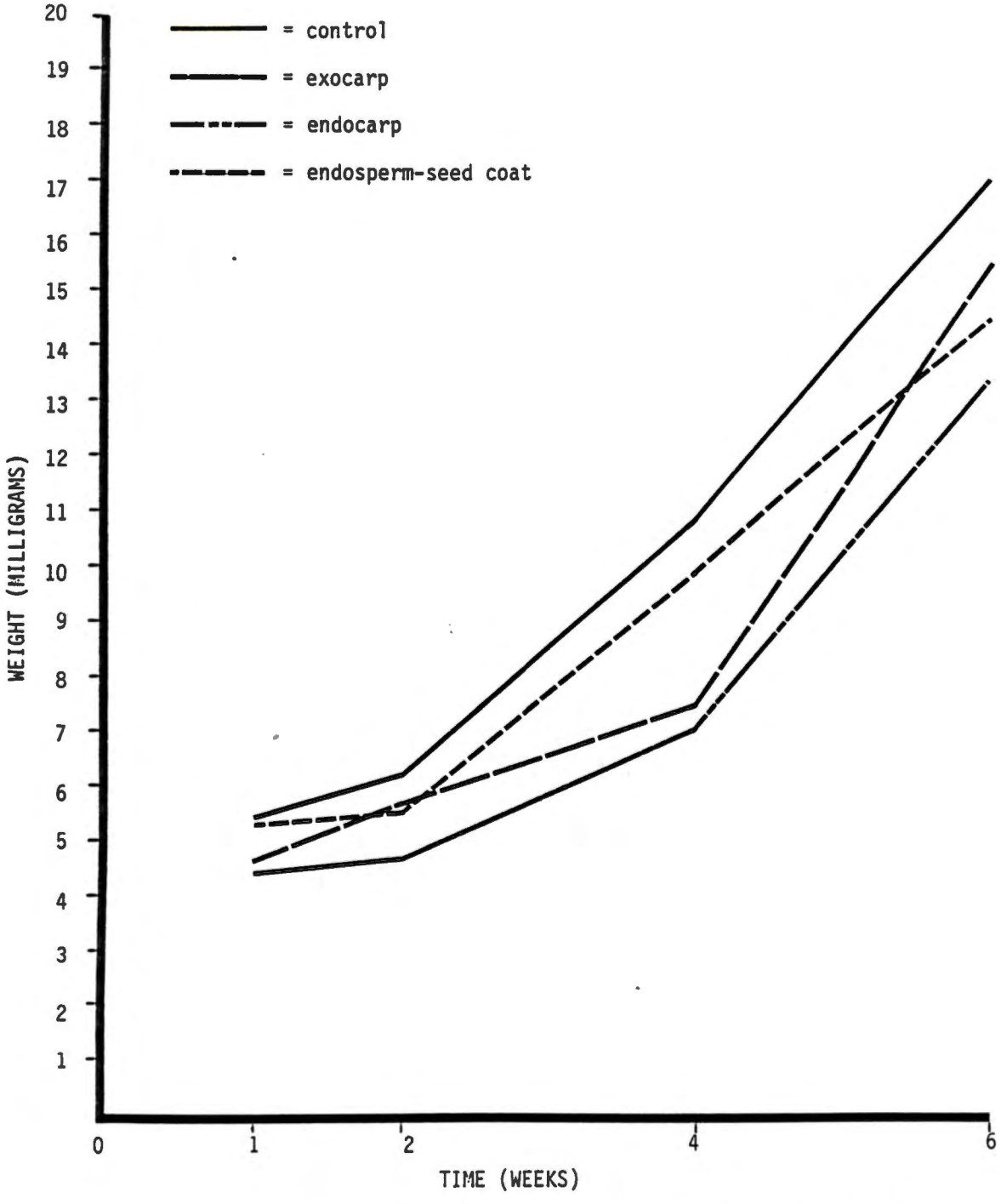

Figure V-6. Oven Dry Weight of Control and Green Extract Treated Seedlings.

a strong effect. Differences among the exocarp, endocarp, and endosperm-seed coat treatments, however, were not significant when tested at the 0.05 percent level (Appendix C).

Dry weight growth of seedlings cultured in extracts derived from green fruits versus those grown in extracts from stratified fruits was also compared statistically to gauge the impact of prolonged moist, low temperature exposure on the inhibiting influence of each extract type. Results obtained from these tests failed to show any significant difference between the experiments. Dry weight accumulation in green extract treated plants was essentially the same as those plants cultured in media containing the corresponding stratified extract. The same pattern occurred when controls were compared (Appendix C).

This outcome, while indicative of total cell growth, does not fully illustrate the differential impact of stratified and non-stratified extracts on cell elongation and seedling morphology. The following discussion highlights morphological and developmental differences which appeared noteworthy.

Root development. The elongation of root systems on mountain silverbell seedlings was found to be severely impeded by non-stratified extract treatments (Figure V-7, Table V-4). Although the overall effect of green extract culturing on root system development would at first seem to be an intensification of those effects produced earlier with stratified extracts, several important

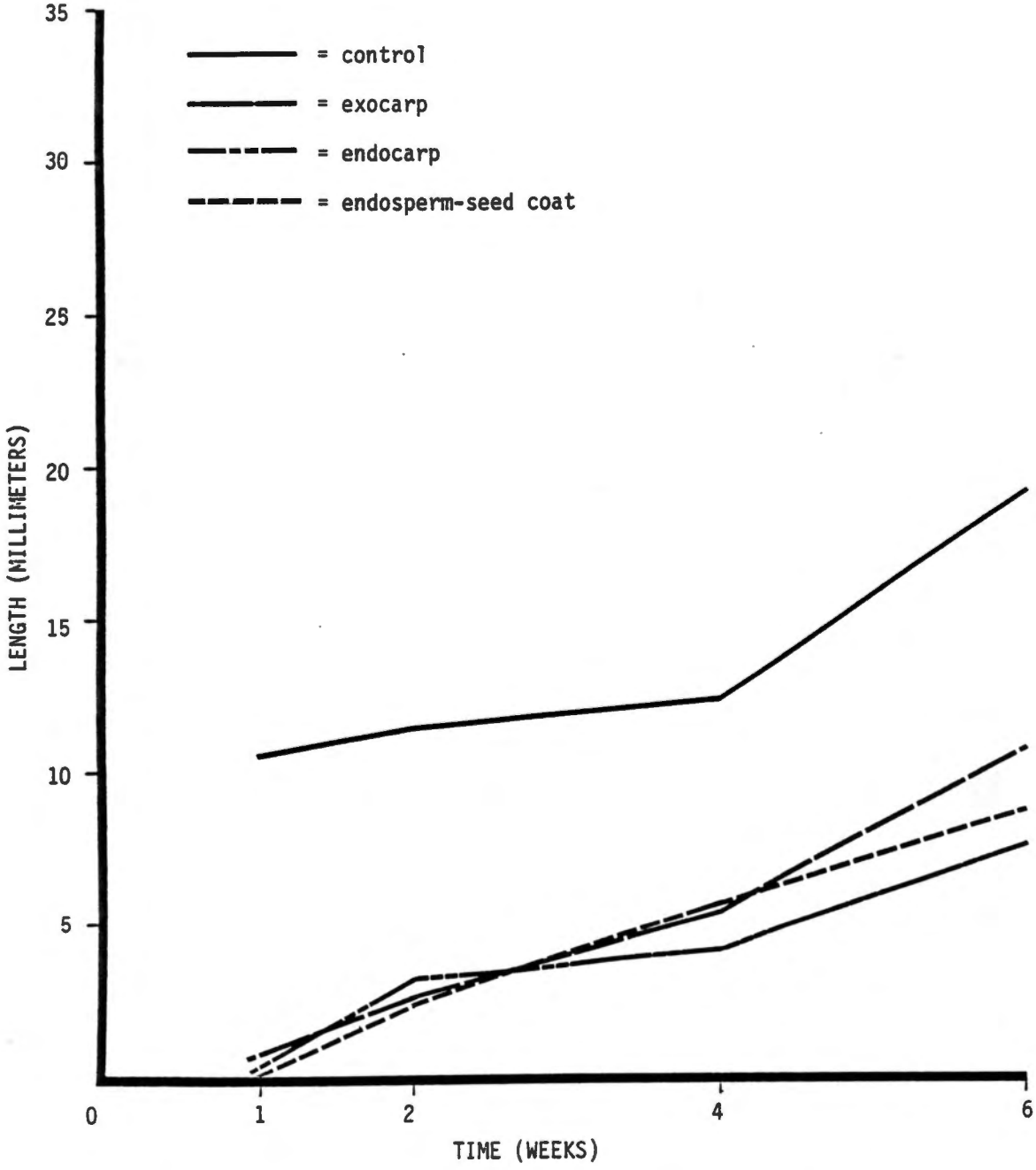

Figure V-7. Length of Longest Root Produced on Control and Green Extract Treated Seedlings.

Table V-4. Number of Secondary and Tertiary Roots Produced on Control and Green Extract Treated Seedlings.

Weeks in Culture	Control		Green Extract Treatment					
			Exocarp		Endocarp		Endosperm - Seed Coat	
	Secondary	Tertiary	Secondary	Tertiary	Secondary	Tertiary	Secondary	Tertiary
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	3.00	0.60	2.80	0.00	1.60	0.00	2.80	0.00
6	6.20	0.60	5.20	0.40	3.00	0.00	4.80	0.20

abnormalities were in evidence. The portion of the embryos in direct contact with the green extracts showed indications of tissue damage within 24 hours after culturing. Injury to the peripheral region of the radicle and lower hypocotyl resulted in the discoloration of the surrounding medium probably due to oxidized phenolics. Further evidence of injury was apparent several days later as the destroyed cells themselves began to turn brown and necrotic.

Shortly after the initiation of germination, all extract treated seedlings began to produce callus tissue apparently in response to wounding. The amount and pattern of callus formation differed with treatment type. Seedlings grown in exocarp extracts reacted by producing relatively large spherical masses which entirely enveloped the radicle. In some instances, callus projected several millimeters above the level of the media adjacent to the cotyledons. In media containing extracts from the endocarp and endosperm-seed coat, damage was less visible consisting entirely of spindle shaped swellings of moderate thickness.

The degree of injury, perceived solely on the basis of hyperplastic callus formation, did not in all cases correlate well with rootlet development. Seedlings treated with exocarp extract, for example, displayed large callus masses, yet these ultimately gave rise to a rather complex root system second only to the controls in number and length of rootlets produced.

Development of the seedling axis. Observation of the morphological development of seedlings cultured in green, non-stratified extract revealed a reduction in total seedling length when compared with stratified extract treated seedlings. Although much of this decline could be attributed to diminished rootlet length, a portion of it was also caused by reduced stem elongation above the level of the cotyledons. The diminished capacity for seedling elongation may have been related to a lessening of the ability of the seedlings to obtain vital nutrients from the culture media resulting from a smaller rooting area, or perhaps the disruption of vascular tissues caused by the injury and callus formation. A part of each seedling's growth potential could have also been redirected to produce callus. Growth curves (Figure V-8) show the suppressive effect of green extracts on axial elongation over a 6 week period.

These experiments have demonstrated that extracts derived from both non-stratified and naturally stratified fruit and seed tissues failed to prevent rapid and complete embryo germination at the applied concentrations. This suggests, therefore, either the complete absence of germination inhibiting substances in the media, or insufficient levels of such substances necessary to prevent the initiation of the greening process.

Although germination was not prevented, the study showed that extracts exerted a suppressive influence on seedling dry weight growth and morphological development. Since extracts from stratified fruits were generally found to be less morphologically debilitating

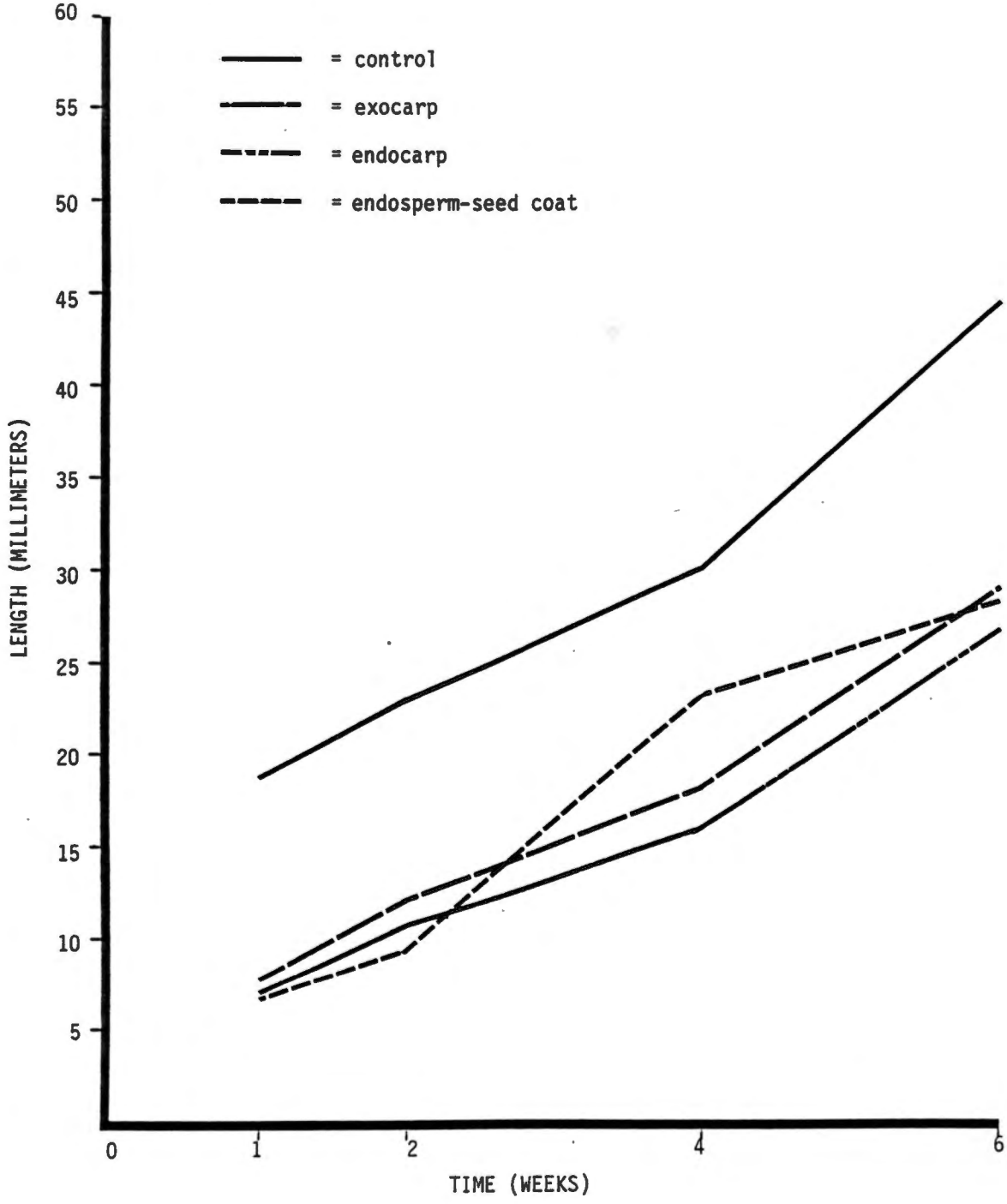

Figure V-8. Major Axial Length of Control and Green Extract Treated Seedlings.

than green, and because they failed to induce wound callus formation, it would appear that prolonged cold exposure led to the partial degradation of the perturbing substances. Significant amounts of precipitation, common in the mixed mesophytic forest, probably also promoted a high degree of leaching and the dispersion of some of the chemicals into the soil below.

Being that these experiments relied on external applications of extract, it is unclear if under forest conditions, soluble chemicals are involved in the delayed germination of mountain silverbell embryos or whether they interfere with subsequent seedling growth. However, because fruits lying on the forest floor are continually exposed to a variety of environmental factors which promote the destruction or dissolution of these chemical substances, their intervention would appear unlikely.

Personal observation indicates that the breakdown of tissues occurs very slowly during the overwintering phase of stratification. Until March, the primary barriers of the hard seed coverings remain intact and the chemicals contained within the fruits, may at that time, exist in concentrations high enough to be mildly inhibiting. With the advent of mild temperatures in the spring, tissue decomposition takes place at an accelerated rate due to the activity of soil microbes and other biotic agents. Not only is it likely that the barriers of hardseededness are overcome during these processes, but it is also probable that any residual chemical constituents within them are also degraded to the point where they become inconsequential.

Morphological Dormancy

Morphological dormancy, involving underdeveloped or undifferentiated embryos, may be partly responsible for delayed germination in silverbell. In most species germination cannot take place until the embryos fill the seed cavity. Warm and cold stratification, which occurs naturally in the environment, and which has been recommended by propagators, may promote the continued enlargement of the seed and embryo after abscision from the parent plant. This, however, has never been investigated. To test the hypothesis, seed and embryo development in green, non-stratified fruits was compared with those in fruits exposed to natural overwintering.

Test for Morphological Dormancy

In the southern Appalachians, fruits of mountain silverbell reach mature size 8 to 12 weeks after fertilization. In most populations this occurs during the month of July. Beginning in mid July, 1983, approximately 500 non-abscised, green fruits were gathered at random from trees in the Stratton Meadow area of the Cherokee National Forest. One hundred seeds were excised from these fruits and the number of seeds per fruit, seed weight, and seed length recorded. Embryos were also extracted from the seeds, paying particular attention to their development. Embryo length, fresh weight, and dry weight (oven dried 24 hours at 105° C) were obtained, as well as endosperm-seed coat weight. These same procedures were followed

for fruits collected in mid September (non-abscised) and again in mid November (abscised).[10]

To determine if natural stratification fosters the *in situ* growth of seeds and embryos, a fourth fruit collection from Stratton Meadow was made in late March, 1984. One hundred seeds and embryos were extracted from these fruits and their development compared with the non-stratified.

Development of seeds and embryos in non-stratified green fruits. Though the fruits of mountain silverbell get no larger after mid summer, the internal structures continue to grow. Both seeds and embryos exhibited a steady increase in length from July through September. Close examination showed that in September most of the embryos had completely filled the seed cavity and in 26 percent of the cases, embryo length actually exceeded that of the seed. Embryos of this type were found to possess folded cotyledons. This trend continued into November, even after fruit abscision, at which time 45 percent of the embryos were longer than the seeds enclosing them (Figure V-9; Appendix C).

There was also a corresponding increase in total seed and embryo weight over the same period (Figures V-10 and V-11). The greatest portion of this weight increase can be attributed to growth of the nutritive endosperm. In July, endosperm tissues were relatively

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[10]The November fruits were considered non-stratified because they had not been exposed to a protracted cold period.

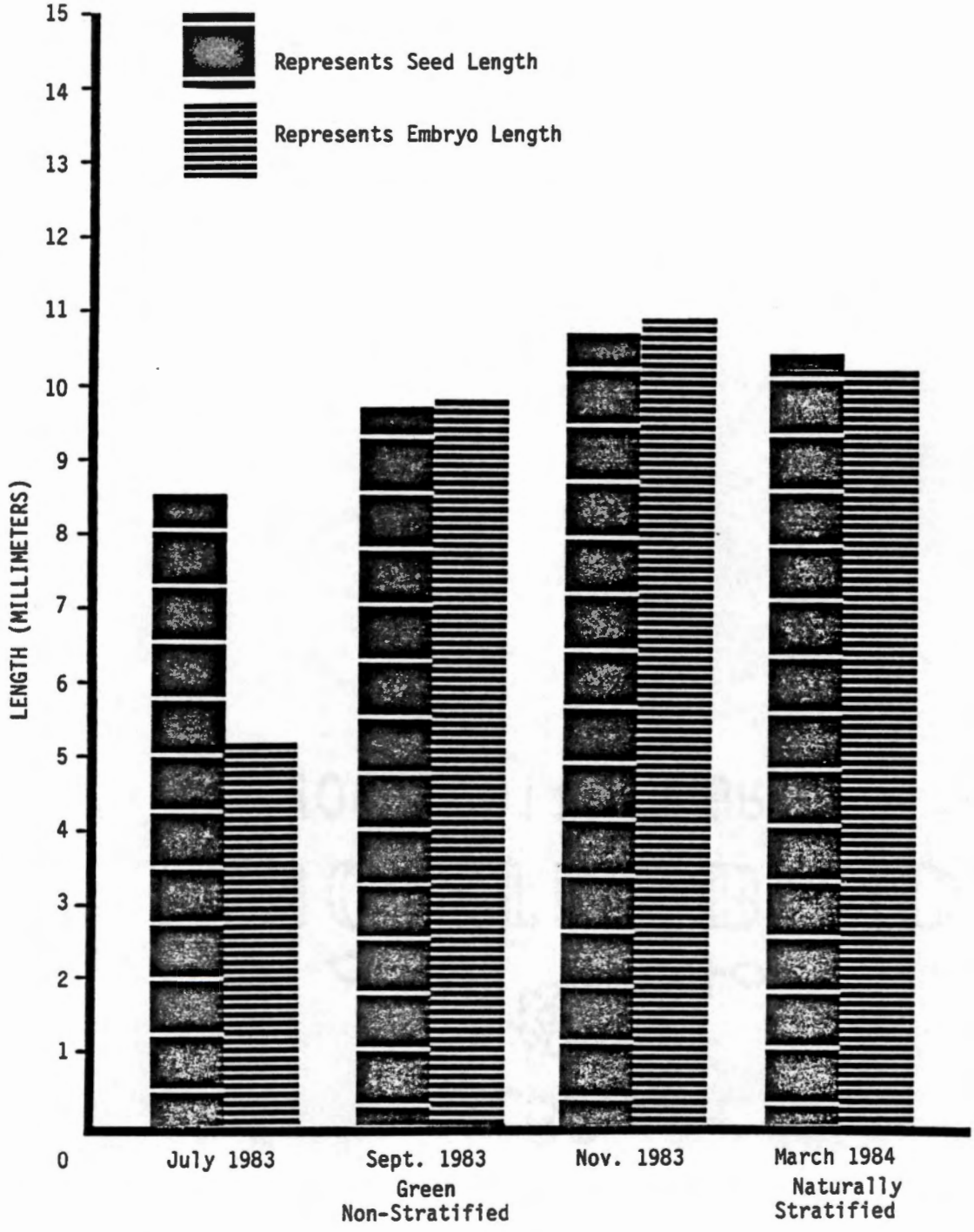

Figure V-9. Length of Seeds and Embryos Excised from Green and Stratified Fruits.

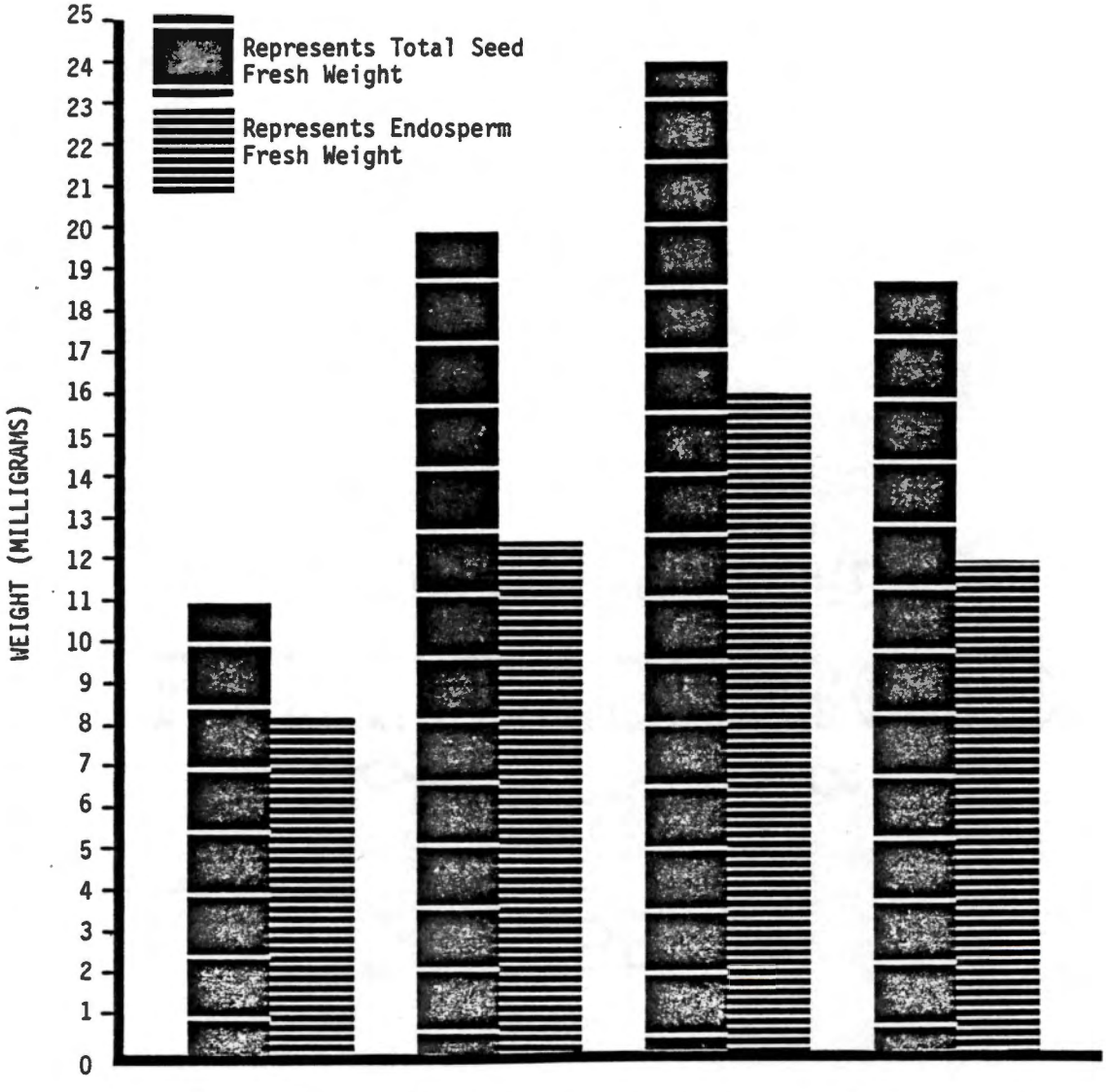

Figure V-10. Fresh Weight of Seeds and Endosperm Tissue Excised from Green and Stratified Fruits.

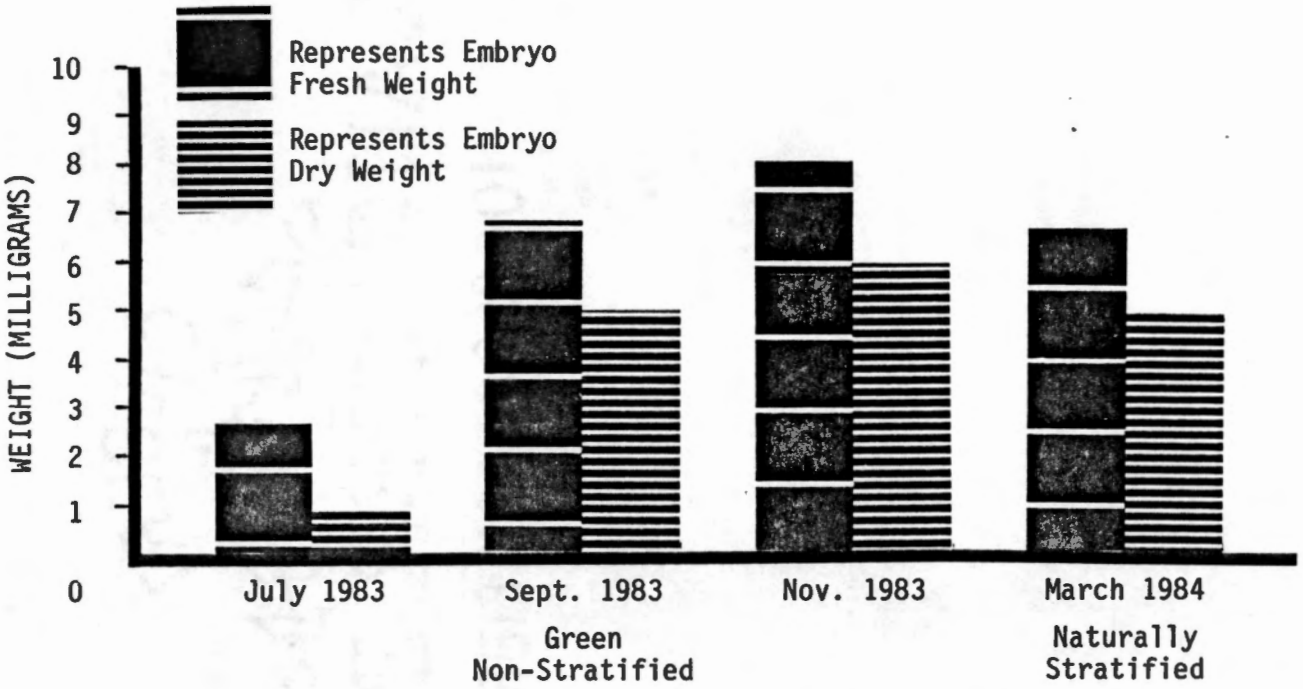

Figure V-11. Fresh and Dry Weight of Embryos Excised from Green and Stratified Fruits.

thin and pliant, but reexamination in September showed an increase in volume and transformation to a more solid state. This likely represents the conversion of soluble food storage reserves to insoluble forms (Krugman et al., 1974; Bonner, 1975, 1976).

Mountain silverbell, like most species which produce fruits that overwinter on the ground, showed a marked loss in seed and embryo moisture content as ripening progressed. This is considered by seed scientists to represent a natural defense mechanism to lessen the effect of freezing temperatures (Crocker and Barton, 1953; Hartmann and Kester, 1983). The moisture content of the endosperm (green weight basis) decreased from 48 percent in July to about 25 percent in both September and November. A similar pattern was observed in the embryos. Here moisture content decreased from an average high of 69 percent in July and leveled off to approximately 25 percent in September and November.

It would appear that a large portion of the reduction in seed moisture content occurs concomitantly with, and is related to, the development of hardseededness. In July, the vascular connection between the fruit and parent plant is intact. Despite a porous endocarp, which permits moisture loss to the surrounding environment, moisture levels remain relatively high due to this vascular continuity. As fruit maturity progresses and vascular integrity begins to break down, moisture content drops significantly. Complete moisture loss in the internal structures is prevented, however, by the rapid lignification of the endocarp.

Additional tests verified these observations. Succulent green fruits collected in July and subjected to conditions of low humidity at laboratory temperature possessed highly dessicated seeds and embryos within a week. Further indication of the permeability of early season fruits was demonstrated by placing them in a solution of red dye. The dye readily penetrated the external coverings and reached the seed, in all cases, within 4 weeks.

In September and November, after the formation of the abscision layer and hardening of the endocarp, air drying and dye penetration were inhibited for the 8 week duration of the test.

Development of seeds and embryos in stratified fruits. Naturally stratified fruits gathered in March from Stratton Meadow contained seeds and embryos that were shorter and lighter in weight than those from non-stratified fruits collected in September and November. These losses are illustrated in Figures V-9, V-10, and V-11 and Appendix C. Because seed moisture content was previously found to stabilize at about 25 percent as early as September and remain that way through the stratification period, the length and weight reductions that were observed in March cannot be explained on the basis of moisture loss. The dense endocarp would, in all certainty, prevent such an occurrence. One likely cause is low level seed respiration that normally takes place over time, even when external seed coverings interfere with oxygen availability.

More important than respirational losses were losses caused in internal contamination. Partial degradation of the endosperm,

and to a lesser degree embryo tissue, was observed in 22 percent of the stratified seeds. Since overt contamination was essentially absent from freshly collected, non-stratified lots, it would appear that one effect of overwintering was to provide enough time and the proper environment for the gradual intensification of infection leading to the slow degradation of the internal structures. The spread of internal pathogens from seed to seed, however, would likely progress at an even faster rate if it were not for the combination of low temperature, low moisture content, and their distinct segregation within the woody matrix of the endocarp.

These experiments indicate that morphological underdevelopment of embryos does not play a direct role in delaying the germination of silverbell. Seeds and embryos grow rapidly throughout the summer under ambient forest conditions and are fully developed by the time the bulk of the fruit crop abscises in mid autumn. Warm and cold stratification, recommended in the literature, is therefore not necessary for their continued enlargement (at least in the mountain form of the tree). Such exposures may even be detrimental due to prolonged low-level respiration and the insidious growth of endogenous contaminants.

Physiological Deep Dormancy

Although seeds of mountain silverbell reach full size by mid autumn without stratification, this does not necessarily mean that the embryos inside are capable of germination. For decades

it has been known that the embryos of many species, particularly in temperate regions, are held in a dormant state until an unfavorable balance between growth promoting and growth inhibiting hormones is overcome by exposure to cold treatments. This form of dormancy, termed physiological deep dormancy, is thought to represent a specialized survival mechanism which prevents germination in the autumn just prior to the onset of freezing temperatures. Exposure of the seeds to cold winter conditions instead causes a shift in hormonal concentrations so that embryos emerge from dormancy and germinate when temperatures are more suitable for seedling establishment (Evenari, 1949; Nikolaeva, 1969; Leopold and Kriedemann, 1975; Bidwell, 1979).

The contention by previous investigators, that the fruits of the piedmont-coastal plain form of silverbell undergo a mandatory cold stratification of several months duration, suggests that the primary benefit of low temperatures may be in overcoming physiological barriers such as those described above (Giersbach and Barton, 1932; Bonner and Mignery, 1974; Dirr, 1983). This, however, has not been demonstrated conclusively. Neither has it been for the mountain silverbell, a fall fruiting species adapted to cool montane climates. This tree's particular adaptation to colder sites may further add an ecological basis for the low temperature requirement of the fruit in preparation for germination.

In vitro culturing of excised embryos is a commonly recognized method used to detect the occurrence of physiological deep dormancy. Although embryos under the influence of this type of dormancy are

nearly always capable of germination when transferred to an agar medium, the growth and development of the resultant seedlings is delayed and abnormal due to residual hormonal imbalances. Such seedlings are referred to as "physiological dwarfs" because they typically exhibit stunted epicotyls which results in a rosette-like growth pattern (Crocker and Barton, 1953; Nikolaeva, 1969; Kozlowski, 1979).

Test for Physiological Deep Dormancy

To test for physiological deep dormancy in mountain silverbell, a series of *in vitro* trials were made at various stages of embryo maturity. It was reasoned, that if present, embryos from non-stratified fruits should yield dwarf seedlings while those from fruits exposed to natural overwintering conditions should grow normally.

The non-stratified fruits used in these tests were obtained from the Stratton Meadow area and consisted of three separate collections made during July, September, and November, 1983. Twenty seeds were removed from the fruits of each respective seedlot on the day following their collection, and the excised embryos aseptically cultured on Knop's medium as described earlier *in vitro* experiments. Fruits produced during the same year, but which had undergone natural stratification on the forest floor, were harvested from Stratton Meadow in March, 1984. Embryos derived from these were then immediately cultured and used for comparison.

The non-stratified embryos and the naturally stratified controls were monitored over a 6 week period and data detailing their length and dry weight growth recorded at periodic intervals. To better understand the comparative response to the two treatments, mean separation techniques were used. The data evaluated were seedling dry weights after 6 weeks.

Growth of non-stratified and stratified embryos. Hypocotyl and cotyledonary greening was observed in the majority of July collected embryos, but complete germination, marked by both radicle and plumule elongation, was present in only 15 percent (3 of 20 embryos). Although germination rates and seedling growth was generally poor (Figures V-12 and V-13; Appendix C), those that did germinate showed none of the symptoms characteristic of physiological deep dormancy.

The use of embryos obtained from the September and November seedlots led to a clear improvement in germinative performance and subsequent seedling development. Each of these germinated readily within 1 week and showed a normal growth pattern throughout the course of the trial. Seedling dry weight accumulation after 6 weeks in culture was significantly greater in both September and November than in July ($pr > f = 0.0001$).

When analyzed separately, November collected embryos were found to perform even better than the September group. Even though germination rates were similar, the November seedlings exhibited

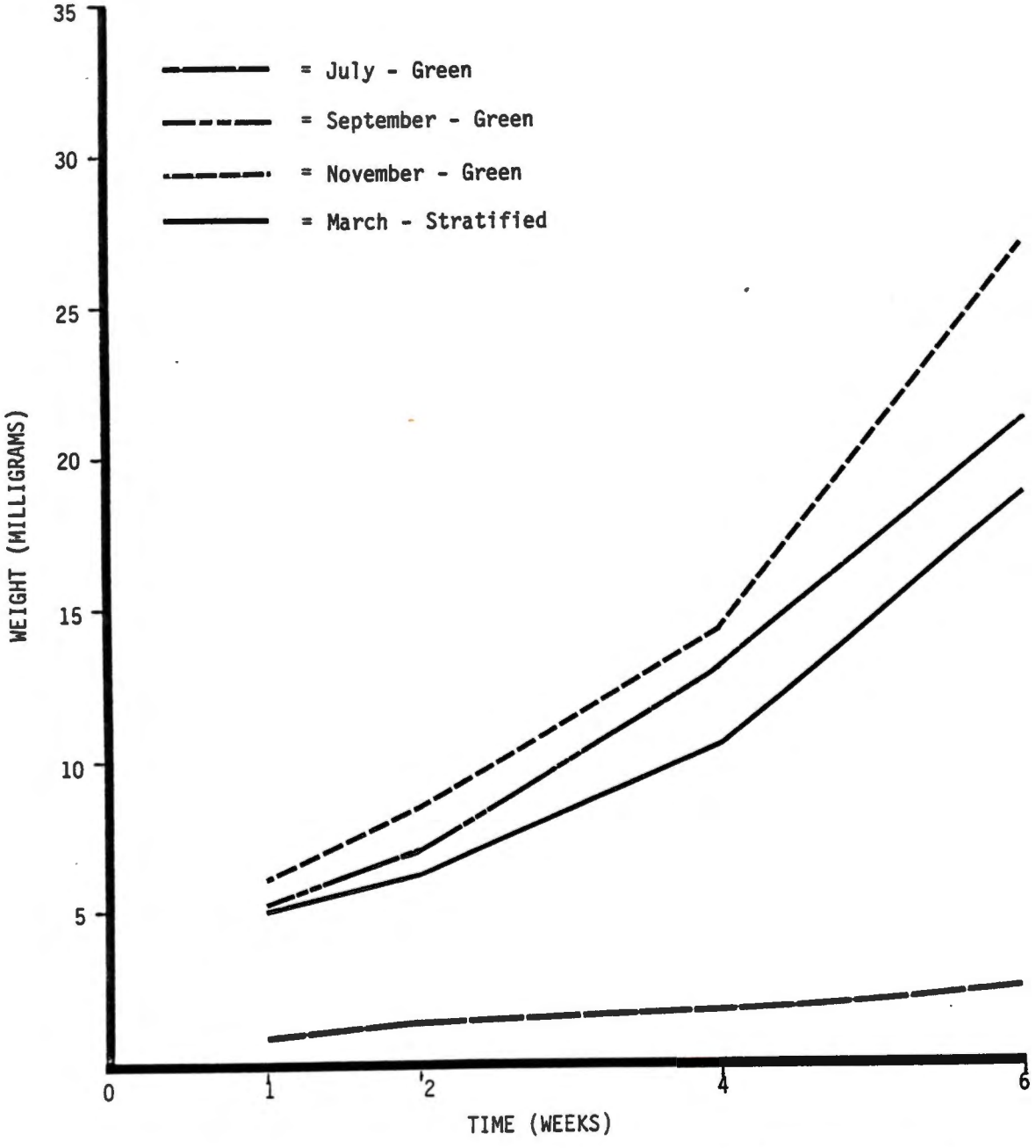

Figure V-12. Oven Dry Weight of Seedlings Derived from Green and Stratified Embryos

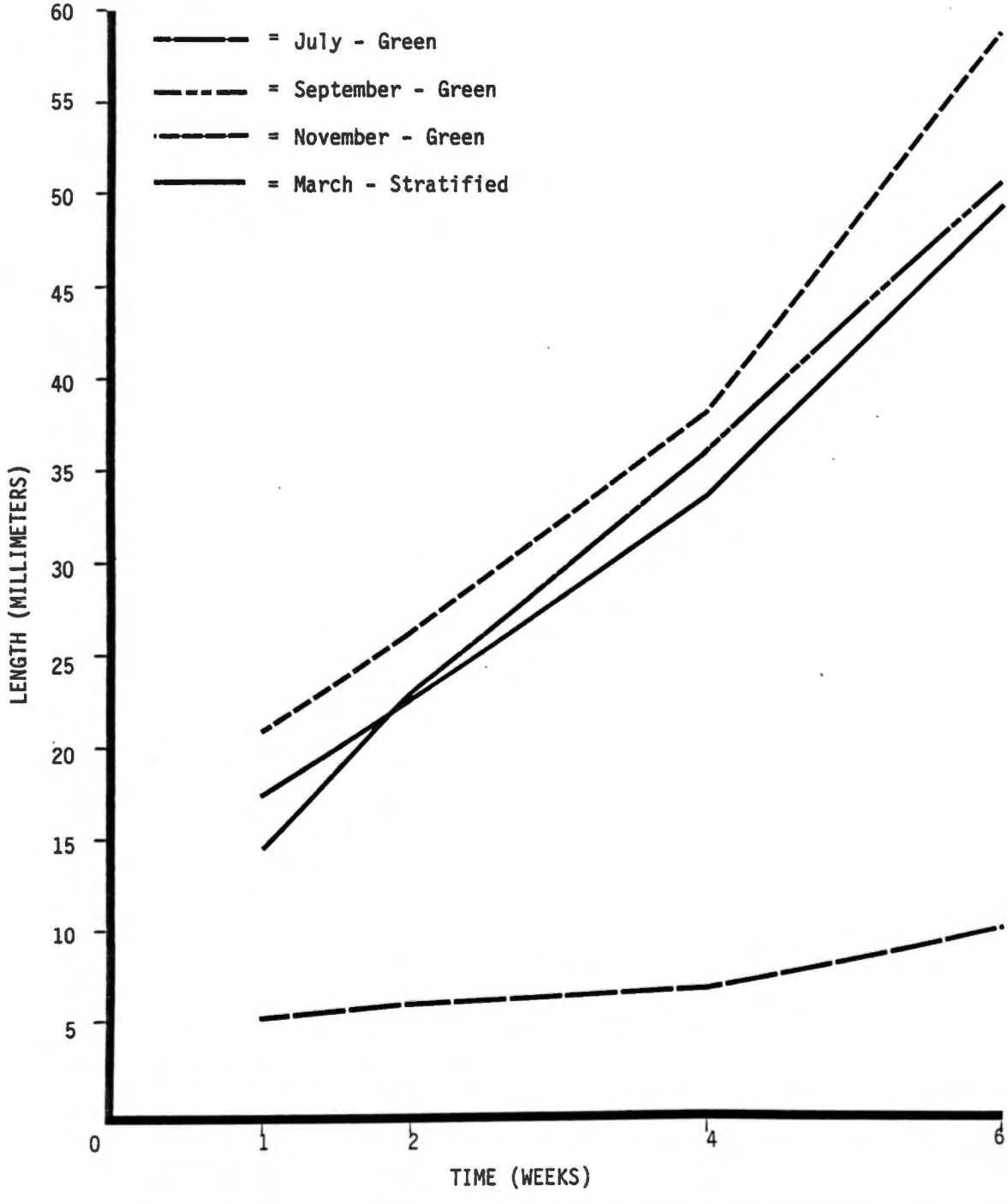

Figure V-13. Length of Seedlings Derived from Green and Stratified Embryos.

nearly a 16 percent gain in length and a 27 percent gain in dry weight after 6 weeks in culture. This increase in seedling dry weight over the September seedlot was also found to be statistically significant ($pr > f = 0.0057$; Appendix C).

While it was known from previous experiments that stratified embryos were capable of germination and development into normal, vigorous seedlings when cultured, these trials illustrated by way of comparison that equal or better results could be obtained by employing non-stratified embryos collected in early to mid autumn. Growth rates of stratified control seedlings were found to consistently lag behind those of both September and November in real terms (Figures V-12 and V-13).

The absence of dwarf, rosette-like growth patterns in the seedlings derived from green, non-stratified embryos clearly suggests that deep physiological dormancy is not involved in delaying the germination of mountain silverbell. This finding is particularly noteworthy when viewed in the context of *in vitro* propagation since it indicates that vigorous seedlings can be derived from excised embryos without first having to expose them to a time consuming cold stratification.

Seedling growth in relation to embryo development. The previous experiments indicate that germination and seedling development are not influenced by physiological deep dormancy. They are, however, very closely tied to the physical dimensions of the embryo at the time of culturing. In July, embryo lengths and weights were at

a comparatively low level reflecting their underdevelopment. It was not unexpected, therefore, that they were, for the most part, unable to germinate normally when cultured. The progressive increase in embryo development that took place *in situ* in September and November led to a corresponding improvement in their growth potential. Best seedling growth occurred in November when the embryos reached maximum size.[11]

Overwintering seemingly represented a biological cost to the embryos. Although 100 percent viable, the reduction in bulk due to long-term respiration and endogenous contamination was likely responsible for their reduced growth rates in culture.

Dormancy and the Role of Natural Stratification

The successful germination and growth of excised embryos from fall collected fruits indicates that the embryos themselves are not fundamentally dormant at the time of fruit abscision and are not bound by any morphological or deep physiological constraints. Since inhibiting chemicals also do not seem to present a major impediment to germination, dormancy in this species appears to be related to those physical barriers which temporarily restrict the access of water to the quiescent seed. Previous dye penetration and water scarification tests have already established that the bony endocarp is such a barrier.

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[11]The 40 seedlings derived from non-stratified embryos collected in September and November were subsequently transferred to soil and grew normally under greenhouse conditions.

Mechanical dormancy, similarly related to hard seed coverings, could further contribute to a germination delay by preventing the emergence of the embryo once hydration has been completed. The occurrence of this mechanism, while far less certain than physical barriers, would appear plausible because of the sutureless nature of the endocarp.

Although several months of stratification are adequate to bring about germination in most species exhibiting physical and mechanical dormancy, the amount of time necessary to promote the emergence of silverbell embryos has been found to vary considerably among seedlots (Giersbach and Barton, 1932; Bonner and Mignery, 1974). There was also evidence to suggest strong germinative variability among individual fruits. The cause or causes underlying this erratic behavior has never been critically evaluated and remains largely a matter of speculation.

Under conditions of artificial stratification, where silverbell fruits are kept buried in a moist, non-sterile medium, erratic germination could be accounted for by varying endocarp density and variable placement of the seeds within the matrix of the endocarp. Such configuration would permit water to access the embryos at different rates. It would also influence the relative effectiveness of microbial decomposers in reducing mechanical barriers of the endocarp walls since the walls are not uniform.

With natural stratification, not only might the anatomical variability of the fruit affect the rate at which hydration and

decomposition take place, but so might the depth at which the fruits lie within the forest litter. During the month of March, 1984, it was observed that fruits sitting on the surface of the duff material were similar in outward appearance to freshly fallen fruits. Even after several months of exposure to the elements, very little external tissue decomposition or moistening could be detected. On the other hand, fruits buried in the moist leaves were noticeably sodden indicating the possibility of embryo hydration.

In an attempt to judge the efficacy of natural stratification in overcoming physical and mechanical dormancy, and also to better understand the erratic germination of mountain silverbell seed, a series of cutting tests were conducted throughout the spring and early summer of 1984. Fruits used in the test were gathered from the ground at the Stratton Meadow collection area in late March, May, and June. Each lot consisted of 100 fruits. Half were collected from the surface while the other half were obtained from deep within the moist litter layer. Individual fruits were then sectioned longitudinally using the high speed electric grinding wheel. Through this process the degree of tissue decomposition and the ingress of moisture into the internal fruit structures could be more accurately evaluated.

Fruits of the March collection were considered to have been exposed to a minimum of 4 months of stratification since the vast majority of fruits fall to the ground by the end of November. Even after 4 months, sectioning revealed no visual alteration of the hard endocarp in fruits collected from the surface of the litter

layer. Similarly, no water infiltration could be detected. All of the seeds remained unimbibed and tightly bound within their woody enclosures.

The fruits which had been buried within the wet leafy matter had become porous enough, in some instances, to allow moisture to penetrate to the level of 8 seeds. Each of these was positioned shallowly within the endocarp near the outer surface of the fruit. Although swelling was not discernible, partial hydration was indicated by color and textural changes in the endosperm material. Seeds exposed to water were transformed from their normal yellow-white hue, to pure white. The endosperms also became noticeably spongy and pliant when compared with their former semi-cornified state. Total seed moisture content determinations (including both endosperm and embryo) confirmed these visual observations. Moisture content of hydrated seeds was found to have increased to 47.7 percent, nearly doubing the average of 26.4 percent for the more deeply imbedded, non-hydrated seeds.

In May, after approximately 6 months of exposure, fruits lying on the surface of the forest floor began to show obvious outward signs of deterioration. The presence of fungal mycelia on the partially eroded exocarps of many fruits helped to explain the nature of the external decomposition. With the exception of the presence of fungi, however, these fruits appeared in all other respects, like those more deeply buried fruits which had been studied 2 months earlier during the month of March. The endocarp material

was still solid and rather impervious, allowing moisture penetration to only 15 of the most peripherally located seeds. Moisture content of these seeds was found to average 46.3 percent while non-hydrated seeds averaged 28 percent.

Buried fruits were characteristically in a much more advanced state of decomposition. In all but a few instances, the four-winged exocarp had completely disintegrated, making them difficult to locate in the litter. At this stage of stratification, the rate of deterioration of the endocarp itself was found to be surprisingly variable and was probably related to the varying hardness of the material. In some fruits, large pieces of the woody enclosure could be easily broken off with the thumbnail while in many others only the very outer surfaces could be barely stripped away. In spite of this heterogeneity, all seeds, regardless of depth within the endocarp, were hydrated. Moisture content was found to be 52 percent for the entire lot.

Along with increased moisture content and the advent of warmer spring temperatures came the first indications of significant seed contamination. Even though only 19 of a total of 73 seeds were involved (26 percent), the severity of the necrosis in 12 of those seeds (16.4 percent) would have, in all probability, rendered them incapable of germination.

A high level of seed hydration and moderate ambient temperatures would seem adequate to initiate germination of non-contaminated seeds, but a careful survey of 1/100 hectare at Stratton Meadow

showed extremely low germination among the hundreds of fruits examined. Only two recently germinated seedlings could be found. Because the fruit casing from which they emanated were still attached to the young plants it was clear that, in both cases, germination occurred from the peripheral region of fruits whose endocarps had become very friable. Such poor overall germinative performance would suggest that what Nikolaeva (1969) termed "mechanical dormancy" might still have the capacity to strongly limit embryo emergence in a large portion of the previous year's fruit crop well into the growing season.

The final collection from Stratton Meadow, in June, was made only with great difficulty because of heavy and continual red squirrel predation. Virtually all fruits at or near the surface of the leaf litter had either been stripped of their seeds or cached away so their condition could not be adequately evaluated. The only intact fruits available were those deeply buried in the duff. As a result of having been exposed to the forces of microbial activity for an extended period of time, these fruits were generally more decomposed than ones examined a month earlier. Internally, though, seed moisture content was identical to the May collection stabilizing at 52 percent.

Contamination levels among hydrated seeds continued to increase markedly over time. Of 82 seeds examined, 25 (30.5 percent) were judged to be no longer viable while another 30 (36.6 percent) exhibited lesser degrees of necrotic spotting. The remaining 27 (32.9 percent) appeared to be completely sound.

Successful germination in the wild remained at very low levels even though temperatures appeared adequate for growth. Only one newly germinated seedling over a 1/100 hectare sampling area was observed during the month of June.

The results of these trials have shown that natural stratification has the potential to eliminate the physical and mechanical barriers to germination in mountain silverbell. However, because environmental conditions and the structural makeup of individual fruits is highly variable, so is the rate at which germination can take place.

Field observations indicate that fruits which abscise in early or mid autumn soon become covered with leaf-fall. Since leaves effectively retain moisture, fruits buried within them tend to become hydrated more quickly than those sitting on the surface of the litter layer. With the coming of warm spring temperatures, fruits encorporated in the litter layer are also more prone to decomposition because the incidence of microbial activity is much higher in this type of environment than at the surface. In this way, mechanical restrictions are more effectively overcome.

How rapidly germination barriers are eliminated is further controlled by the density of the endocarp and how deeply encased the seeds are within that endocarp. Pervious fruits buried in the moist litter and containing seeds close to the periphery of the endocarp wall seem to be able to germinate more readily since the physical and mechanical impediments are less severe. It was from

three fruits of this type that germination was first observed in May and June.

Most fruits, nevertheless, have more deeply held seeds and possess endocarps that are remarkably resistant to microbial erosion. Such persistent mechanical barriers seem to be highly disadvantageous and may be very important in helping to explain the low germination percentages observed in mountain silverbell. Although physical barriers to water availability are eventually relieved, seeds which remain in a hydrated state and unable to germinate allows destructive pathogens enough time to reach lethal levels. This is particularly true as the summer progresses and the ambient temperature increases. In June, for example, fully 2/3 of the seeds examined were either kileld or appeared in jeopardy of losing their viability. If this trend were to continue, a large percentage of the total seed crop would be lost by the end of the growing season.

Other very important factors which may be involved in reducing germination, but not analyzed in the current study, include the potentially negative interactive influence of high seed moisture content and persistent coverings in inhibiting embryo respiration. It is well known that the embryo's oxygen requirement normally increases substantially upon hydration due to an increase in metabolic activity. The possibility exists that some viable, non-contaminated seeds may, in effect, suffocate before emergence can occur. High saturation levels, resulting in the leaching of soluble food reserves or food mobilizing enzymes from the seed, could likewise play a deleterious role and need to be explored further.

CHAPTER VI

SUMMARY AND DISCUSSION

Despite mountain silverbell's high potential as an ornamental and for timber products, a very limited number of seedlings are available on the commercial market. The same holds true for the ornamentally attractive piedmont-coastal plain form of the tree which bears nearly identical fruits. Low germination percentages and erratic germination rates over a prolonged period make conventional seed propagation difficult and expensive and likely accounts for the relative unavailability of seedlings. Current nursery practices, as Dirr (1983) has stated, commonly involve "fall planting" and a great deal of "patience."

In light of these difficulties, a study was undertaken to use previously unexplored means to promote the early germination of mountain silverbell seeds. The primary goal of the study was to attempt to hasten germination so that seedlings suitable for out-planting could be obtained within a year's time.

Initial experiments focused on the most obvious impediment to germination; the barrier of the bony, sutureless endocarp. Chemical, water, and mechanical scarification, each of which has been successful in bringing about rapid germination in a wide array of hardseeded species, proved ineffective with mountain silverbell. Extreme variability in endocarp density and thickness among seedlots and even

among individual fruits resulted in unequal penetration of the chemical scarifiers. This made it impossible to predetermine effective exposure intervals. Additional problems with seed contamination arose when water or mechanical scarification procedures were used and resulted in complete failures in germination tests.

The feasibility of *in vitro* propagation was also examined because of the success of this technique in other species having germination problems similar to those found in silverbell. Seeds from fruits harvested in mid winter were removed from their restrictive enclosures using a high-speed grinding wheel and then surface sterilized to rid them of naturally occurring contaminants. Next, embryos were excised from the seeds, sterilized and finally cultured in closed test tubes containing agar-based Knop's medium.

One hundred percent germination over a 7 day period resulted from these procedures regardless of seed source. Six weeks later the seedlings were large enough to transfer to soil. After a brief period of acclimation, growth continued at appreciable rates. Within a year the seedlings averaged more than a meter in stem height. Subsequent field plantings demonstrated the capacity of *in vitro* propagated seedlings to adapt to climatic extremes of the outside environment.

The rapid and complete germination of excised, cultured embryos contrasts with the extremely delayed and erratic germination rates reported from both the wild, and under nursery conditions. These findings suggest that *in vitro* propagation allowed prompt germination

to take place largely as a result of its capacity to circumvent naturally occurring dormancy mechanisms. Because there is very little substantive information available to document the specific causes underlying dormancy in this species, the beneficial role which *in vitro* culturing plays can only be understood through a better comprehension of those dormancy mechanisms.

The earliest observers believed that the lignified endocarp was the primary impediment to germination. Even so, they failed to conduct tests to determine whether this was due to a physical blocking of water to the seed, or if it was the result of the endocarp mechanically limiting embryo expansion after seed hydration had occurred. Inhibiting chemicals located in the external coverings could have interfered, but this also was not closely scrutinized. In each of these cases, however, complete removal of the embryo from the enclosures would appear to be advantageous in promoting germination.

Later reports, which pointed to the absolute necessity of subjecting fall collected fruits to natural overwintering (or artificial stratification designed to mimic overwintering) in order to obtain germination, introduced the possibility that additional endogenous germination inhibiting factors may be involved. Even though stratification does have the ability to gradually overcome the physical, mechanical, and chemical barriers referred to above, it is more commonly prescribed as a presowing treatment to relieve morphological and physiological forms of dormancy.

The existence of morphological and physiological dormancy in mountain silverbell, and the necessity of stratification to relieve them, is unknown but highly plausible. It is, therefore, questionable as to whether excised embryos can be successfully cultured without such pre-treatment.

Because of this basic uncertainty, a second objective was established to provide propagators and seed scientists with insight into the most likely source(s) of dormancy in mountain silverbell. It was hoped that such information would, at the same time, serve to better clarify the beneficial role that stratification might play and encourage further research in this area.

Chemical dormancy was the first mechanism examined. To test for the presence of germination inhibiting chemicals, separate extracts were derived from each of the various fruit and seed layers and incorporated into Knop's growth medium. Excised embryos were then cultured in the mixtures and their responses monitored over a 6 week period. Extracts from both non-stratified green and naturally stratified fruits were included to detect any lessening of inhibitor activity that might be associated with the stratification treatment.

Experiments failed to show germination inhibition in any of the extract cultures at the applied concentrations. Germination occurred in 100 percent of the embryos over a 7 day period. Although germination was not prevented, extracts were shown to exert a suppressive influence on seedling dry weight growth and morphological

development. Growth abnormalities were manifested most clearly in seedlings grown in extracts derived from green, non-stratified fruits.

Because the trials relied on external applications of extract in the context of an *in vitro* system, it was not possible to say with certainty whether, under natural conditions, chemicals prevent germination or interfere with seedling growth. However, since fruits lying on the forest floor are continually exposed to high levels of leaching and microbial decomposition, it was concluded that their long-term intervention would be unlikely.

To investigate the possibility of morphological dormancy, a series of cutting tests were undertaken. Seed and embryo growth were monitored both before and after exposure to natural stratification in an attempt to detect changes in embryo development. Results showed that after spring flowering, length and weight parameters increased steadily throughout the summer but then began to slowly taper off by mid autumn. Maximum development was attained in November at a time corresponding with the highest incidence of fruit abscision. Over the same period, moisture content decreased until finally stabilizing at approximately 25 percent in September.

After undergoing stratification, seed and embryo lengths and weights continued to decline but weight losses appeared more significant. Since moisture content of the stratified tissues was found to be 25 percent, equalling prestratification levels, the weight losses could not have resulted from water reductions alone.

The source of the reductions was associated primarily with endogenous contaminants which partially eroded the seeds in about 1/4 of the sample population. Low level seed respiration may have also played a small rôle in reducing weight, particularly in seeds where contamination was not apparent.

It was concluded that morphological underdevelopment does not contribute to dormancy in mountain silverbell. Seeds and embryos attain full size in the fall under ambient forest conditions, so a subsequent period of stratification is not necessary to promote their *in situ* development. Moreover, stratification may even reduce viability because of the encroachment of endogenous contamination and loss of seed mass.

The presence of deep physiological dormancy was suspected as a potential cause of germination inhibition in mountain silverbell. Even though seeds of this type appear completely developed, a period of overwintering or stratification is necessary to rectify an unfavorable balance between growth inhibiting and growth promoting hormones. Deep physiological dormancy is easily detected in *in vitro* culture because embryos under its influence give rise to seedlings with dwarfed and otherwise abnormal growth patterns.

To test for the occurrence of this dormancy type, standard embryo culturing techniques were employed. The growth of excised non-stratified embryos was compared to that of stratified. It was reasoned that if hormonal imbalances existed, embryos from green fruits would yield dwarf seedlings while those from naturally stratified fruits would grow normally.

Seedlings obtained from non-stratified embryos collected in September and November were found to equal or outperform the stratified in terms of length and dry weight accumulation. The lack of dwarfing in the non-stratified group clearly suggests that deep physiological dormancy is not a factor in delaying the germination of mountain silverbell and that prolonged stratification to overcome it is therefore not required.

The same results also showed that seedling development (in the context of *in vitro* culture) is closely related to the physical dimensions of the embryo from which it was derived. Best overall growth occurred in November precisely at the time when embryos reached their maximum size.

The absence of morphological and deep physiological dormancy in the embryo, plus the apparent lack of significant chemical inhibitors in the fruit and seed coverings, indicate that the most likely source of delayed germination is related to the bony, sutureless endocarp. Because of its structure, it was felt that the endocarp constituted a physical barrier to the ingress of water to the embryo. It was also suspected that this same layer could further delay germination for a time by mechanically restricting expansion once water became available.

Since natural overwintering or stratification is presently considered requisite for germination, its beneficial function is most likely tied to its ability to gradually break down one or more of these impediments within the endocarp. Determining whether both

are involved, as well as how rapidly they are eliminated under natural conditions, was the aim of the last set of experiments.

The results of cutting tests made during the latter stages of the natural stratification process demonstrated that the physical barriers to moisture availability remain largely intact throughout the cold portion of overwintering. In March, however, after approximately 4 months on the forest floor, this began to abate. At the same time, it became evident that the rate at which the physical impediments were being relieved depended very much upon the anatomical makeup of each individual fruit and also its relative position within the leaf litter. The only embryos receiving an external supply of water were those few that were shallowly placed within the matrix of the endocarp and which were deeply buried within the moisture-conserving duff material.

This same general pattern was observed in May. Externally, fruits at the surface level remained quite dry, but buried fruits were saturated. Moisture content of whole seeds contained within buried fruits were also found to be much higher, nearly doubling that of seeds excised from fruits found at the surface.

While long-term exposure in a moist environment undoubtedly led to the diminution of physical barriers and contributed to an increase in the percentage of seeds being hydrated, the indirect effect of higher temperature cannot be overlooked. With the advent of warm spring weather came a corresponding increase in the activity of decomposers. These organisms were responsible for noticeably

increasing the friability of the endocarp tissue in a portion of the fruit crop which likely fostered the infiltration process.

Even though seeds and embryos became hydrated by late spring and the endocarps no longer posed a physical barrier, germination was observed in only three cases over a 1/100 H. sampling area. In each instance seedlings arose only from peripherally located embryos where the endocarp was thinnest. However, since the vast majority of seeds are much more deeply embedded, it was suspected that the persistent bony coverings mechanically restricted embryo emergence well into the growing season. Probably as a result of their failure to germinate promptly, contaminants were allowed to reach levels high enough to kill about 1/3 of the seeds examined in late June.

Aside from combined physical and mechanical dormancy mechanisms, and the attending problem of contamination, other factors may delay or totally prevent germination from occurring. These include, inadequate embryo respiration due to the interaction of high moisture content and persistent coverings, and also the possible leaching of soluble food materials or food mobilizing enzymes late in the stratification process. Each of these needs to be investigated further.

Ecological Implications

Upon first impression, the inability of mountain silverbell embryos to germinate rapidly would appear to be highly counterproductive

in terms of survival. Each year, a large portion of the seedcrop is consumed by squirrels as the fruits overwinter on the forest floor. Countless more seem to lose much of their vitality due to prolonged low level seed respiration. Still others are gradually overcome by contaminants.

Nevertheless, closer scrutiny indicates that this delaying mechanism actually contains elements which are extremely advantageous for survival in a temperate environment. Because mountain silverbell is a fall fruiting species, immediate germination might result in many of the seedlings being killed or severely injured by freezing temperatures that would soon follow. Instead, hard coverings allow the quiescent seeds to survive through the cold winter months so that germination can take place when growing conditions are more favorable. The extremely heterogeneous nature of the fruits, furthermore, does not allow germination to occur all at once. Instead, emergence is prolonged over a period of at least several months. This delay would likely be beneficial by permitting seedling establishment and survival despite erratic growing conditions. Although the first emerging seedlings may be killed by early frosts, drought, or other environmental factors, a sufficient number of seeds remain viable to ensure adequate reproduction.

While not directly observed in the current study, reports from several other sources indicate that germination delays of 2, or even up to 3 years, are possible in some seedlots under certain environmental conditions. Such an occurrence would seem to provide

for the build up of a seed reserve in the soil allowing for continued reproduction even during unusual years when a total crop failure occurs.

Implications for Propagators

From the results of the study on dormancy mechanisms, it would appear that the *in vitro* culturing of excised mountain silverbell embryos is successful because it effectively removes the barriers to water availability and likely allows free expansion of the embryo once growth is initiated. Other benefits may include improved conditions of embryo aeration, a readily available source of nourishment from the growth medium, and the ability to remove naturally occurring contaminants. The absence of morphological under-development or residual hormonal imbalances in the embryos from fall collected fruits further eliminates the need for time consuming stratification treatment prior to culture.

In vitro propagation of individual embryos represents a reliable means of obtaining small quantities of seedlings in a far shorter period of time than is currently possible with any other conventional seed propagation method. It is, however, not without drawbacks. The process is highly labor intensive, and the specialized facilities and equipment required (transfer hood, autoclave, glassware, etc.) are costly. Economic considerations may not justify its use unless these are already on hand. Commercial feasibility would be greatly enhanced through the development of a multiplication regimen where

very high rates of clonal material could be mass produced in a relatively short period of time. Selecting the optimum hormonal levels for such manipulations, though, would require additional experimentation.

LITERATURE CITED

LITERATURE CITED

Artmann, J. O. 1935. Common Forest Trees of the Norris Lake Region. Tennessee Valley Authority. 48 pp.

Atwater, B. R. 1980. Germination, Dormancy, and Morphology of the Seeds of Herbaceous Ornamental Plants. Seed Sci. and Tech. 8:523-73.

Ayres, H. B. and W. W. Ashe. 1905. The Southern Appalachian Forests. U.S. Geological Survey Professional Paper No. 37, Washington, D.C. 291 pp.

Baily, L. H. 1935. The Standard Cyclopedia of Horticulture. 3 vols. Macmillan Co., New York.

Barr, A. J., J. H. Goodnight, J. P. Sall, W. H. Blair, D. M. Chilko, J. T. Helwig, and J. A. Council. 1979. SAS User's Guide. SAS Institute, Inc. Raleigh, NC. 494 pp.

Barry, J. B. 1917. Georgia Forest Trees. Ga. St. Coll. of Agric., Univ. of Ga., Athens. 68 pp.

Bean, W. J. 1973. Trees and Shrubs Hardy in the British Isles (Eighth Edition), 2 vols. John Murray Pub., London.

Bewley, J. D. and M. Black. 1985. Seeds: Physiology of Development and Germination. Plenum Press, New York. 367 pp.

Bonner, F. T. 1975. Maturation of Black Cherry Fruits in Central Mississippi. U.S.F.S. Res. Note SO-205. 4 pp.

Bonner, F. T. 1976. Fruit Maturation in Hardwoods. U.S.F.S. Res. Note SO-121. 5 pp.

Bonner, F. T., B. F. McLemore, and J. P. Barnett. 1974. Presowing Treatment of Seed to Speed Germination. In: Seeds of Woody Plants in the United States. U.S.D.A. Agr. Handbook No. 450. Washington, D.C. pp. 126-135.

Bonner, F. T. and A. L. Mignery. 1974. Halesia carolina var. carolina L., Carolina Silverbell. In: Seeds of Woody Plants in the United States. U.S.D.A. Agr. Handbook No. 450. Washington, D.C. pp. 441-442.

Bidwell, R.G.S. 1979. Plant Physiology (Second Edition). Macmillan Pub. Co., Inc., New York. 726 pp.

Borthwick, H. A., S. B. Hendricks, E. H. Toole, and V. K. Toole. 1954. Action of Light on Lettuce Seed Germination. Bot. Gaz. 115:205-225.

Braun, E. L. 1950. Deciduous Forests of Eastern North America. Blakiston Co., Philadelphia. 596 pp.

Burd, S. 1976. Untitled. Unpublished M.S. Thesis, Univ. of Ga., Athens.

Chester, E. W. 1966. A Biosystematic Study of the Genus Halesia Ellis (Styracaceae). Unpublished Ph.D. Dissertation, Univ. of Tenn. 299 pp.

Coker, W. C. and H. R. Totten. 1945. Trees of the Southeastern United States. Univ. of N.C. Press, Raleigh. 419 pp.

Crocker, W. 1906. Role of Seed Coats in Delayed Germination. Bot. Gaz. 42:265-291.

Crocker, W. 1916. Mechanics of Dormancy in Seeds. Am. Jour. Bot. 3:99-120.

Crocker, W. and L. V. Barton. 1953. Physiology of Seeds. Chronica Botanica Co., Waltham, Mass. 267 pp.

Dirr, M. A. 1977. The Silverbells. American Nurseryman 146(3): 12-13, 42-46.

Dirr, M. A. 1983. Manual of Woody Landscape Plants (Third Edition). Stipes Pub. Co., Champaign, Ill. 826 pp.

Doran, W. L. 1957. Propagation of Woody Plants by Cuttings. Univ. of Mass. Coll. of Agric., Exp. Sta. Bull. No. 491. pp. 40.

Esau, K. 1977. Anatomy of Seed Plants (Second Edition). John Wiley and Sons, Inc., New York. 550 pp.

Evenari, M. 1949. Germination Inhibitors. Bot. Rev. 15:153-194.

Evenari, M. 1965. Light and Seed Dormancy. Ency. Plant Phys. 15(2):804-841.

Gibson, H. H. 1913. American Forest Trees. Hardwood Record, Chicago. 708 pp.

Giersbach, J. and L. V. Barton. 1932. Germination of Seeds of the Silver Bell, Halesia carolina. Contrib. Boyce Thompson Instit. 4-5:27-36.

Grant, J. A. and C. L. Grant. 1943. Trees and Shrubs for Pacific Northwest Gardens. Pacific Books Pub., Palo Alto, Ca. 323 pp.

Halward, R. E. 1965. Propagation of Difficult, Unusual, and Rare Plants. Proc. Int. Plant Prop. Soc. 15:206-207.

Hare, R. C. 1981. Nitric Acid Promotes Pine Seed Germination. U.S.F.S. Research Note SO281:1-2.

Harrar, E. S. 1967. Hough's Encyclopedia of American Woods. 16 vols. Robert Speller and Sons, New York.

Hartmann, H. T. and D. E. Kester. 1983. Plant Propagation (Fourth Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J. 727 pp.

Heit, E. C. 1967. Propagation from Seed Part 6: Hardseededness--A Critical Factor. Am. Nurseryman 125(10):10-12, 88-96.

Hesse, C. O. and D. E. Kester. 1955. Germination of Embryos of Prunus Related to Degree of Embryo Development and Method of Handling. Proc. Amer. Soc. Hort. Sci. 65:251-64.

Holmes, J. S. 1911. Forest Conditions in Western North Carolina. N.C. Geol. and Econ. Surv. Bull. 23:1-111.

Holmes, J. S. 1954. Common Forest Trees of North Carolina (Seventh Edition). N.C. Dept. of Conservation and Development, Raleigh. 169 pp.

Hora, B. 1981. The Oxford Encyclopedia of Trees of the World. Oxford Univ. Press, Oxford. 288 pp.

Hunt, F. A. 1984. National Register of Big Trees. American Forests 90(11):29-39.

Isely, D. (Ed.). 1965. Rules for Testing Seeds. Assoc. Offic. Seed Anal., Lake Mills, Iowa. 112 pp.

Jack, J. G. 1894. Patience with Germinating Seeds. Garden and Forest 7:135-136.

Keeler, H. L. 1912. Our Native Trees and How to Identify Them. (Eighth Edition). Charles Scribner's Sons, New York. 533 pp.

Kramer, P. J. and T. T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, New York. 811 pp.

Krugman, S. L., W. I. Stein, and D. M. Schmitt. 1974. Seed Biology. In: Seeds of Woody Plants in the United States. U.S.D.A. Agr. Handbook No. 450. Washington, D.C. pp. 5-40.

Lammerts, W. E. 1942. Embryo Culture, An Effective Technique for Shortening the Breeding Cycle of Deciduous Trees and Increasing Germination of Hybrid Seed. Amer. Jour. Bot. 29:166-171.

Laurie, A. 1930. Use of Peat in the Greenhouse. Ag. Exp. Sta., Mich. St. Coll. Ag., East Lansing. Special Bull. No. 194, pp. 21-24.

Lawrence, G.H.M. 1951. Taxonomy of Vascular Plants. Macmillan Pub. Co., New York. 823 pp.

Lemmon, R. S. 1955. The Silver-bell. Flower Grower 42(4):86, 135.

Leopold, A. C. and P. E. Kriedemann. 1975. Plant Growth and Development (Second Edition). McGraw-Hill, Inc., New York. 545 pp.

Lin, C. F. and A. A. Boe. 1972. Effects of Endogenous and Exogenous Growth Regulators on Plum Seed Dormancy. Jour. Amer. Soc. Hort. Sci. 97:41-44.

Lipe, W. and J. C. Crane. 1966. Dormancy Regulation in Peach Seeds. Science 153:541-42.

Martin, A. C. 1946. The Comparative Internal Morphology of Seeds. Amer. Midl. Nat. 36:513-660.

Martin, G. C., H. Forde, and M. Mason. 1969. Changes in Endogenous Growth Substances in the Embryo of Juglans regia During Stratification. Jour. Amer. Soc. Hort. Sci. 94:13-17.

Miller, F. H. 1938. Brief Narrative Descriptions of the Vegetative Types in the Great Smoky Mountains National Park. Report to the Superintendent, Great Smoky Mountains National Park. 17 pp. (typewritten)

Miller, H. A. and H. E. Jaques. 1972. How to Know the Trees (Second Edition). William C. Brown Co. Pubs., Dubuque, Iowa. 302 pp.

Nikolaeva, M. G. 1969. Physiology of Deep Dormancy in Seeds. Israel Program for Scientific Translations, Jerusalem. 220 pp.

Nikolaeva, M. G. 1977. Factors Affecting the Seed Dormancy Pattern. In: The Physiology and Biochemistry of Seed Dormancy and Germination. A. A. Khan (Ed.). North Holland Pub. Co., Amsterdam. pp. 51-76.

Norstog, K. 1979. Embryo Culture as a Tool in the Study of Comparative and Developmental Morphology. In: Plant Cell and Tissue Culture. W. R. Sharp, et al. (Eds.). Ohio St. Univ. Press, Columbus. pp. 179-202.

Peattie, D. C. 1950. A Natural History of Trees. Houghton Mifflin Co., Boston. 548 pp.

Pijl, L. van der. 1969. Principles of Dispersal in Higher Plants. Springer-Verlag, Berlin. 153 pp.

Radford, A. E., H. A. Ashles, and C. R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. Univ. of N.C. Press, Chapel Hill. 1183 pp.

Ramaley, F. 1899. Seedlings of Certain Woody Plants. In: A Biosystematic Study of the Genus Halesia Ellis (Styracaceae). E. W. Chester (Ed.). Unpublished Ph.D. Dissertation, Univ. of Tenn. p. 15.

Record, S. J. and R. W. Hess. 1949. Timbers of the New World. Yale Univ. Press, New Haven. 640 pp.

Rehder, A. 1913. Neue oder Kritische Geholze. In: A Biosystematic Study of the Genus Halesia Ellis (Styracaceae). E. W. Chester (Ed.) Unpublished Ph.D. Dissertation, Univ. of Tenn. p. 157.

Reveal, J. L. and M. J. Seldin. 1976. On the Identity of Halesia carolina L. (Styracaceae). Taxon 25(1):123-140.

Ridley, H. N. 1930. Dispersal of Plants Throughout the World. L. Reeve and Co., Ltd., London. 744 pp.

Rudnicki, R. 1969. Studies on Abscisic Acid in Apple Seeds. Planta 86:63-68.

Rudolf, P. O. 1969, 1970. Observations and Data Filed. U.S.F.S. N. Cent. For. Exp. Stn., St. Paul, Minn.

Salisbury, F. B. and C. W. Ross. 1978. Plant Physiology (Second Edition). Wadsworth Pub. Co., Inc., Belmont, Ca. 422 pp.

Sargent, C. S. 1921. Notes on North American Trees VIII. Jour. Arnold Arboretum 2:164-174.

Schroeder, E. M. 1935. Dormancy in Seeds of Benzoin aestivale L. Contrib. Boyce Thompson Instit. 7:411-419.

Shanks, R. E. 1954a. Reference Lists of Native Plants of the Great Smoky Mountains. Botany Dept., Univ. of Tenn. 17 pp. (mimeographed)

Shanks, R. E. 1954b. Climates of the Great Smoky Mountains. Ecology 35:354-361.

Sondheimer, E., D. S. Tzou, and E. C. Galston. 1968. Abscisic Acid Levels and Seed Dormancy. Plant Phys. 43:1443-1447.

Spongberg, S. A. 1976. Styracaceae Hardy in Temperate North America. Jour. Arnold Arboretum 57:54-73.

Stevenson, G. B. 1967. Trees of the Great Smoky Mountains National Park. Great Smoky Mtns. Nat. Hist. Assoc. 32 pp.

Stupka, A. 1964. Trees, Shrubs, and Woody Vines of Great Smoky Mountains National Park. Univ. of Tenn. Press, Knoxville. 186 pp.

Sudworth, G. B. 1897. The Forest Flora and Conditions of Middle and East Tennessee. In: The Forests of Tennessee (G. B. Sudworth and J. B. Killebrew, Eds.) Pub. House M.E. Church, Nashville, Tenn. p. 18.

Van Dersal, W. R. 1938. Native Woody Plants of the United States--Their Erosion Control and Wildlife Values. U.S.D.A. Misc. Pub. No. 303. Washington, D.C. 362 pp.

Villiers, T. A. 1972. Seed Dormancy. In: Seed Biology, Vol. 2. T. T. Kozlowski (Ed.). Academic Press, New York. pp. 220-282.

Villiers, T. A. 1975. Dormancy and the Survival of Plants. Arnold Pub., London. 185 pp.

Villiers, T. A. and P. F. Wareing. 1965. The Possible Role of Low Temperature in Breaking the Dormancy of Seeds of Fraxinus excelsior. Journ. Exp. Bot. 16(48):519-532.

Vines, R. A. 1960. Trees, Shrubs, and Woody Vines of the Southwest. Univ. of Texas Press, Austin. 1104 pp.

Wharton, M. E. and R. W. Barbour. 1973. Trees and Shrubs of Kentucky. Univ. Press of Ky. 582 pp.

Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. Ecol. Monog. 26:1-80.

Williams, P. M., J. D. Ross, and J. W. Bradbeer. 1973. The Abscisic Acid Content of the Seeds and Fruits of Corylus avellana L. Planta 110:303-10.

Wright, R.C.M. 1973. The Complete Handbook of Plant Propagation. Macmillan Pub. Co., Inc., New York. 191 pp.

Wyman, D. 1953. Seeds of Woody Plants. Arnoldia 13:41-60.

Wyman, D. 1971. Wyman's Gardening Encyclopedia. Macmillan Co., New York. 1222 pp.

APPENDICES

APPENDIX A

UNUSUAL OCCURRENCES IN SEED AND FRUIT OF MOUNTAIN SILVERBELL

1. polyembryony--two embryos per seed; the radicle of the upper embryo nested between the cotyledons of the lower (two occurrences)
2. tricotyledonous embryos (two occurrences)
3. fruit containing five developed seeds, four proxmal and one distal (one occurrence); three proxmal and two distal (two occurrences)

APPENDIX B

IN VITRO CULTURING METHODOLOGY

Knop's Medium Formulation

Knop's Medium as Modified by Nitsch and Steeves

Knop's Stock X2		B5 Minor Elements Solution	
$Ca(No3)2 \cdot 4H2O$	1.000 gm.	$H2SO4$	0.500 ml.
$KNO3$	0.250 gm.	$MnC12 \cdot 4H2O$	2.500 gm.
$MgSO4 \cdot 7H2O$	0.250 gm.	$H3BO3$	2.000 gm.
$KH2PO4$	0.250 gm.	$ZnSO4 \cdot 7H2O$	0.050 gm.
De-ionized $H2O$	1000 ml.	$CoC12 \cdot 2H2O$	0.030 gm.
		$CuC12 \cdot 2H2O$	0.015 gm.
		$Na2MoO4 \cdot 2H2O$	0.025 gm.
		De-ionized $H2O$	1000 ml.

refrigerate

Ferric Citrate Stock Solution

$FeC6H5O7 \cdot 5H2O$	2.5 gm.
De-ionized $H2O$	100 ml.

Heat gently to dissolve, then refrigerate.

Medium - 1 Liter

Knop's solution X2	500 ml.	Mix the Knop's solution and water. Stir. Add the B5 minor elements, ferric citrate, sucrose, and stir until the sucrose dissolves. Adjust the pH to 5.5. Heat almost to boiling and add the agar slowly with continuous stirring until it dissolves.
De-ionized H20	500 ml.	
B5 minor elements	0.5 ml.	
Ferric citrate	0.4 ml.	
Sucrose, 2%	20.0 gm.	
Bacto-Agar 0.8%	8.0 gm.	

Sterilization Procedures for Halesia carolina Seed

Materials:

Sterilized
- 1000 ml. double distilled water in (2) 500 ml. flasks
- (2) 250 ml. flasks (cover opening with aluminum foil)
- (10) deep culture dishes with glass covers in place

Also
- (2) 10 ml. graduated cylinders
- (2) strips of "Para-film"
- 70 percent ethanol in squeeze bottle
- chalice containing 95 percent ethanol for flaming
- forceps
- timer
- "Kimwipes"
- marking pen
- 20 to 30 excised silverbell seeds

Procedures:

1. While the water and glassware are cooling (autoclave water and glassware separately to reduce condensation), add 10 ml. chlorox to one of the 10 ml. graduated cylinders. Next add 2 ml. of 0.1N hydrochloric acid to the second 10 ml. graduated cylinder. Cover both of these promptly with aluminum foil.

Under Laminar Flow Hood (thoroughly wipe down hood and everything to be put in it with 70 percent ethanol).

2. Remove the foil from one of the 250 ml. flasks. Add the 10 ml. chlorox.

3. Next add 200 ml. sterilized, double distilled water to produce a 5 percent chlorox solution. Agitate thoroughly.

4. Carefully decant 100 ml. of the chlorox solution into each of two sterilized culture dishes. Label.

5. Using fresh glassware, repeat steps 2, 3, and 4 to produce a dilute solution of hydrochloric acid. Label.

6. Add approximately 100 ml. of sterilized, double distilled water to each of the six remaining culture dishes. Label.

Sterilization

7. Add 10 to 15 seeds to the first culture dish containing the 5 percent chlorox solution. Agitate 3 minutes.

8. Flame corceps and quickly transfer the seeds to a culture dish containing sterilized, double distilled water. Agitate briefly.

9. Flame forceps and quickly transfer the seeds to a culture dish containing the dilute hydrochloric acid solution. Agitate 15 seconds.

10. Flame forceps and quickly transfer to a fresh sterilized, double distilled water bath. Agitate.

11. Finally, flame forceps and quickly transfer seeds to a third sterile water bath. Seal cover with "Para-film."

12. Repeat steps 7 through 11 for the second group of 10 to 15 seeds.

13. Store the two sealed culture dishes containing the sterilized seeds in the refrigerator for 48 hours to soften the endosperm.

Excision, Sterilization, and In Vitro Culture of Halesia carolina Embryos

Materials:

Sterilized - 1000 ml. double distilled water in (2) 500 ml. flasks
- (25) deep Petrie dishes
- (1) deep Petri dish containing approximately (20) 5 or 7 cm. circular filter papers.
- (2) 250 ml. flasks (cover opening with aluminum foil)

Also
- (2) 10 ml. graduated cylinders
- 70 percent ethanol in squeeze bottle
- chalice containing 95 percent ethanol for flaming
- forceps, scalpel, thin blade spatula
- dissecting microscope
- time
- "Kimwipes"
- marking pen
- appropriate culture medium
- 20 to 30 sterilized, hydrated silverbell seeds

Procedures:

1. While water and glassware are cooling (autoclave water and glassware separately to reduce condensation), add 2 ml. chlorox to one of the 10 ml. graduated cylinders. Next add 2 ml. of 0.1N hydrochloric acid to the second 10 ml. graduated cylinder. Cover both of these promptly with aluminum foil.

Under Laminar Flow Hood (thoroughly wipe down hood and everything to be put in it with 70 percent ethanol).

2. Remove the foil from one of the 250 ml. flasks. Add 2 ml. chlorox.

3. Next add 200 ml. sterilized, double distilled water to produce a 1 percent chlorox solution. Agitate thoroughly.

4. Carefully decant the contents into each of five sterilized deep Petri dishes. Label. (Do not add too much solution because these must be agitated later.)

5. Using fresh glassware, repeat steps 2, 3, and 4 to produce a dilute solution of hydrochloric acid. Label.

6. Add about the same volumes of sterilized, double distilled water to the remaining 15 deep Petri dishes.

Embryo Excision

7. Wipe down microscope and stage with 70 percent ethanol, flame forceps and add a filter paper circle.

8. Remove a seed from the sterile water bath and place it on the filter paper.

9. Flame forceps and scalpel. Carefully make a shallow, longitudinal incision through the fleshy endosperm taking care not to injure the embryo. Peel back endosperm and tease out embryo.

10. Flame forceps and place the embryo in one of the Petri dishes containing sterilized, double distilled water.

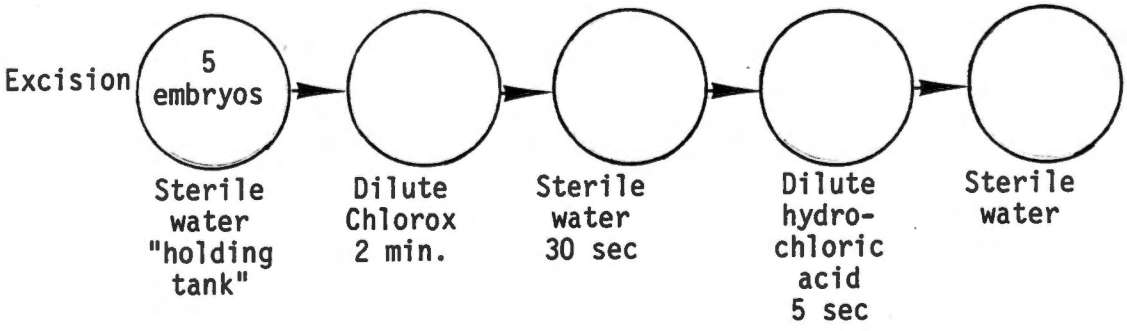
11. Repeat steps 8, 9, and 10 until five embryos have been excised. (Note that the same Petri dish containing the first excised embryo, can be used as a "holding tank" for up to five embryos. This prevents their dissication.)

12. Once five embryos have been procured, remove them from the sterile water bath with flamed forceps and place them in a Petri dish containing the chlorox solution. Agitate for 2 minutes.

13. Flame forceps and quickly transfer the embryos to a Petri dish containing sterile water. Agitate for 30 seconds.

14. Flame forceps, and quickly transfer the embryos, this time to a Petri dish containing the hydrochloric acid solution. Agitate for 5 seconds.

15. Finally, flame forceps, and quickly transfer to a Petri dish containing fresh, sterilized water.



Embryo Culture

16. For each transfer, remove an individual embryo from the final water bath and place it on a fresh filter paper circle to remove excess water.

17. Flame spatula and use normal procedures to transfer the embryo to the appropriate culture medium. Continue until all five embryos are cultured.

18. Repeat steps 7 through 17, excising and culturing five embryos each time. Proceed until the supply of seed is exhausted.

APPENDIX C

INVESTIGATION OF DORMANCY MECHANISMS

Table C-1. Summary of Growth Parameters for Control and Stratified Extract Treated Seedlings.[a]

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				REPLICATE 1 - Control			
1	$\bar{x}$	20.70	11.60	6.80	2.70	29.24	5.32
	sx	7.70	7.31	1.15	1.64	5.82	0.30
2	$\bar{x}$	25.70	16.10	7.50	11.90	44.04	6.68
	sx	4.40	2.92	0.35	2.75	13.32	1.06
4	$\bar{x}$	33.30	19.30	7.60	21.80	73.04	10.34
	sx	7.88	8.17	3.42	3.36	12.37	1.39
6	$\bar{x}$	57.30	30.50	9.10	33.10	139.60	18.18
	sx	10.75	7.26	3.85	3.52	17.98	2.10
				Exocarp Extract			
1	$\bar{x}$	8.10	1.60	4.70	0.00	11.62	4.32
	sx	0.74	0.22	0.45	0.00	2.91	0.33
2	$\bar{x}$	16.00	7.40	6.50	6.00	24.42	5.08
	sx	5.93	4.66	1.54	4.78	8.09	0.72
4	$\bar{x}$	28.40	16.70	6.60	20.00	65.54	9.50
	sx	4.04	3.96	2.22	4.40	9.67	0.70
6	$\bar{x}$	41.20	20.30	8.80	29.00	108.06	15.76
	sx	9.24	4.91	1.75	5.16	19.18	1.97

Table C-1 (continued)

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				Endocarp Extract			
1	$\bar{x}$	11.20	5.80	4.90	0.00	12.98	4.34
	sx	5.15	4.91	0.22	0.00	3.28	0.38
2	$\bar{x}$	13.00	5.80	5.60	6.70	22.22	4.90
	sx	3.74	3.90	1.39	2.88	2.23	0.65
4	$\bar{x}$	24.40	12.70	6.20	18.30	53.14	7.94
	sx	5.28	4.97	2.80	3.07	6.66	0.76
6	$\bar{x}$	39.80	18.60	7.00	29.90	99.08	13.80
	sx	8.11	7.24	2.55	2.45	17.14	2.03
				Endosperm - Seed Coat Extract			
1	$\bar{x}$	6.90	1.40	3.90	0.00	9.20	3.98
	sx	0.74	0.22	0.55	0.00	1.97	0.50
2	$\bar{x}$	16.10	7.10	4.70	5.00	22.34	5.62
	sx	4.17	3.93	0.57	4.32	4.39	0.96
4	$\bar{x}$	24.40	12.80	5.00	17.70	48.80	7.64
	sx	5.87	5.37	0.35	2.64	6.99	0.63
6	$\bar{x}$	37.30	14.50	5.50	25.00	88.22	13.02
	sx	9.30	5.29	1.27	5.46	15.65	1.82
				REPLICATE 2 - Control			
1	$\bar{x}$	20.50	7.60	5.90	3.00	23.58	5.08
	sx	6.64	2.63	0.96	1.46	5.88	0.71
2	$\bar{x}$	22.80	12.10	7.00	13.00	39.74	6.64
	sx	8.66	5.73	0.87	5.10	6.67	0.45
4	$\bar{x}$	37.10	19.60	7.80	22.90	76.50	10.64
	sx	6.16	6.22	2.56	2.33	11.98	1.29
6	$\bar{x}$	44.30	21.30	9.20	32.90	121.90	17.50
	sx	15.43	4.07	4.04	4.22	28.12	3.17

Table C-1 (continued)

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				Exocarp Extract			
1	$\bar{x}$	11.90	4.50	5.50	0.40	15.56	4.58
	sx	4.76	1.77	1.46	0.55	5.46	0.55
2	$\bar{x}$	16.90	7.70	6.40	11.30	28.70	5.50
	sx	8.18	5.75	1.29	4.10	6.36	0.54
4	$\bar{x}$	27.60	12.50	6.50	19.20	63.70	9.76
	sx	3.83	4.14	0.93	4.02	4.56	0.76
6	$\bar{x}$	34.70	14.80	8.00	27.20	98.30	14.50
	sx	4.48	3.47	1.12	2.86	9.27	1.14
				Endocarp Extract			
1	$\bar{x}$	14.00	6.90	4.70	0.50	15.00	4.94
	sx	4.31	3.20	0.57	0.50	3.33	0.24
2	$\bar{x}$	16.60	8.90	5.10	9.50	34.86	5.68
	sx	5.18	4.56	1.39	5.43	11.61	0.61
4	$\bar{x}$	25.00	12.20	5.60	20.60	61.84	9.86
	sx	4.53	4.72	1.14	2.68	8.52	0.91
6	$\bar{x}$	34.20	14.00	5.80	26.90	103.64	14.32
	sx	4.32	4.36	0.76	3.23	1.74	0.47
				Endosperm - Seed Coat Extract			
1	$\bar{x}$	8.00	2.10	3.90	0.20	9.56	4.20
	sx	1.54	1.34	0.41	0.45	3.72	0.60
2	$\bar{x}$	11.20	3.30	4.50	6.10	24.84	5.60
	sx	0.97	1.40	0.87	4.34	4.38	0.26
4	$\bar{x}$	16.50	5.70	5.20	18.40	49.82	7.96
	sx	5.21	1.89	1.09	3.05	6.11	1.01
6	$\bar{x}$	26.10	9.80	5.30	25.00	80.86	12.74
	sx	5.61	2.17	0.91	4.08	13.07	1.65

[a]The means shown are the averages from 5 seedlings at each time interval.

Table C-2. Summary of Contrast Statements for Seedling Dry Weight Values at 6 Weeks and Treatment Type (Stratified Extracts).[a]

Contrast	df.	SS.	F.	PR>F
Treatment 1 vs. 2,3,4	1	55.30	16.25	0.0003**
Treatment 2 vs. 3,4	1	8.75	2.57	0.1186
Treatment 3 vs. 4	1	3.36	0.99	0.3275

**highly significant

[a]Treatment 1 = control
Treatment 2 = stratified exocarp extract
Treatment 3 = stratified endocarp extract
Treatment 4 = stratified endosperm-seed coat extract

Table C-3. Summary of Growth Parameters for Control and Green Extract Treated Seedlings.[a]

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				REPLICATE 1 - Control			
1	$\bar{x}$	18.80	10.60	5.50	3.80	24.44	5.42
	sx	2.75	3.63	0.94	1.04	5.70	0.41
2	$\bar{x}$	23.00	11.50	5.70	13.00	36.10	6.24
	sx	2.87	2.62	0.84	2.37	8.50	0.56
4	$\bar{x}$	30.10	12.30	6.30	20.90	60.14	10.86
	sx	5.49	2.75	0.76	4.64	12.11	1.70
6	$\bar{x}$	44.50	19.00	8.20	27.90	103.16	17.08
	sx	5.30	2.85	3.47	3.27	16.21	1.06
				Exocarp Extract			
1	$\bar{x}$	7.80	0.60	4.20	0.10	14.42	4.62
	sx	0.57	0.22	1.15	0.22	3.33	0.50
2	$\bar{x}$	12.30	2.50	4.50	4.20	25.80	5.66
	sx	3.42	2.21	0.35	2.80	3.86	0.57
4	$\bar{x}$	18.20	5.50	5.40	14.00	45.18	7.56
	sx	6.00	2.72	0.42	7.48	15.63	1.94
6	$\bar{x}$	29.00	10.70	7.50	23.40	92.22	15.44
	sx	5.47	4.24	4.51	7.21	20.73	2.69
				Endocarp Extract			
1	$\bar{x}$	7.10	0.30	4.40	0.00	10.86	4.42
	sx	0.42	0.27	0.55	0.00	2.50	0.33
2	$\bar{x}$	10.80	3.10	4.50	2.70	22.74	4.76
	sx	1.35	1.98	0.50	3.55	6.24	0.87
4	$\bar{x}$	16.00	4.20	5.20	11.40	44.86	7.10
	sx	4.20	1.75	1.26	10.85	24.39	2.42
6	$\bar{x}$	26.80	7.70	6.40	21.10	87.50	13.42
	sx	2.66	1.40	2.86	6.86	12.63	3.11

Table C-3 (continued)

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				Endosperm-Seed Coat Extract			
1	$\bar{x}$	6.90	0.00	4.20	0.00	12.20	5.32
	sx	0.22	0.00	0.76	0.00	2.63	0.69
2	$\bar{x}$	9.20	2.30	4.90	6.20	20.26	5.54
	sx	2.88	2.66	0.74	5.09	8.71	0.71
4	$\bar{x}$	23.30	5.70	5.30	20.50	55.26	9.92
	sx	6.25	2.89	2.11	11.83	10.37	2.18
6	$\bar{x}$	28.30	8.70	6.00	26.40	87.24	14.52
	sx	4.62	3.23	1.27	3.04	10.64	1.72

[a]Measurements represent values of 5 seedlings at each time interval.

Table C-4. Summary of Contrast Statements for Seedling Dry Weight Values at 6 Weeks and Treatment Type (Green Extracts).[a]

Contrast	df.	SS.	F.	PR>F
Treatment 1 vs. 2,3,4	1	25.74	7.57	0.0097**
Treatment 2 vs. 3,4	1	7.20	2.12	0.1554
Treatment 3 vs. 4	1	3.03	0.89	0.3528

**highly significant

[a]Treatment 1 = control
Treatment 2 = green exocarp extract
Treatment 3 = green endocarp extract
Treatment 4 = green endosperm-seed coat extract

Table C-5. Summary of Contrast Statements for Seedling Dry Weight Values at 6 Weeks and Treatment Type (Stratified vs. Green Extracts).[a]

Contrast	df.	SS.	F.	PR>F
Stratified vs. Green Treatment 1	1	1.60	0.47	0.4978
Stratified vs. Green Treatment 2	1	0.26	0.08	0.7856
Stratified vs. Green Treatment 3	1	1.09	0.32	0.5755
Stratified vs. Green Treatment 4	1	6.40	1.88	0.1797

[a]Treatment 1 = control
Treatment 2 = exocarp extract
Treatment 3 = endocarp extract
Treatment 4 = endosperm-seed coat extract

Table C-6. Summary of Seed and Embryo Measurements.[a]

		Seed Length (mm.)	Embryo Length (mm.)	Seed Fresh Weight (mg.)	Embryo Fresh Weight (mg.)	Embryo Dry Weight (mg.)	Endosperm Fresh Weight (mg.)	Embryo Moisture Content %
July	$\bar{x}$	8.53	5.23	10.87	2.64	0.83	8.24	68.99
	sx	1.23	1.17	1.95	0.84	0.42	1.41	13.42
Sept.	$\bar{x}$	9.72	9.80	19.19	6.78	5.04	12.40	25.33
	sx	0.76	0.85	2.74	1.08	0.78	2.09	8.01
Nov.	$\bar{x}$	10.72	10.92	24.00	8.05	5.98	15.95	25.58
	sx	1.20	1.15	3.71	1.19	0.77	2.85	5.29
March	$\bar{x}$	10.44	10.23	18.55	6.61	4.91	11.94	25.27
	sx	1.21	1.31	3.00	1.17	0.77	2.10	5.29

[a]Measurements represent values of 100 seeds and embryos at each time interval.

Table C-7. Summary of Growth Parameters for Seedlings Derived from Green and Stratified Embryos.[a]

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				July-Green			
1	$\bar{x}$	5.10	0.00	3.60	0.00	4.32	0.88
	sx	0.22	0.00	1.90	0.00	0.13	0.94
2	$\bar{x}$	6.00	0.20	4.10	1.20	7.88	1.30
	sx	1.97	0.45	0.22	2.68	7.96	1.16
4	$\bar{x}$	6.90	1.20	3.40	1.30	7.24	1.64
	sx	2.70	1.89	0.65	2.91	3.47	0.86
6	$\bar{x}$	10.30	2.80	4.00	4.10	13.02	2.38
	sx	10.50	5.46	0.94	9.17	15.52	1.97
				September-Green			
1	$\bar{x}$	14.60	6.40	5.30	2.10	13.14	5.44
	sx	4.14	2.19	0.45	1.78	5.89	1.11
2	$\bar{x}$	23.10	12.30	7.00	10.60	39.42	7.24
	sx	7.48	4.52	0.35	1.92	4.62	0.78
4	$\bar{x}$	36.00	12.60	5.90	25.50	81.56	13.12
	sx	5.79	3.66	0.89	1.77	8.93	1.76
6	$\bar{x}$	50.80	22.40	7.70	30.10	132.50	21.32
	sx	4.13	4.20	2.14	3.47	7.66	1.00
				November-Green			
1	$\bar{x}$	20.90	11.70	5.60	3.90	22.50	6.16
	sx	5.71	5.39	1.02	1.14	6.18	0.77
2	$\bar{x}$	26.30	16.80	6.20	14.10	57.76	8.54
	sx	6.40	4.16	0.57	4.04	6.64	0.37
4	$\bar{x}$	38.20	18.90	6.00	25.90	85.84	14.28
	sx	3.23	4.84	0.79	2.90	8.37	1.14
6	$\bar{x}$	58.90	23.90	6.10	34.00	172.66	27.06
	sx	8.62	3.54	1.75	1.22	29.25	4.88

Table C-7 (continued)

Week		Major Axis (mm.)	Radicle (mm.)	Longest Cotyledon (mm.)	Longest Leaf (mm.)	Green Weight (mg.)	Dry Weight (mg.)
				March-Stratified			
1	$\bar{x}$	17.30	6.70	5.60	2.50	22.16	5.18
	sx	2.97	2.61	1.14	2.00	4.15	0.32
2	$\bar{x}$	22.90	12.70	5.60	12.50	37.22	6.26
	sx	3.03	2.82	5.60	3.69	9.47	0.61
4	$\bar{x}$	33.80	19.20	6.10	22.90	74.74	10.58
	sx	9.53	7.34	0.96	3.23	17.20	1.26
6	$\bar{x}$	49.30	28.20	7.60	33.20	139.00	18.82
	sx	5.43	5.50	1.91	3.03	11.51	1.93

[a]Measurements represent values of 5 seedlings at each time interval.

Table C-8. Summary of Contrast Statements for Seedling Dry Weight Values at 6 Weeks and Date of Embryo Collection.[a]

Contrast	df.	SS.	F.	PR>F
Collection 1 vs. 2,3,4	1	13.54	1.67	0.2149
Collection 2 vs. 3,4	1	1585.59	195.37	0.0001**
Collection 3 vs. 4	1	82.37	10.15	0.0057**

**highly significant

[a]Collection 1 = March (stratified control)
Collection 2 = July (non-stratified)
Collection 3 = September (non-stratified)
Collection 4 = November (non-stratified)

VITA

Paul Conrad Durr was born in Englewood, New Jersey on February 10, 1954. In June, 1972 he graduated from Ramapo High School in Franklin Lakes, New Jersey and entered Muhlenberg College in September of that year. The author transferred to The University of Tennessee, Knoxville in September, 1975 and received a Bachelor of Science Degree in Forestry in June, 1979.

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