GROWTH AND DIEBACK OF UNDERPLANTED NORTHERN RED OAK SEEDLINGS UNDER VARIOUS LIGHT AND MOISTURE CONDITIONS

by

Thomas James Tworkoski

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APPROVED:

D. J. Parrish, co-chairman

D. Wm Smith, co-chairman

J. L. Hess

T. L. Sharik

D. M. Orcutt

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Blacksburg, Virginia

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INTRODUCTION

Northern red oak (Quercus rubra L.), an important commercial tree for furniture manufacturing, veneer, and pulp, is difficult to establish (Farmer 1975, Loftis 1979) and on many sites is being replaced naturally by commercially less desirable species (Phillips 1963, Trimble and Hart 1961). Difficulty in reestablishing red oak is largely caused by insufficient advance regeneration prior to harvest (Carvell and Tryon 1961) and by slow growth following canopy removal (Trimble 1974).

Advance understory regeneration must be present prior to canopy removal in order to maintain northern red oak as a major component of future stands (Arend and Scholtz 1969). Such advance regeneration may be difficult to establish, because acorn production varies each year and because of predation of acorns by wildlife. Consequently, tree harvests may need to be delayed up to 20 years to insure adequate accumulation of acorns and the resulting oak regeneration (Sander 1972). Planting seedlings in the understory (underplanting), may prevent such costly delays. Recent evidence has shown that red oaks can survive when planted in the understory (Smith, unpublished results). With such a practice, genetically-improved seedlings could have potential for use in a more flexible rotation. Underplanting

also could enable red oak stands to be established on appropriate sites that do not presently have a significant red oak overstory.

Oaks respond differently to various light and moisture conditions (Carvell and Tryon 1961) which, in turn, are related to the canopy cover. For example, following a harvest, oak regeneration may succumb to species that grow more rapidly in the clearings (Fowells 1965). Conversely, oak seedlings may undergo dieback at low light intensities.

Dieback is an apparently physiological (nonpathological) phenomenon involving the death of part or all of the shoot that results in a decrease in total height. In oaks stems will often resprout following dieback resulting in plants with root systems much older than stems (Merz and Boyce 1956). These resurgent individuals, termed seedling sprouts, are an important type of advance regeneration. The ultimate cause of poor stem growth and dieback is presumably stress (Seidel 1972) and the consequent disturbance of physiological processes. It would be valuable, therefore, to examine growth patterns and physiological responses of red oak seedlings to those light and moisture conditions that may be encountered under a canopy or upon canopy removal. Such information regarding response to canopy cover would be of benefit in formulating the silvicultural system for an

individual stand. It would also be of interest to compare growth of red oak with that of white oak (Q. alba L.) and white pine (Pinus strobus L.) because these three species occupy similar ecological niches (Powells 1965).

The objectives of this study were to: (1) determine the effects of light and moisture and their interactions on shoot growth and stem dieback in northern red oak seedlings; (2) quantify the changes that may occur during a growing season in carbohydrate reserves and auxin levels in northern red oak seedlings under different light and moisture conditions; and (3) examine the effect of canopy removal on carbohydrate reserves and growth of underplanted northern red oak seedlings during the first growing season after canopy removal and to compare such growth with that of white oak (Q. alba L.) and eastern white pine (Pinus strobus L.).

LITERATURE REVIEW

Establishment of Oaks for Advanced Regeneration

Northern red oak is difficult to establish and maintain on many sites. Even areas previously occupied by northern red oak have shifted to stands of less desirable and more tolerant species, such as dogwood (*Cornus florida* L.), hornbeam (*Carpinus caroliniana* Walt.), beech (*Fagus grandifolia* Ehr.), and red maple (*Acer rubrum* L.) (Tryon and Carvell 1958, Merz and Boyce 1958, Trimble and Hart 1961). Such failures often result from inadequate quantity or size of advanced oak regeneration (Beck 1980). In order to insure stand regeneration of red oak, large numbers of advanced regenerants must be present in the understory before the final harvest (Merz and Boyce 1956, Carvell and Tyron 1961). Failure of red oak to establish advanced regenerants may be aggravated by the number of acorns destroyed by animals and a hiatus of 2 to 5 years between heavy seed crops (Fowells 1965). Several silvicultural systems aimed at establishing necessary regeneration have been examined.

In Europe, oaks are naturally regenerated over a period of 20 to 40 years after an initial cut that increases the amount of vacant growing space (Evans 1982). Under this shelterwood cutting system, up to six additional light cuttings are necessary to allow more light onto the forest

floor. Alternatively, Korstian (1962) suggested a 2- or 3-cut shelterwood may be well-suited for regenerating even-aged oak stands. However, Beck (1980) reported that shelterwood cuts did not foster establishment of new oak seedlings, nor did they promote survival and growth of older seedlings. In another study, small openings (1/10 acre) such as might occur in a group selection resulted in abundant oak regeneration (Merz and Boyce 1958). However, the still-heavy shading and the moisture competition in very small openings, which occur under single tree selection, are likely to limit survival and growth of oaks (Musselman and Gatherum 1969). These studies demonstrate the importance of canopy shading and root competition in survival and growth of red oak seedlings. Once seedlings have germinated, additional crown exposure to sunlight is necessary for longterm survival (Lorimer 1981). If too little sunlight is available, red oak seedlings will likely succumb to more shade-tolerant species (Carvell and Tryon 1961).

Clearcutting has not been successful for northern red oak stand regeneration (McGee and Hooper 1970). Following one clearcut, oaks were a minor component of stand regeneration, even though 67% of the overstory had been oak (Merz and Boyce 1958). Apparently, oak regenerants were unable to compete successfully with faster-growing species. Loftis

(1979) found that planting oaks after clearcutting was not successful for regenerating oak stands even when competition from undesirable species was controlled. In contrast, large oaks (45-60 cm tall) have been successfully established in clearcuts in northern France (Evans 1982). However, costs of establishment were undoubtably high, because at least one weeding each year was necessary. Without large investments to control competition following a clearcut, red oak regeneration seems to be sure to fail (Powells 1965). In addition, oak regenerants must be at least 2.5 cm in diameter (ground line) if they are to survive following canopy removal (Sander 1971).

In general, silvicultural techniques to promote natural northern red oak regeneration have not been successful, nor has planting following a clearcut. Underplanting prior to harvest, however, may be a viable means for obtaining advanced northern red oak regeneration (Smith, unpublished data). In addition to providing adequate amounts of regeneration, underplanting may permit greater flexibility in the harvesting schedule, control the size and composition of regeneration, and introduce genetically superior planting

stock.

Effects of Light and Moisture on Growth of Oak Regenerants

As previously mentioned, once sufficient advanced oak regeneration has been established, the amount of canopy present and the degree of canopy removal is important to continued seedling survival and growth. Den Uyl (1972) noted that red oaks need early release from shading, because they seldom survive in the intermediate or suppressed crown class. The presence of an overstory will also tend to increase the competition for available soil moisture. Even with sufficient light, as available soil moisture decreases, photosynthesis decreases in oaks (Musselman and Gatherum 1969). Partial or complete canopy removal may be necessary to obtain the minimum of 30% full sunlight required by red oak for adequate photosynthesis (Phares 1971), since light intensity in the understory of a hardwood stand may be less than 20% full sunlight. Initially, soil moisture will be more available to seedlings following canopy removal. Therefore, both the shading of seedlings and the competition for moisture that are imposed by the overstory may be critical limiting factors in northern red oak survival and growth.

Microenvironmental conditions such as light availability have a significant impact on oak seedling growth and

persistence in the understory (Carvell and Tryon 1961). Under a full forest canopy, average height growth of oaks is very slow with up to 85% of the total seedling growth occurring during the first season of a six year period (Beck 1970). Generally, seedlings grown in reduced light have thin stems with reduced vascular tissues and weakly lignified tracheary elements (Zimmermann and Brown 1974). Shade also apparently induces or promotes dieback (Sander 1972, Lorimer 1981) with subsequent resprouting (Arend and Scholtz 1969). If light intensities remain low, sprout regeneration will decrease (Vogt and Cox 1970). The interruption of height growth associated with the top kill may allow competing vegetation to overtake dieback seedlings (Brenneman 1980). Consequently, the oak component of stand regeneration will become smaller. It is possible, however, that dieback provides a mechanism for seedling survival, perhaps through maintenance of an appropriate shoot/root balance.

Stem and root dry matter accumulation and root elongation are also predictably slowed under reduced sunlight (Zimmermann and Brown 1974). Farmer (1975) found that the dry weight of red oaks grown under 25% full sunlight was only 30% of those grown in full sun. This lower weight was accompanied by a shift in the distribution of dry matter to the leaves resulting in a higher shoot/root ratio. Longmann

and Coutts (1974) also found such a shift: under 20% full sunlight, shoot dry matter production was not affected, but root elongation and dry matter accumulation were greatly reduced. These findings are in general agreement with those of Shirley (1929) who found leaf area and leaf dry matter increased while woody cell production decreased at lowered light intensity, producing succulent, leafy growth. Larger leaf areas will presumably increase a plant's ability to intercept light and thus improve its chances for survival in low light. While promotion of leaf area is potentially beneficial, low light has a simultaneous adverse effect on oak regeneration in its diminution of root and secondary growth. Presumably, extensive root systems and ample xylem would favor interspecific competition by faster growth of oak seedlings (Arend and Scholtz 1969). The morphogenetic shift toward shoot growth under heavily shaded conditions, then, must represent the plant's strategy for survival. Periodic dieback of the shoot may be a necessary facet or desirable consequence of this strategy.

Available soil moisture as well as sunlight can be increased by canopy removal. Carvell and Tryon (1961) found that, when adequate light is available for oak growth, the next most limiting factor becomes soil moisture. For example, on ridges, upper slopes, or southerly aspects in West

Virginia, moisture was limiting and red oaks could not grow beyond the seedling stage even with adequate sunlight. Carvell and Tryon suggested that on more moist sites, larger crown openings should be provided toward the end of a rotation to provide sufficient sunlight for seedling survival; but, less canopy removal might be desirable on drier sites. Gatherum et al. (1963) found that drought survival of nursery-grown seedlings increased as light intensity increased up to, but not above, 3500 footcandles (35% full sunlight). Moisture stress appears to act as does light deprivation in that, in addition to slowing stem growth, moisture stress may also cause an imbalance in the shoot/root ratio. Larson and Whitmore (1970) found that, under moisture stress, new shoots developed but no new roots appeared. This growth differential was followed by a decrease in the number and diameter of stem vessels. Absorption area of roots and water conducting capacity of stems may thus be reduced by water stress.

Conditions of recurrent moisture stress appear to favor dieback (Zimmermann and Brown 1974). It appears, therefore, that available soil moisture as well as light intensity may

strongly influence growth and dieback of oak regeneration.

Effects of Light and Moisture on Other Physiological Processes

The foregoing discussion of growth suggests that the amount of canopy covering oak regenerants affects the quantity and distribution of photosynthate within a seedling. Northern red oaks are able to carry on photosynthesis in dimmer light and at lower moisture levels than many species (Ferrel 1953, Kramer and Decker 1944), but the two environmental factors interact such that photosynthetic rates decrease with decreasing soil moisture (Bourdeau 1954, Musselman and Gatherum 1969). Sunlight directly affects photosynthesis and photorespiration and thus affects the plant's overall carbohydrate economy including synthesis and assimilation of cell wall material (Zimmermann and Brown 1974). For example, at 5% full sunlight, height growth was limited because of decreased photosynthate in *Populus tremuloides* (Farmer 1963). Reduced carbohydrate levels may also contribute to plant mortality. Studying northern red oak trees, Staley (1965) found that decline resulted from diminished availability of carbohydrates. Diminution of carbohydrate reserves was due to defoliation or moisture stress. Subsequent mortality then resulted from extreme moisture stress.

In addition to stem and root elongation, secondary xylem growth may also be a major sink for available carbohydrates in red oak. Nearly 75% of the water in oaks move in the current year's xylem (Longmann and Coutts 1974), suggesting that when more photosynthate is used in the production of new vessels, more water can be moved. But, as suggested in the growth studies above, the use of carbohydrate for secondary growth may be at the expense of primary growth. When there is sufficient xylem to accommodate water transport beyond some minimal, maintenance levels, height growth may be triggered in oaks (Oliver 1978). If photosynthate is limiting secondary xylem growth, seedlings may be more susceptible to moisture stress. The relationships between stem/root growth, carbohydrate levels, current year xylem growth, and light or moisture conditions are not well known.

Hormones are important in growth and development, and, therefore, should not be overlooked as a possible cause of growth reductions or dieback. A hormonal imbalance can cause growth reduction in many systems. For example, by clipping apical meristems, Blake (1981) induced a decline in endogenous auxin levels in Eucalyptus that resulted in shoot dieback. Such shifts in hormone concentrations can result from phytochrome-mediated responses to light quality and

from reduced light and moisture in the understory. Under a forest canopy, light energy is minimal at 670 nm (red) and maximal at approximately 730 nm (near-infrared) (Kramer and Kozlowski 1979). The high levels of far-red light convert phytochrome from one biologically active form (Pfr) to another (Pr) (Smith 1975). Pr may influence hormonal levels by inhibiting IAA-oxidase or by enhancing reactions that produce gibberellins (Nitsch 1963). Thus, heavy shade may increase stem growth by increasing auxin levels due to decreased IAA-oxidase activity. In contrast, shade may lower IAA levels as a direct result from reduced meristematic activity (IAA production is thought to take place primarily in the apical meristem) and thus indirectly from light or moisture constraints (Zimmermann and Brown 1974).

Because IAA is an important stimulant of cell elongation (Salisbury and Ross 1978), auxins will likely play an important role in oak stem growth and/or dieback. IAA is also important in preventing senescence of some systems (Jacobs 1979). As stem senescence apparently involves the same general degenerative changes found in oak stem dieback, endogenous IAA levels may be related to dieback. Low levels of auxin in the stem may result in an altered hormonal gradient that contributes to initiation of stem abscission (Salisbury and Ross 1978). Reduced auxin levels under light

and moisture stress may therefore partially account for oak stem dieback. Arend and Scholz (1969) postulated that interruption of the auxin flow from apical meristems following crown deterioration initiates sprouting of axillary buds.

In addition to influencing stem growth and dieback, auxins are believed to play a role in apical dominance (Salisbury and Ross 1978). Decreased auxin production in apical buds resulting from light, moisture, or nutritional constraints may cause the loss of apical dominance. Such a loss may help explain the bushy, umbrella-shaped crowns commonly found in oaks that grow in the understory for long periods (Beck, personal communications). Similar plate-shaped crowns occur in Norway spruce (Picea abies (L.) Karst.) grown under heavy shade (Greis and Kellomaki 1981). Greis and Kellomaki attributed this crown form to decreased leader growth and to an increased growth of laterals.

Studies have shown hormone levels are of great importance during seedling growth. Farmer (1975) proposed that hormonal balance and not moisture availabiltiy was responsible for episodic growth in northern red oak. Larson (1970) found root growth in red oak to be more dependent on hormones supplied from an actively growing stem than on photosynthate availability. Because stem elongation appears to

be strongly influenced by auxins (Kramer and Kozlowski 1979) and because auxin metabolism can be changed by light and moisture, study of the effect of canopy removal on auxin levels may be useful in considering the ability of northern red oak seedlings to survive and grow.

Stem Dieback in Other Species

Dieback of small and large branches in the crowns of Norway maples (Acer platanoides L.) has been recently reported (Manion 1981). Manion proposed that age, root girdling, and biotic agents predisposed maples to dieback but that abiotic factors, such as drought, promoted such crown thinning. Staley (1965) included dieback of small upper crown twigs and their replacement by sprouts as symptoms of decline in mature red and scarlet oaks (Q. coccinea Muenchh.). Like Manion, Staley attributed dieback to a number of interrelated causes that reduce the amount of photosynthate produced, destroy weakened organs, and cause moisture stress. Thus, external factors that reduced production and availability of carbohydrates were largely responsible for decline. Working with black cottonwood (Populus trichocarpa Torr. and Gray), Heilman et al. (1972) also attributed stem mortality to reduced stored food reserves.

Blake (1981) reported stem dieback of coppice stools of Eucalyptus trees. He found no difference in stored carboh-

ydrate levels between seedlings that had or had not died back. However, Blake found that exogenous applications of IAA to decapitated stems maintained cell membrane integrity of cells adjacent to the cut and also prevented dieback. He attributed top kill to leakage of phenols from vacuoles. These phenols can have a toxic effect on cells and may act as cofactors in the destruction of auxin. Dieback of primary crown units and epicormic shoot development of Eucalyptus obliqua L. Herit. was reported by Kile et al. (1981). This dieback was attributed to a combination of factors, with drought as the primary cause and Armillaria spp. infection of stems as a contributing factor. Sapwood starch was lower in trees dying back than those not dying back. However, this difference was attributed to a reduction in crown area. Thus, starch reduction was an effect and not a cause of dieback.

MATERIALS AND METHODS

Two related experiments were performed in this study. In one experiment, northern red oak seedlings were greenhouse-grown under three light levels and two moisture levels. In a second experiment, seedlings of northern red oak and two other species that were planted in 1977 and 1978 under a mixed hardwood canopy were exposed to three levels of canopy removal in 1981. Survival, growth/dieback, and carbohydrate content of roots and stems of seedlings from greenhouse and field studies were determined.

Greenhouse Study

Twelve-hundred 2-0 bare-root northern red oak seedlings were obtained from Musser Nursery (Indiana, Pennsylvania). Very large or small plants were culled in order to obtain a more uniform group of 1080 seedlings. The seedlings were planted in 20 liter, plastic pots (2 seedlings/pot) on 1 April 1981. A mixture of alluvium and A1 horizon of a Calvin silt loam; clayey, mixed, mesic Typic Hapludult from a forested site in Montgomery County, Virginia was passed through a 1 cm screen and used as the growth medium. Soil tests indicated no macronutrient deficiencies (Table A8). Pots were placed under three levels of light (0, 63, and 92% shade) to simulate the range of light intensities seedlings might encounter in the field. Light intensity was regulated

by single layers of polypropylene shade cloth (Hummert Seed Co.; St. Louis, Missouri). Temperature under the three light intensities was measured with a hygro-thermograph (Bendix Corp.).

Within each light treatment, two levels of soil moisture were imposed. Soil water potential was monitored periodically using psychrometers (Chanel-Constantan Thermocouple Psychrometer/Hygrometer; Wescor, Inc.). Psychrometers were calibrated by submerging thermocouples in NaCl solutions of known water potential. Each thermocouple juncture was cleaned after submergence. Dew point depression was used for estimating water potential. All psychrometers had essentially the same calibration equation: Water Potential= (-.66) + (1.2) (microvolt reading corrected for temperature). Six pots containing psychrometers within each light and moisture treatment combination (36 psychrometers total) were used to monitor moisture conditions. Between waterings, soil was allowed to dry to -0.3 (+/- 0.1) or -2.0 (+/- 0.3) Mega Pascals (MPa) representing a range from readily to less available water (Larcher 1975). From 1 April until 1 July, the minimal moisture stress was defined as -1.0 instead of -0.3 MPa. Slow seedling growth, small leaf areas, and low evapotranspiration kept all pot soil above the -1.0 MPa level. Consequently, effects of moisture

treatment were not observed until after 15 June 1981. When the predetermined soil moisture limit was attained, plants were immediately rewatered to field capacity (water draining from pot bottoms as an indication). Beginning in mid-June, seedlings under minimal moisture stress were watered at intervals of 1 to 2 weeks while greatly-stressed seedlings were watered less frequently at intervals of 3 to 5 weeks.

Throughout the study (15 April to 1 October), seedling heights were recorded each week. Diameters at the root collar were measured on 15 April, 1 July, and 1 October. On 1 and 15 May, 1 and 15 June, 1 July, 1 August, 1 September, and 1 October, five seedlings were randomly selected within each replication of each treatment for destructive sampling. Current-season cambium activity, leaf area, and carbohydrate levels of roots and shoots were determined for each seedling (see below). The terminal 5 cm from the tallest living branch from each seedling was removed, placed in liquid nitrogen, lyophilized and stored at 0 C for IAA extraction and analysis. However, this amount of tissue subsequently proved to be too small for detection of auxin. Therefore, an evaluation of several IAA purification techniques have been reported in lieu of the originally-intended auxin quantification.

For measurements of cambium activity, a stem section was cut from each harvested seedling at a point 5 cm above the root collar. The width of the current year's xylem ring was measured after the stem had been cleanly recut on a microtome. Ring width in two directions (90 degrees to each other) was measured under a dissecting microscope. The two values were averaged to give one value per stem.

Total leaf numbers and areas were recorded for each group of five harvested seedlings. Leaf areas were measured with and automatic area meter (Hayashi Denko Co., Ltd.; Tokyo, Japan).

For stem carbohydrate analysis, a sample was taken from each of the five harvested seedlings. A 6-cm segment was cut from the stem midpoint, dried 24 hr at 90 C, and ground to pass a 40 mesh screen. The five stem segments from each treatment replication were pooled for carbohydrate analysis. Roots were sampled similarly. Total non-structural carbohydrates (TNC) were then assayed from two 200 mg powdered subsamples per replication. Each subsample was extracted and partially hydrolyzed in boiling .2 N sulfuric acid for 30 min. The hydrolysates were cooled and then neutralized with phosphate buffer, and Clarase (Miles Laboratories) was added to each. Clarase contains invertase, maltase, and diastase for digestion of non-structural carbohydrates. The

tissue was then incubated in Clarase for 48 hr at 27 C and assayed colorimetrically for TNC (Smith 1969). P-hydroxybenzoic acid hydrazide (PABAH) was added to an aliquot for colorimetric analysis of reducing sugars with an autoanalyzer (Technicon) (Davis 1976). The two TNC values for each treatment replication were averaged to give a single value for statistical analysis.

Statistical analysis for differences between light and moisture treatments utilized a 2 X 3 factorial design for each sampling period. Differences at the .05 alpha level were considered significant, and treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie 1960).

Field Study

In addition to the greenhouse study, a field experiment was conducted with northern red oak, white oak (Quercus alba L.) and white pine (Pinus strobus L.). Seedlings of each species had been planted in 1977 and 1978 under a mixed hardwood canopy. Twelve plots with a basal area from 23.0 to 27.5 m^2/ha were selected at the Reynold's Homestead Research Facility (Critz, Virginia). These plots were randomly scattered across the intervening slopes between two major ridges. Elevations of the plots were from 314 to 335 meters. Stand composition included yellow-poplar (Lirioden-

dron tulipifera L.), maples (Acer spp.), oaks (Quercus spp.), beech (Fagus grandifolia Ehrh.), Virginia pine (Pinus virginiana Mill.), and loblolly pine (Pinus taeda L.). The understory was composed of small hardwood reproduction, such as maple and oak, and honeysuckle (Lonicera japonica L.). Madison fine sandy loam and Louisa loam were the two major soils on these slopes.

Each plot received one of three levels of canopy removal: (1) none; (2) moderate, with approximately 11.5 m^2 residual basal area; and (3) clearfelling. These levels of canopy removal resulted, respectively, in 90, 70, and 0 % canopy cover during the growing season as measured by a spherical densiometer (Lemmon 1956). Treatments consisted of the three levels of canopy removal and the three species in an unbalanced and nested design. Differences at the .05 alpha level were considered significant, and means were separated by Harvey's procedure (Harvey 1975).

Heights of underplanted seedlings were measured monthly, and diameters were taken at the end of the growing season. On 1 November 1981, five saplings from each replication within each treatment were harvested for subsequent TNC analysis. In addition, five northern red oak wildlings were harvested from full canopy sites adjacent to the no canopy removal treatment. For carbohydrate and survival

comparisons, the plot was the experimental unit; so statistical analysis was a completely randomized design with 4 replications. Square root arc-sine transformations on percent survival were performed prior to statistical analysis.

RESULTS

The results of this study will be presented for the field experiment and then the greenhouse experiment. In the field experiment, complete, partial, and no canopy removal corresponded to 0, 70, and 90% canopy coverage over the forest floor. Field results will deal first with individual species' response to canopy coverage. Comparisons among species will then be made. In the greenhouse experiment, shade cloth provided 0, 63, and 92% screening of available light; and two levels of soil moisture were imposed on each light treatment. Greenhouse studies dealt only with northern red oak. Those results will be presented first for height growth and then for the other measured parameters.

Field Study

Canopy removal treatments were associated with significant differences in height growth only during August and October (Table 1). However, most height growth occurred during the early part of the growing season (Tables 2, 3, and 4). Consequently, canopy removal treatments had little effect on total seasonal height growth for any species investigated. It was not until late in the growing season that the new environmental conditions resulting from canopy removal treatments appeared to affect seedling performance.

Table 1. Significance of F values[1] for height and diameter growth due to species and canopy removal treatments in the field experiment in Patrick County, Virginia.

Dependent Variable	Independent Variable		
	Canopy Removal	Species/ Treatment	Interaction
Height			
April	ns	*	*
May	ns	*	*
June	ns	*	*
July	ns	ns	ns
August	*	ns	ns
September	ns	*	ns
October	*	ns	ns
Spring (Apr-Jun) Total	ns	*	*
Summer (Jul-Oct) Total	*	ns	ns
Entire Season	ns	*	*
Diameter			
Entire Season	ns	*	*

[1] * designates significance and ns designates non-significance at .05 alpha level.

Table 2. Mean height and diameter growth of planted white oak, northern red oak, and white pine seedlings in no canopy removal treatment areas of the field experiment in Patrick County, VA.

Measurment Period	Species/Treatment			
	WC[1]	RO	WO	WP
	Height Increment (cm)			
Apr	clipped	0.8 a[2]	1.0 a	3.6 a
May	11.7 a	1.1 c	1.0 c	3.8 b
Jun	1.5 a	0.4 a	0.4 a	0.8 a
Jul	0.4 a	0.1 a	0.2 a	0.4 a
Aug	0.3 a	0.2 a	0.4 a	0.4 a
Sep	0.1 a	0.4 a	0.2 a	0.3 a
Oct	0.1 a	0.1 a	0.1 a	0.3 a
Spring (Apr-Jun)	13.2 a	2.2 c	2.4 c	8.1 b
Summer (Jul-Oct)	0.8 a	0.7 a	0.8 a	1.1 a
Entire Season	14.1 a	3.0 c	3.3 c	9.5 b
	Diameter Increment (mm)			
Entire Season	2.1 a	-0.2 b	-0.2 b	0.3 b

[1] WC=white oak seedlings, clipped to the root collar prior to bud break, RO=red oak, WO=white oak, WP=white pine.

[2] Within row, means followed by the same letter are not significantly different at alpha level .05 (Harvey's Procedure). Number of replications: WC=37; RO=60; WO=41; WP=74.

Table 3. Mean height and diameter growth of planted white oak, northern red oak, and white pine seedlings in partial canopy removal treatment areas of the field experiment in Patrick County, VA.

Measurment Period	Species/Treatment			
	WC[1]	RO	WO	WP
	Height Increment (cm)			
Apr	clipped	1.1 a[2]	1.6 a	2.8 a
May	11.1 a	0.5 b	0.5 b	1.9 b
Jun	1.2 a	0.9 a	0.3 a	0.6 a
Jul	0.5 a	0.3 a	0.1 a	0.4 a
Aug	0.1 a	0.2 a	0.1 a	0.1 a
Sep	0.1 ab	-0.2 a	0.2 ab	0.5 b
Oct	0.1 a	0.0 a	0.4 a	0.2 a
Spring (Apr-Jun)	12.3 a	2.5 b	2.4 b	5.3 b
Summer (Jul-Oct)	0.6 a	0.3 a	0.4 a	0.9 a
Entire Season	13.1 a	2.9 b	3.2 b	6.4 b
	Diameter Increment (mm)			
Entire Season	2.8 a	0.3 b	-0.3 b	0.0 b

[1] WC=white oak seedlings, clipped to the root collar prior to bud break, RO=red oak, WO=white oak, WP=white pine.

[2] Within row, means followed by the same letter are not significantly different at alpa level .05 (Harvey's Procedure). Number of replications: WC=33; RO=50; WO=39; WP=87.

Table 4. Mean height and diameter growth of planted white oak, northern red oak, and white pine seedlings in complete canopy removal treatment areas of the field experiment in Patrick County, VA.

Measurment Period	Species/Treatment			
	WC[1]	RO	WO	WP
	Height Increment (cm)			
Apr	clipped	-2.8 a[2]	-2.4 a	2.6 a
May	8.5 a	-0.2 b	1.5 b	0.6 b
Jun	3.5 a	2.3 a	1.2 b	-0.9 c
Jul	1.6 a	1.5 a	0.5 a	0.0 a
Aug	1.5 a	0.5 b	0.3 b	0.3 b
Sep	0.3 a	0.3 a	0.2 a	0.8 b
Oct	0.3 a	-0.4 c	-0.7 bc	0.2 a
Spring (Apr-Jun)	12.0 a	-0.8 b	0.2 b	2.2 b
Summer (Jul-Oct)	3.3 a	2.3 a	1.1 b	1.0 b
Entire Season	15.4 a	1.2 b	0.6 b	3.4 b
	Diameter Increment (mm)			
Entire Season	3.8 a	0.8 b	-0.7 c	-0.8 c

[1] WC=white oak seedlings, clipped to the root collar prior to bud break, RO=red oak, WO=white oak, WP=white pine.

[2] Within row, means followed by the same letter are not significantly different at alpha level .05 (Harvey's Procedure). Number of replications: WC=38; RO=61; WO=41; WP=50.

The ranking of species by seasonal height growth (Tables 2,3 and 4) was the same regardless of harvest treatment; the greatest height growth (13.1 to 15.4 cm) occurred with white oak that had been clipped on 15 April 1981. However, by the end of one growing season, the height of clipped white oak was still less than it was prior to clipping. By season's end, clipped white oaks averaged 14 cm in contrast to their average height of 35 cm before clipping. White pine was intermediate in height growth, with 1981 growth increments ranging from 3.4 to 9.5 cm. Red and unclipped white oaks grew least (a range of .6 to 3.3 cm); their total seasonal growth usually did not differ significantly from each other. Although the ranking of species height growth was consistent within each canopy removal treatment, the amount of growth differed. This difference in species performance is reflected in numerous significant species X treatment interactions (Table 1). Individual species performance was therefore compared within each canopy removal treatment, followed by comparisons within species and among canopy removal treatments.

Height Growth Within Canopy Treatments

Under 90% canopy (no-cut treatment), most growth in all species occurred between 1 April and 1 July when temperatures rarely exceeded 32 C and soil moisture was plentiful

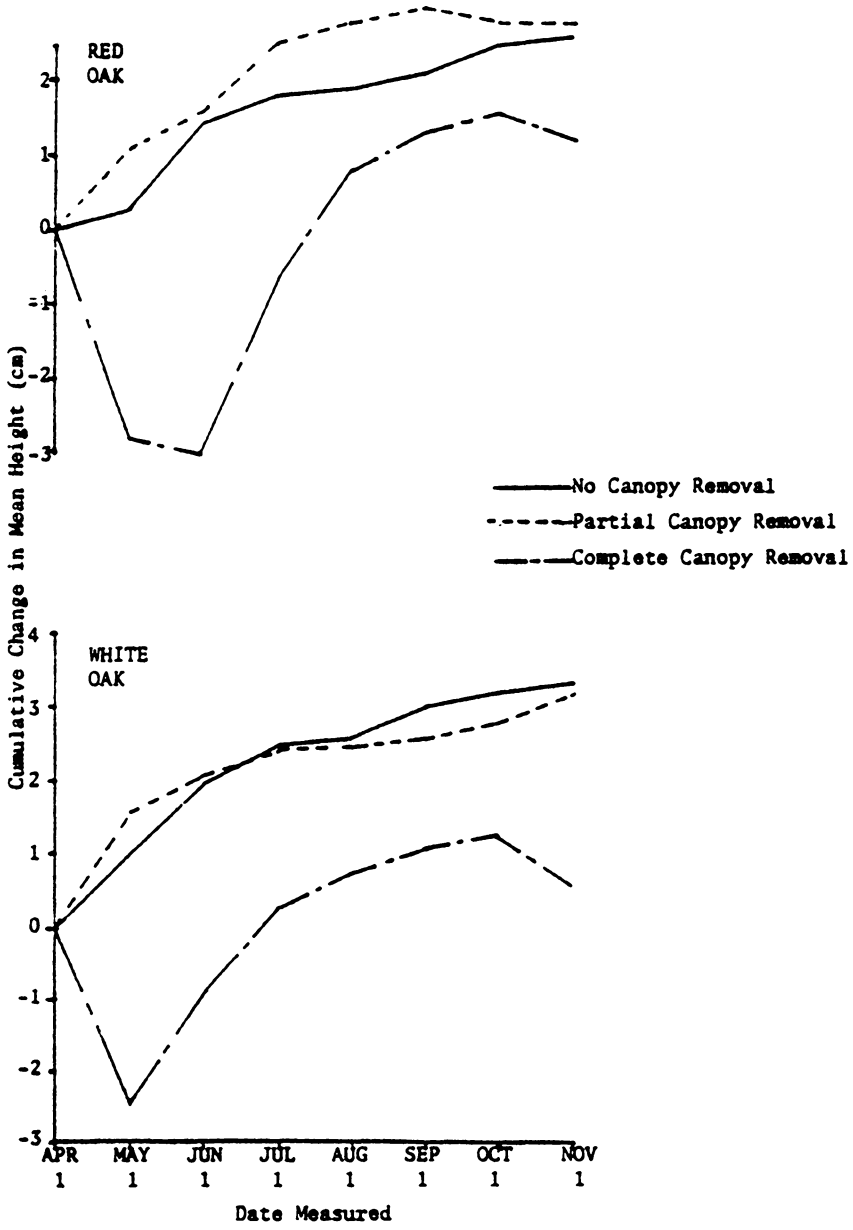

Figure 1. Cumulative mean height growth of underplanted northern red oak and white oak grown in the field after complete, partial, and no canopy removal.

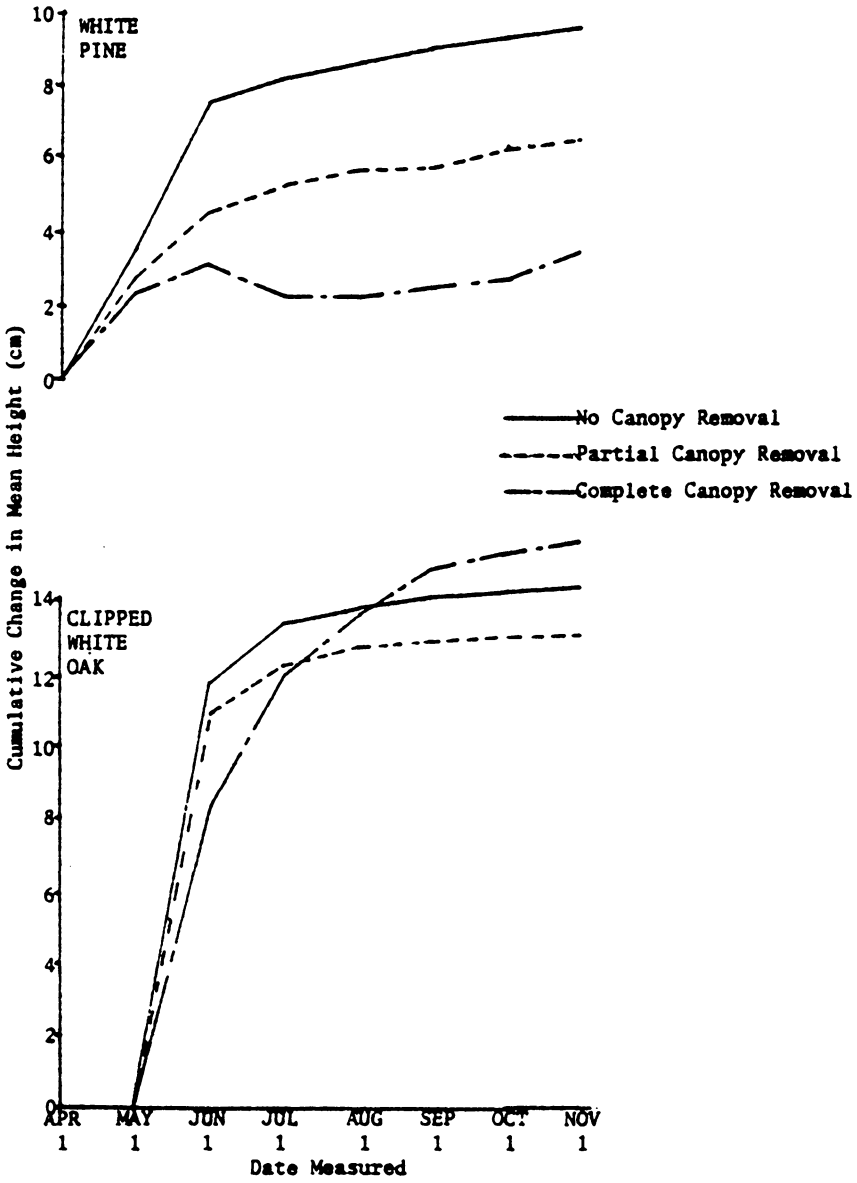

Figure 2. Cumulative mean height growth of underplanted white pine and clipped white oak grown in the field after complete, partial, and no canopy removal.

(Figures 1 and 2). Greatest height growth occurred during May, when clipped white oak seedlings grew 11.7 cm, intact (i.e., unclipped) white oaks grew 1 cm, and white pines grew 3.8 cm (Table 2). This spring growth represented 70% of the total seasonal height growth in the oaks and 90% in white pine. From 1 July to the end of the growing season, heights increased approximately 1 cm for all species.

Seedlings under 70% canopy cover (partial-cut treatment) exhibited growth patterns (Table 3) that were similar to seedlings under 90% canopy. More than 70% of their total seasonal height growth occurred between 1 April and 1 July. As in the no-cut treatment, clipped white oak seedlings grew approximately 11 cm from 15 April to 1 June (listed as the May increment). In contrast to no-cut, where maximum growth of unclipped seedlings occurred in May, the partial cut treatment showed greatest monthly height growth during April. In all species, less than 1 cm height growth occurred from 1 July until the end of the growing season. An average decrease in height (dieback) of red oaks and white oaks was measured during September. Frequently, stem dieback was accompanied by sprout growth from buds at a lower point on the stem. This sprout growth was rapid and could mask height loss due to dieback during any particular month. Sprouting from the root collar area was also observed in

white oak, but not measured unless dieback was to ground level.

In the clearcut (0% canopy cover), considerable shoot dieback occurred with intact oaks during April (Table 4). Dieback was followed by the largest monthly height increase (1.5 to 2.3 cm) that was observed with intact oaks. Large height growth (8.5 cm) also occurred in clipped white oak during May, which was the month after clipping. In general, with greater losses of shoot (due to dieback or clipping), ensuing sprout growth was proportionately greater. As under 70% and 90% canopy cover, white pine displayed greatest growth during Spring.

Height Growth of Individual Species:

Canopy Treatment Effects

Clipped white oak seedlings displayed similar seasonal growth patterns under all treatments (Table 5). Regardless of percent canopy cover, more than 70% of the total seasonal height growth occurred from 15 April (date of clipping) to 1 July, with the greatest monthly height growth observed during May (8.5 cm). Early in the growing season, clipped white oak had less total growth in the clearcut than on the shaded sites (Table 5). However, clearcutting resulted in significantly greater growth of clipped white oak during the summer than other treatments. This late growth compensated

Table 5. Mean height and diameter growth of clipped white oak within each canopy removal treatment area in the field experiment in Patrick County, VA.

Measurement Period	Treatment		
	NC[1]	PC	CC
	----Height Increment (cm)----		
Apr	-37.4 a[2]	-40.8 a	-27.9 b
May	11.7 a[3]	11.1 a	8.5 a
Jun	1.5 a	1.2 a	3.5 b
Jul	0.4 a	0.5 a	1.6 b
Aug	0.3 a	0.1 a	1.5 b
Sep	0.1 a	0.1 a	0.3 a
Oct	0.1 a	0.1 a	0.3 a
Spring (Apr-Jun)	13.2 a	12.3 a	12.0 b
Summer (Jul-Oct)	0.8 a	0.6 a	3.3 b
Entire Season	14.1 a	13.1 a	15.4 b
	----Diameter Increment (mm)----		
Entire Season	2.1 a	2.8 ab	3.8 b

[1] NC=no canopy removal; PC=partial canopy removal; CC=complete canopy removal.

[2] The negative numbers during April represent the stem height that was lost due to clipping.

[3] Within row, means followed by the same letter are not significantly different at the .05 alpha level (Harvey's Procedure). Number of replications: NC=37; PC=33; CC=38.

for the smaller amount of spring growth. As a result, the seasonal height growth of clipped white oak was significantly greater in the clearcut.

White pine and intact oaks responded differently than clipped white oaks to complete canopy removal. On average, intact red and white oak stems died back (as indicated by negative height increments) by as much as 3 cm early in the growing season (Table 6 and 7). Although dieback reoccurred during October, most height changes for intact oaks after 1 June were positive. The most rapid monthly height growth of intact oaks occurred immediately following the initial stem dieback. In clearcuts, this sprout growth was vigorous and exceeded corresponding non-sprouting growth of oak seedlings that were under no- and partial-cut treatments (Figure 1). Consequently, total seasonal height or diameter growth was not significantly different among canopy removal treatments for either red oak or white oak (Tables 6 and 7). Red oaks grew 2.3 cm in June after a mean 3.0 cm height loss during April and May. Similarly, intact white oaks grew 1.5 cm in May following a mean 2.4 cm height dieback in April (Table 6). Such rapid sprout growth following dieback is reminiscent of the surge in height growth observed in white oaks immediately after clipping.

Table 6. Mean height and diameter growth of non-clipped white oaks within each canopy removal treatment in the field experiment in Patrick County, VA.

Measurement Period	Treatment		
	NC[1]	PC	CC
	----Height Increment (cm)----		
Apr	1.0 a[2]	1.6 a	-2.4 a
May	1.0 a	0.5 a	1.5 a
Jun	0.4 a	0.3 a	1.2 b
Jul	0.2 a	0.1 a	0.5 a
Aug	0.4 a	0.1 a	0.3 a
Sep	0.2 a	0.2 a	0.2 a
Oct	0.1 a	0.4 a	-0.7 b
Spring (Apr-Jun)	2.4 a	2.4 a	2.2 a
Summer (Jul-Oct)	0.8 a	0.4 a	1.0 a
Entire Season	3.3 a	3.2 a	3.4 a
	----Diameter Increment (mm)----		
Entire Season	-0.2 a	-0.3 ab	-0.8 b

[1] NC=no canopy removal; PC=partial canopy removal; CC=complete canopy removal.

[2] Within row, means followed by the same letter are not significantly different at the .05 alpha level (Harvey's Procedure). Number of replications: NC=41; PC=39; CC=41.

Table 7. Mean height and diameter growth of northern red oaks within each canopy removal treatment area in the field experiment in Patrick County, VA.

Measurement Period	Treatment		
	NC[1]	PC	CC
	----Height Increment (cm)----		
Apr	0.8 a[2]	1.1 a	-2.8 a
May	1.1 a	0.5 a	-0.2 a
Jun	0.4 a	0.9 ab	2.3 b
Jul	0.1 a	0.3 a	1.5 b
Aug	0.2 a	0.2 a	0.5 a
Sep	0.4 a	-0.2 b	0.3 a
Oct	0.1 a	0.0 a	-0.4 a
Spring (Apr-Jun)	2.2 a	2.5 a	-0.8 a
Summer (Jul-Oct)	0.7 a	0.3 a	2.3 b
Entire Season	3.0 a	2.9 a	1.2 a
	---Diameter Increment (mm)---		
Entire Season	-0.2 a	0.3 ab	0.8 a

[1] NC=no canopy removal; PC=partial canopy removal; CC=complete canopy removal.

[2] Within row, means followed by the same letter are not significantly different at the .05 alpha level (Harvey's Procedure). Number of replications: NC=60; PC=50; CC=61.

As in the partial-cut treatment, white pines on clearcut sites had their largest monthly growth increment during April (2.6 cm) (Table 8). After April, white pine growth was usually small, while clipped white oaks were exhibiting their greatest growth. Also, in contrast to oaks, white pines had a net negative height increment of 0.9 cm (Table 4) during June. The height loss resulted from attack of white pine weevil (Pissodes strobi Peck.). This insect also contributed to the 40% mortality of white pine grown in the clearcut areas (Table 9). In the clearcut area, white pine displayed significantly more September growth than did trees in partial or full shade. September.

In contrast to unclipped oaks, total growth of white pine was significantly affected by canopy removal treatment. White pine growing in no cut areas had greatest height growth, most of which occurred early in growing season (Table 8). This height growth was accompanied by the greatest diameter growth (0.3 mm). In partial and clearcuts, growth was reduced by white pine weevil. However, even when not attacked by the weevil, white pines in clearcuts displayed small amounts of early growth compared to fully shaded seedlings. White pine in a clearcut showed greater height growth later than earlier in the season. However, in contrast to the oaks, this growth was not sufficiently great to compensate for reduced earlier growth.

Table 8. Mean height and diameter growth of white pines within each canopy removal treatment in the field experiment in Patrick County, VA.

Measurement Period	Treatment		
	NC[1]	PC	CC
	----Height Increment (cm)---		
Apr	3.6 a[2]	2.8 a	2.6 a
May	3.8 a	1.9 ab	0.6 b
Jun	0.8 a	0.6 a	-0.9 b
Jul	0.4 a	0.4 a	0.0 b
Aug	0.4 a	0.1 a	0.3 a
Sep	0.3 a	0.5 ab	0.8 b
Oct	0.3 a	0.2 a	0.2 a
Spring (Apr-Jun)	8.1 a	5.3 ab	2.2 b
Summer (Jul-Oct)	1.1 a	0.9 a	1.0 a
Entire Season	9.5 a	6.4 ab	3.4 b
	----Diameter Increment (mm)---		
Entire Season	0.3 a	0.0 a	-0.8 a

[1] NC=no canopy removal; PC=partial canopy removal; CC=complete canopy removal.

[2] Within row, means followed by the same letter are not significantly different at the .05 alpha level (Harvey's Procedure). Number of replications: NC=74; PC=87; CC=50.

Table 9. Survival of clipped and intact white oak, white pine, and northern red oak within each canopy removal treatment of the field experiment in Patrick County, VA.

Species	Treatment		
	NC[1]	PC	CC
	% survival		
White Oak (clipped)	90	87	95
White Oak (intact)	98	87	98
Red Oak	90	89	97
White Pine	100	97	59
	initial number of seedlings		
White Oak (clipped)	41	38	40
White Oak (intact)	42	45	42
Red Oak	67	56	63
White Pine	74	90	85

[1] NC=no canopy removal; PC=partial canopy removal; CC= complete canopy removal.

Diameter Growth

Diameter growth was significantly greater in clipped white oaks than in intact white oaks or other species (Tables 2, 3, and 4). Under 90% canopy cover, new diameter growth averaged 2.1 mm while an average negative increment occurred with intact seedlings and white pine grew only 0.3 mm. Clipped white oak diameter growth reflected total diameter measurement as the initial diameter was zero due to clipping. With increasing sunlight, diameter growth of clipped white oak increased (2.8 mm under 90% shade vs. 3.8 mm in clearcut). On average, diameter increment of intact oak or white pine increased slightly or decreased under the partial cut harvest treatment. Negative diameter increments (-0.7 mm in intact white oak and -0.8 mm in white pine) in clearcut areas reflected stem dieback to ground level and white pine weevil attack, respectively.

TNC Levels

Statistical analysis indicated canopy removal treatments had no effect on total nonstructural carbohydrate (TNC) levels of roots (22.6 to 28.1% of dry matter) or shoots (8.2 to 9.7% of dry matter) of northern red oak (Table 10). (The other two species were not analyzed for TNC.) Shoot dry weight of seedlings growing in a clearcut was significantly greater than seedlings growing under

Table 10. Carbohydrate and dry weights [1] of northern red oaks sampled from each canopy removal treatment in the field experiment in Patrick County, VA.

Harvest Treatment	Variable			
	Dry Weight (g)		TNC (% d.w.)	
	Shoot	Root	Shoot	Root
No Cut	12.7 B[2]	40.3 A	9.7 A	26.1 A
Partial Cut	10.3 B	33.1 A	8.2 A	22.6 A
Clear Cut	24.8 A	68.1 A	8.6 A	28.1 A
Wildling (no cut)	16.2	42.5	8.6	16.3

[1] Values are means of 5 red oak seedlings per replication, randomly selected from each harvest treatment. Wildlings were growing naturally on nearby sites.

[2] Means within a column followed by the same letter are not significantly different at the .05 alpha level (Duncan's Procedure).

either full or partial canopy. No statistically significant differences in root dry weights due to canopy removal treatments were found, but the means suggested some greater growth in clearcuts.

In general, dry weights and shoot TNC of wildling northern red oaks were similar to those of planted red oak seedlings under a complete canopy. However, root TNC levels were approximately 40% lower in wildlings than in any planted red oak seedling.

Greenhouse Study

During Spring, (1 April through 1 July), height growth of the newly-transplanted seedlings was greatest under 92% shade (averaging 2.6 cm). Under full sunlight, more than 20% of the stems died back, resulting in an average height loss of up to 3.9 cm (Figure 3, Tables 11 and 12). Seedlings grown under 63% shade displayed small positive growth (ranging from 0.4 to 1.9 cm) during the Spring. Based on soil psychrometer readings, moisture treatments did not become effective until late June; possibly due to small leaf areas and relatively low temperatures. Thus light, but not moisture, had a significant effect on height growth during April and May (Table A1).

During April, seedlings growing under 63% and 92% shade averaged 0.3 and 0.7 cm growth, respectively (Table 12). In

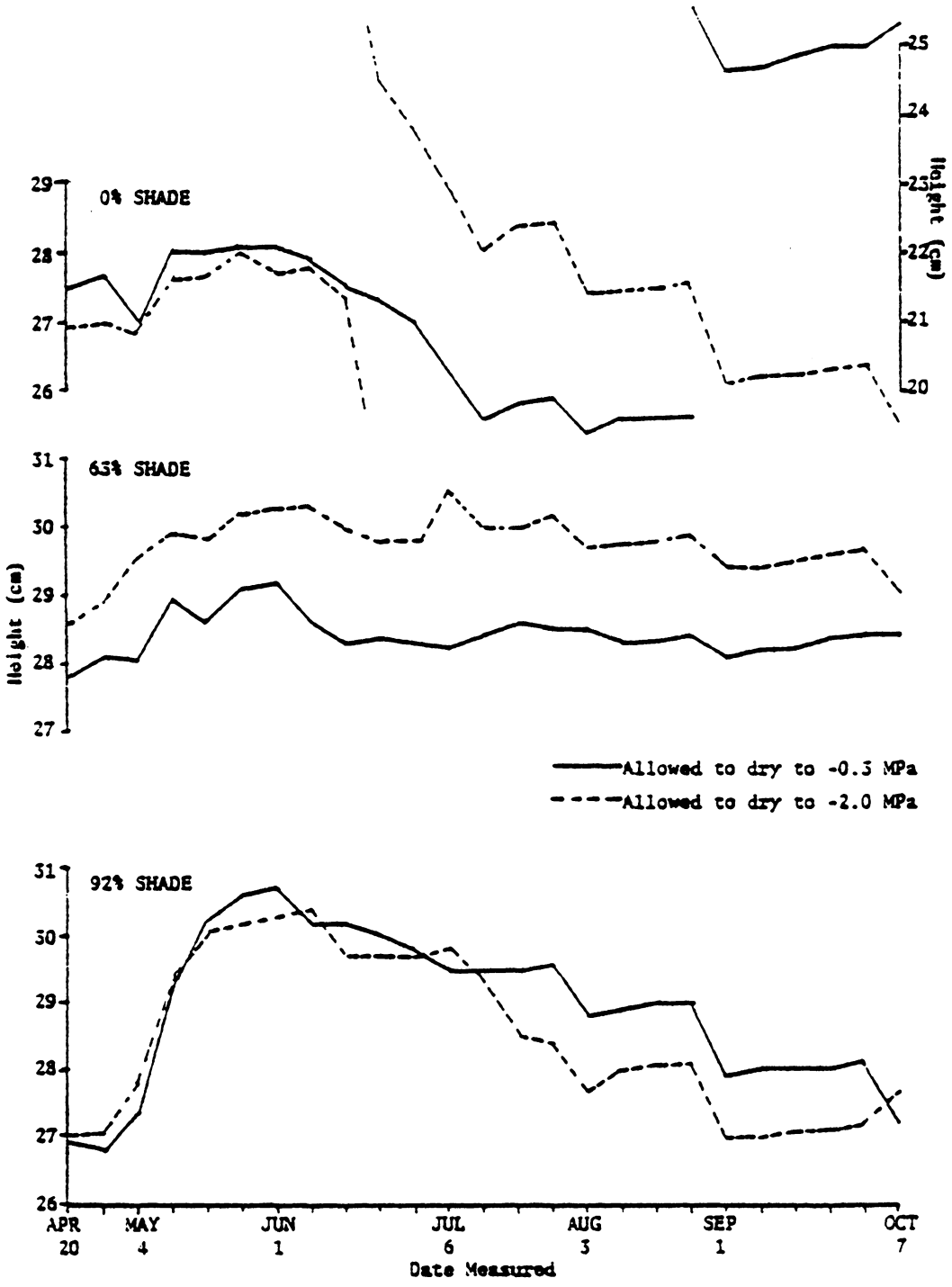

Figure 3. Weekly changes in mean heights of northern red oaks grown in a greenhouse under 0% (top), 63% (middle), and 92% (bottom) shade while being allowed to dry to -0.3 or -2.0 MPa.

Table 11. Cumulative height of potted 2-0 red oak seedlings within each light and moisture combination in the greenhouse.[1]

Measurement Date	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	Total Height (cm)					
Apr 20	27.5 a	------	27.8 a	------	26.9 a	------
May 4	27.9 a	------	28.0 ab	------	27.4 ab	------
Jun 1	28.1 b	------	29.2 ab	------	30.7 a	------
Jul 6	26.3 b	23.0 c	28.2 ab	30.5 a	29.5 ab	29.8 a
Aug 3	25.4 b	21.5 c	28.5 ab	29.7 a	28.8 ab	27.7 ab
Sep 1	24.7 b	20.1 c	28.1 ab	28.4 a	27.9 ab	27.0 ab
Oct 7	25.4 a	19.5 b	28.4 a	29.1 a	27.2 a	27.7 a

[1] Means within a row that are followed by the same letter are not significantly different at the .05 alpha level.

Table 12. Mean height and diameter growth of potted 2-0 northern red oak seedlings within each light and moisture combination in the greenhouse.[1]

Measurement Period	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	Height (cm)[2]					
Apr	-0.2 a	-----	0.3 a	-----	0.7 a	-----
May	1.1 b	-----	1.2 b	-----	3.3 a	-----
Jun	-0.9 a	-----	-0.6 a	-----	-0.5 a	-----
Jul	-0.4 a	-0.6 ab	0.4 a	-0.8 ab	-0.1 a	-2.3 b
Aug	0.4 a	0.2 a	0.0 a	0.3 a	0.2 a	0.4 a
Sep	0.6 a	0.2 a	0.4 a	0.4 a	0.4 a	0.3 a
Spring (Apr-Jun)	0 c	-2.0 d	0.9 bc	1.9 abc	3.5 a	2.9 ab
Summer (Jul-Oct)	0.6 ab	0.2 ab	0.8 a	-0.1 ab	0.5 ab	-1.6 b
Entire Season	0.6 a	-1.8 b	1.7 a	1.8 a	4.0 a	1.3 a
	Diameter (mm)					
Spring	0.1 b	0.0 b	0.1 b	0.1 b	0.3 a	0.1 ab
Summer	0.3 ab	0.2 ab	0.4 a	0.1 b	0.1 b	0.1 b
Entire Season	0.3 a	0.2 a	0.4 a	0.2 a	0.3 a	0.2 a

[1] Means within a row that are followed by the same letter are not significantly different at the .05 alpha level.

[2] Negative means are the result of stem dieback of individual seedlings within a treatment.

contrast, seedlings under 0% shade died back for a mean net height loss of 0.2 cm. In May, 92%-shaded seedlings exhibited significantly larger growth than other light treatments. Seedlings under 92% shade averaged 3.1 cm growth compared to 1.3 cm for 63%-shaded and 1.2 cm for unshaded seedlings (Table 12).

June was an unusual month in that all treatments exhibited some dieback. Also during this month, moisture treatments first caused significant differences in height growth (Table A1). Significantly greater stem dieback resulted during moisture stress of seedlings growing with no shade but not under 63% and 92% shade (Table 12). This interaction of moisture and light was statistically significant (Table A1), indicating that the moisture stress treatment did not have the same impact under all light conditions. For example, under moderate (63%) shade, seedlings in the higher moisture soil displayed slightly greater dieback than seedlings that were more stressed.

Summer (1 July through 1 October) height growth of non-shaded seedlings was significantly reduced by moisture stress (Table 12). To a lesser but still significant extent, dieback also occurred in fully shaded (92%), moisture-stressed (allowed to dry to -2.0 MPa) plants (Table 12). Compared to these treatments, the net positive Summer

growth of seedlings under 63% shade and -0.3 MPa moisture was considerable (0.8 cm). Dieback was small and insignificant among non-stressed (allowed to dry to -0.3 MPa) plants, regardless of shade treatment, during the Summer.

In July, moisture but not light affected height growth significantly. Compared to non-moisture stressed seedlings, moisture-stressed seedlings displayed a large amount of stem dieback (Table 12). In addition, the average dieback increment of stressed seedlings increased with increasing shade in July. This significant dieback largely negated the rapid spring growth of heavily-shaded seedlings (Figure 3). Although height growth continued through August and September, no significant differences due to light or moisture treatments were detected (Table A2). Generally, height growth of all treatments varied from 0.1 to 0.6 cm during August and September. No net dieback was observed during these months.

When net heights were examined at the end of the growing season, both light and moisture were found to affect growth (Table 11, Figure 3). Seedlings growing under full sunlight had net negative seasonal increments as a result of dieback: those allowed to dry only to -0.3 MPa exhibited an average loss of 2.1 cm in height while those dried to -2.0 MPa lost 7.6 cm (Table 12). All shade-grown seedlings had a net increase in mean cumulative height of less than 1 cm for

the entire season. The patterns of seasonal growth were markedly different between 63% and 92% shaded seedlings (Figure 3). Moderately-shaded (63%) seedlings grew a small amount in Spring and died back slightly. In contrast, heavily shaded (92%) seedlings displayed considerable growth before dying back. The only statistically significant difference in net height changes at the end of the season resulted from seedlings grown in full sunlight and allowed to dry to -2.0 MPa.

At the time of planting, the mean diameter of all seedlings was 0.4 cm, and average diameter did not differ significantly among treatments. As with height growth, only light treatments had significant effects on diameter growth from April through June (Table A3). However, moisture-stressed seedlings generally showed less diameter growth than did non-stressed seedlings (Table 12). Seedlings growing under 92% shade displayed up to 0.3 mm diameter growth in spring, which was significantly greater than growth of other treatments (0.1 mm or less) (Table 12). Seedlings growing under full sunlight and allowed to dry to -2.0 MPa had no detectable diameter growth in the Spring.

During the Summer months, the diameter growth of seedlings under no or moderate shade was approximately 0.3 mm. This was more than double the 0.1 mm growth of seedlings

under full shade. As with height growth, the rapid early diameter growth of full shaded seedlings was not maintained during the latter part of the growing season. As a result, no difference in diameter growth due to light treatments were found for the entire season (Table 12).

In the greenhouse, survival of seedlings receiving more water was consistently higher across all light treatments (Table 13). Lowest survival (73%) occurred in seedlings growing in full sunlight and allowed to dry to -2.0 MPa, and greatest survival (96%) was seen in seedlings growing under 63% shade and allowed to dry to no more than -0.3 MPa. Evidently, the treatment causing lowest survival was also responsible for greatest dieback. No statistically detectable differences in survival due to light and moisture treatments were found.

The range of dry weights for shoots was 2.3 to 5.4 g and for roots was 4.4 to 8.0 g (Table 14). Root and shoot dry weights under most light and moisture treatments decreased during Spring and then increased for the remainder of the Summer. Seedlings growing under 92% shade and allowed to dry to -2.0 MPa had lower average shoot dry weights in October than they originally had in May (Table 14). In general, most light and moisture treatments had no statistically detectable effect on total amount or distribu-

Table 13. Percent survival of potted 2-0 northern red oak seedlings within each light and moisture combination in the greenhouse.

Shade (%)					
0		63		92	
limit of moisture stress (-MPa)					
0.3	2.0	0.3	2.0	0.3	2.0
Survival (%)					
91.7	73.2	96.1	91.0	92.9	89.3

Table 14. Dry weights of potted 2-0 northern red oak seedlings within each light and moisture combination in the greenhouse.[1]

Measurement Date	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	Shoot Dry Weight (g)					
May 1	11.2 aA	[2]	12.4 aA		13.3 aA	
May 15	11.6 aA		18.5 aA		17.1 aA	
Jun 1	15.1 aA		17.9 aA		19.3 aA	
Jun 15	22.3 aA		18.9 aA		18.1 aA	
Jul 1	20.3 aA	16.7 aA	17.5 aA	16.7 aA	17.5 aA	17.5 aA
Aug 1	18.6 aA	18.7 aA	20.7 aA	17.7 aA	22.2 aA	17.5 aA
Sep 1	22.0 aA	20.4 aA	17.1 aA	21.7 aA	27.1 aA	18.1 aA
Oct 1	22.9 abA	18.9 abA	15.3 abA	24.2 aA	23.3 abA	11.8 bB
	Root Dry Weight (g)					
May 1	25.2 aA		26.8 aA		27.6 aA	
May 15	22.0 aA		26.3 aA		27.9 aA	
Jun 1	21.9 aA		26.7 aA		26.9 aA	
Jun 15	33.8 aA		27.6 aA		29.5 aA	
Jul 1	29.7 aA	24.3 aA	28.6 aA	27.0 aA	26.9 aA	27.9 aA
Aug 1	31.8 aA	26.0 aA	26.7 aA	27.4 aA	30.2 aA	34.2 aA
Sep 1	33.8 aA	34.1 aA	26.2 aA	29.9 aA	40.2 aA	25.9 aA
Oct 1	37.9 aA	37.0 aA	26.7 aA	40.3 aA	36.2 aA	23.0 aA

[1] Means within a row that are followed by the same lower case letter are not significantly different at the .05 alpha level. Means within a column that are followed by the same upper case letter are not significantly different at the .05 alpha level. Each mean represents a sample size of 5 seedlings.

[2] Moisture treatments did not take effect until 1 July 1981. Hence, no means are reported under 2.0 MPa during May or June.

tion of dry matter. However, dry weights of non-shaded seedlings increased by 50 to 100%. In comparison, shaded seedlings generally increased in dry weight by less than 50%. Root/shoot ratios ranged from 1.3 to 2.0 (Table A12). However, no trends due to light or moisture treatments were apparent.

Light treatments had significant effects on root and shoot percent TNC levels in oaks (Tables A5 and A6). Moisture treatments affected only stem TNC percent levels (Table A6). During the first two months of the growing season, total TNC decreased by less than 1 g per seedling under 92% shade; whereas, TNC increased by approximately 1.2 g per seedling when grown with no shading (Table 15). The intermediate shading level resulted in no real change in % TNC by 1 July.

Root percent TNC of non-shaded plants decreased rapidly during May and then began a gradual increase for the remainder of the growing season (Table 15). Seedlings growing in shade also displayed a decline in root carbohydrates during May. However, this depletion of TNC was less rapid and lasted longer than the decline of non-shaded seedlings. Consequently, the growing season nadir for root percent TNC was lower and occurred later with increasing shade. Root percent TNC of seedlings growing under 92% shade decreased

Table 15. Total non-structural carbohydrate percentages of potted 2-0 northern red oak seedlings growing under different light and moisture combinations in the greenhouse.[1]

Measurement Date	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	20
	Shoot Carbohydrate (% d.w.)					
May 1	4.3 aC	[2]	4.1 aB		4.7 aB	
May 15	4.1 abC		3.5 bB		4.5 aBC	
Jun 1	4.2 aC		3.6 aB		3.2 aCD	
Jun 15	5.3 aBC		4.2 abB		3.4 bC	
Jul 1	5.1 aBC	5.4 aB	4.1 abB	4.7 abC	2.8 bD	4.0 abC
Aug 1	5.5 aB	6.2 aAB	4.6 aB	6.1 aB	4.5 aBC	4.8 aBC
Sep 1	7.7 bcA	9.1 aA	7.4 bcdA	8.5 abA	6.5 cdA	6.2 dAB
Oct 1	8.3 aA	8.3 aAB	8.4 aA	8.3 aA	7.2 aA	7.6 aA
	Root Carbohydrate (% d.w.)					
May 1	29.0 aAB		28.8 aA		30.1 aA	
May 15	20.7 aC		21.4 aC		25.9 aA	
Jun 1	20.0 abC		21.2 aC		16.8 bB	
Jun 15	21.8 aC		17.3 aC		19.4 aB	
Jul 1	26.1 aB	20.2 abA	22.4 abBC	21.6 abB	16.2 bB	15.5 bA
Aug 1	25.3 aBC	23.8 aA	19.3 abC	24.4 aAB	14.7 bB	20.9 aBA
Sep 1	26.3 abB	29.4 aA	21.3 abcC	26.5 abA	17.8 cB	18.6 bcA
Oct 1	34.0 aA	27.2 bA	25.3 bcAB	25.7 bcA	17.0 dB	20.6 cdA

[1] Means within a row that are followed by the same lower case letter are not significantly different at the .05 alpha level. Means within a column that are followed by the same upper case letter are not significantly different at the .05 alpha level.

[2] Moisture treatments did not take effect until 1 July 1981. Hence, no means are reported under 2.0 MPa during May or June.

significantly to 17% by the end of the growing season. In contrast, root percent TNC increased after June to at least pre-growing season levels in seedlings grown under 63 and 0% shade. There was a net percent TNC increase from 29 to 34% dry weight with non-shaded seedlings allowed to dry to -0.3 MPa. However, this increase was not statistically significant.

After 1 July, seedlings grown under 92% shade always tended to have significantly less root TNC (15 to 21% dry weight) than those under 0% shade (20 to 34% dry weight). Seedlings under 63% shade were intermediate with 19 to 27% root TNC on a dry weight basis.

Similar to root TNC, the mid-season nadir in shoot TNC was significantly less and occurred later with increasing shade. In contrast to root TNC, stem carbohydrates were much lower and decreased slowly. Also, stem carbohydrates in October were approximately 100% higher (e.g. 4% vs. 8% dry weight) than levels that existed in May, regardless of light or moisture treatment. Within any light treatment, seedlings allowed to dry to -2.0 MPa soil moisture tension generally had higher stem percent TNC than those dried to -0.3 MPa. However, these differences usually were not statistically detectable.

In general, leaf number and leaf area were unaffected by light and moisture treatments (Table A7). Seedlings in 0% shade broke bud at the same rate as shaded seedlings, as indicated by similar numbers of leaves on 1 May. During the first 60 to 75 days of the growing season, leaf area increased within all light treatments. However, shaded seedlings increased leaf area at a faster rate than non-shaded plants (Table 16).

In addition to caliper measurements, seedling diameter growth was also monitored by measuring new xylem width. These diameter increments were significantly affected (according to the overall analysis of variance) by moisture, but not by light treatments. However, no mean separation due to light or moisture was possible with Duncan's MRT (Table 17). In general, seedlings exposed to less moisture stress had more diameter growth; particularly those under 0% and 92% shade. A surge of diameter growth was observed during the first two weeks of June, lagging slightly behind the increase in leaf area growth.

Table 16. Leaf number and leaf area of potted 2-0 northern red oak growing under different light and moisture combinations in the greenhouse.[1]

Measurement Period	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	Leaf Number					
May 1	15 aB	[2]	15 aB		9 aB	
May 15	24 bB		37 aA		31 abA	
Jun 1	42 aA		36 aA		32 aA	
Jun 15	34 aAB		36 aA		29 aA	
Jul 1	38 aAB	28 aA	28 aAB	25 aA	29 aA	36 aA
Aug 1	40 aAB	41 aA	30 abAB	23 bA	35 abA	24 bB
Sep 1	35 aAB	33 aA	27 aAB	30 aA	40 aA	28 aAB
Oct 1	41 aA	31 abA	27 abAB	31 abA	36 abA	21 bB
	Leaf Area (sq cm)					
May 1	25 aB		16 aB		27 aC	
May 15	546 bA		1277 aA		815 abB	
Jun 1	977 aA		1320 aA		1587 aA	
Jun 15	1268 aA		1350 aA		1042 aAB	
Jul 1	1202 abA	667 bA	1162 abA	1089 abA	1193 abAB	1423 aA
Aug 1	889 aA	998 aA	1362 aA	1135 aA	1455 aA	1010 aAB
Sep 1	1255 aA	1111 aA	980 aA	1274 aA	1576 aA	1152 aAB
Oct 1	1238 aA	800 aA	998 aA	1448 aA	1393 aA	785 aB

[1] Means within a row that are followed by the same lower case letter are not significantly different at the .05 alpha level. Means within a column that are followed by the same upper case letter are not significantly different at the .05 alpha level. Each mean represents a sample size of 5 seedlings.

[2] Moisture treatments did not take effect until 1 July 1981. Hence, no means are reported under 2.0 MPa during May or June.

Table 17. Current year xylem ring growth of potted 2-0 northern red oak seedings within different light and moisture combinations in the greenhouse.[1]

Measurement Period	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	Current Xylem Ring Growth (mm)					
May 1	0 aB	[2]	0 aB		0 aB	
May 15	0 aB		.01 aB		.01 aB	
Jun 1	.02 aB		.02 aB		.02 aB	
Jun 15	.20 aB		.21 aA		.21 aA	
Jul 1	.32 aA	.28 aA	.36 aA	.24 aA	.31 aA	.35 aA
Aug 1	.31 aA	.24 aA	.27 aA	.28 aA	.29 aA	.25 aA
Sep 1	.33 aA	.32 aA	.27 aA	.33 aA	.46 aA	.28 aA
Oct 1	.37 aA	.31 aA	.27 aA	.33 aA	.31 aA	.24 aA

[1] Means within a row that are followed by the same lower case letter are not significantly different at the .05 alpha level. Means within a column that are followed by the same upper case letter are not significantly different at the .05 alpha level.

[2] Moisture treatments did not take effect until 1 July 1981. Hence, no means are reported under 2.0 MPa during May or June.

DISCUSSION

Dieback

It is well-established that oak seedlings growing under shade undergo shoot dieback and resprouting (Merz and Boyce 1956). In the greenhouse, dieback was observed in all treatments by 1 June following an early growth period in some treatments. Fully shaded seedlings died back nearly twice as much as moderately-shaded seedlings after 1 June. This larger amount of dieback may have been due to the proportionately greater new growth that occurred early in the growing season; because of insufficient light the plants were presumably unable to maintain the large amount of new growth. In contrast, moderately-shaded seedlings did not grow as tall as fully-shaded ones and died back correspondingly less. There is similarity between these two shade treatments in that the amount of dieback was proportional to current year's growth. Thus, no significant difference in seasonal net growth between shading treatments was found.

Moisture treatments did not differentially induce dieback in shaded treatments in the greenhouse. These moisture treatments differed largely by duration as well as the amount of soil moisture available. At -0.3 MPa and at -2.0 MPa the % soil moisture was 18% and 10%, respectively. Therefore, it seems likely that at a given moisture stress (-0.3 MPa or less), dieback was possible.

The etiolated-like growth induced by full shade was perhaps particularly susceptible to stem dieback. The lower availability of water in the -2.0 MPa treatments may have been compounded by the seedling's inability to obtain water; in shade, root growth is often sacrificed for shoot growth (Grime 1979). Thus, dieback in the shade may be due to the gradual, cumulative effects of environmental stresses on photosynthesis and growth.

In the greenhouse, levels of total non-structural carbohydrates were lower in fully-shaded red oaks than in partially or non-shaded seedlings. TNC levels were lower at the end than the beginning of the study. Such diminution will decrease the potential for growth and increase the susceptibility to stress and disease.

The curtailment of root growth and water absorption under shade can decrease growth of the terminal leader (Zimmermann and Brown 1974). In the field experiment, seasonal height growth of shaded seedlings was less than for seedlings growing in clearcuts. Such arrest of the terminal leader releases lateral branches from previous hormonal suppression (Decker 1962). The flat-topped crowns, which are typical of oak seedlings growing in the understory, may result. With continued light and moisture stress, water-conducting capacity may be further diminished by reductions

of carbohydrates for structural (xylem) or root growth uses to the point where stem dieback may be triggered by drought. In this experiment, neither xylem nor overall diameter growth was affected by shade or moisture treatments. However, in succeeding seasons, secondary growth would likely be reduced by low TNC availability and reduced photosynthesis. In the field experiment, stem dieback of red oak seedlings under shade was observed only in September. These seedlings had lower TNC at season's end than those growing in clearcuts. According to the previously discussed hypothesis, these seedlings could undergo stem dieback in the future.

Stem dieback was also observed in the no-shade environment. In clearcuts and under full sunlight in the greenhouse stem dieback was prominent. Such dieback in northern red oak following clearcutting has also been found by McQuilkin (1974). In contrast to dieback under shade, stem dieback under no shade occurred rapidly, usually early in the growing season and was frequently followed by rapid resprout growth. In both the field and greenhouse, early-season dieback occurred when moisture was plentiful and competition was minimal. Because soil water potential was carefully monitored in the greenhouse and was known to be high in the vicinity of the roots (by psychrometer measure-

ments), it seems likely that adequate moisture was available for absorption. Direct solar radiation was common to no-shade conditions in the field and greenhouse. Hygrothermograph recordings showed afternoon greenhouse temperatures during the growing season to be as high as 35 C. However, it is likely that leaf surface temperatures were greater and may have had detrimental effects on the metabolism of seedlings. For example, mitochondrial and photorespiration may have increased to undesirably high levels at increased temperatures, or the balance of growth regulators may have been disturbed. Photorespiration may also have increased markedly in seedlings exposed to high levels of direct solar radiation. When temperatures exceed 35 C, total respiration may exceed photosynthesis (Kramer and Kozlowski 1979). In addition, high temperatures may have caused short-term drought conditions in succulent, actively-growing stems. In the greenhouse, stem elongation slightly preceeded leaf development and xylem expansion lagged behind both. It is possible that, at that time, transpiration may have been slow and internal water deficits may have resulted from high temperatures. Thus, newly-grown (unhardened) shoots could succumb to the combined stresses. The dieback of the active stem tips would quite likely result in an imbalance that affects the rest of the plant.

It is also possible that "transplanting shock" was partially responsible for dieback under no shade. Seedlings recently removed from a crowded nursery bed or released from the shade of a canopy may be particularly susceptible to desiccation or metabolic disturbance. For example, if freshly transplanted seedlings do not have rapid root growth, probability of survival is poor (Farmer 1975). The exposure to drastic, new environmental conditions that induced stress may partially explain the dieback observed in unshaded seedlings during this experiment.

In contrast to heavy or no shade conditions, dieback was not prevalent under moderate shade. Oak seedlings grown under 63% shade in the greenhouse or under the partial cut treatment (70% canopy cover) in the field exhibited little or no stem dieback. Under moderate shade, a small but positive increase in TNC was observed. It is probable that seedlings grown under moderate shade experienced the least shock and were exposed to the least stressful conditions. It is evident that red and white oak seedlings will have different seasonal growth patterns, depending on light intensity.

Stem dieback in oak may be either an environmentally-imposed "accident" or an internally-regulated senescence process. Senescence may be defined as a normal developmen-

tal process that leads to death of parts or all of a plant (Leopold 1980). Differentiation of xylem elements or the deciduous habit of leaves are examples of senescence. Clearly, stem dieback in oak is not analagous with xylem element differentiation. However, leaf senescence can follow moisture stress. The previously proposed causes of dieback would take effect as a result of continued stress in the understory or disturbance from transplanting or canopy removal. Thus, oak stem dieback may be a genetically-programmed, active survival strategy that occurs only under certain environmental circumstances. Prior to dieback, oak leaves turn yellow as they would in autumn prior to abscission. It is possible that nutrients and metabolites are exported to surviving organs prior to dieback as they are prior to autumn leaf abscission.

Growth

In the field study, winter canopy removal treatments had no statistically detectable effect on growth of unclipped oak seedlings during April and May of the following growing season. According to Kozlowski (1964), shoot growth in oak is strongly dependent on elongation of pre-formed shoots after a rest period (fixed growth). This preformed growth overwinters in a bud before elongating in the next growing season. It is therefore logical that preformed

growth is strongly dependent on environmental conditions at the time it was formed, and this may explain the lack of effect of canopy removal treatments on early growth during the first year. This preformed growth of oaks can be beneficial because it is expressed quickly (often within one month) and occurs at a time when resources will be least limiting. Thus, second year (1981) growth and TNC measurements will more readily reflect long term effects of treatments.

Canopy removal treatments affected height growth of field-grown oak seedlings during June and July. Seedlings growing in clearcuts had significantly greater height growth than those growing under a forest canopy. Russell and Rollins (1969) also found height growth of white oak, black oak, and red oak to be approximately 50% greater with no shade than in partially cut areas with 7 m^2/ha residual basal area. This greater growth was probably due to the increased availability of light. Although available light and moisture were not quantified in the field, competition from the above ground component of the surrounding vegetation did not appear to be great during the first year after clearcutting. In addition to a brighter, wetter environment, rapid growth in the clearcuts during June and July may have resulted from sprouting after stem dieback of the pre-

vious two months. Dieback, however, did not have an overall negative impact on seasonal height growth because sprout growth was rapid later in the growing season. Such rapid growth was consistently observed following stem dieback in both the field and greenhouse or following stem clipping (performed on white oak in the field). Rapid sprout growth has been reported by Sander (1972), McQuilkin (1974), and Ross (1982). For example, McQuilkin (1974) found cumulative heights of white oaks that did not dieback to be the same as those that had, indicating much more rapid growth of sprouts. Lamson (1976) reported sprout growth to be up to 1.6 times faster than that of seedlings.

Rapid growth following clipping or dieback may cause re-establishment of the original biomass distribution between root and shoot. Stimulation might result from changes in metabolites, mineral nutrients, or growth regulators in the roots or in buds in the vicinity of the root collar. Restoration of the original shoot/root ratio following shoot or root clipping has been reported for beans (*Phaseolus vulgaris*) and ryegrass (*Lolium perenne*) (Russell 1977).

Two months after clipping and rapid regrowth, northern red oak seedlings had lower root TNC than seedlings that had not been clipped (Tworkoski *et al.*, unpublished data). Apparently seedlings draw on food reserves to support such

growth. The close proximity of the expanding shoot to stored root carbohydrate may partially account for the rapid growth. Also, lack of competitive above-ground sinks would allow rapid growth of the single meristem.

Canopy removal treatments minimally affected height growth of field-grown oaks after July. Generally, stems elongated by less than one-half cm during each of these months, probably as the result of extension of previously-formed cells. According to Kramer and Kozlowski (1979), oaks frequently undergo late-season shoot growth from current year buds (lammas growth). In this study, second flushes of shoot growth were observed only in oaks that had undergone dieback. Although these flushes were not separately quantified, the additional growth undoubtably contributed to the resprout growth. With the exception of sprouts noted above, no additional flushes of growth were observed after July. Apparently, environmental conditions were not favorable for oak lammas growth in the field or greenhouse study.

In the greenhouse, light and moisture treatments significantly affected red oak height growth. As in the field, seedlings grown under full sunlight died back during the growing season. However, unlike field-grown seedlings, the average height of unshaded, greenhouse-grown seedlings was

consistently less than shaded seedlings due to smaller sprout growth following stem dieback or greater dieback lengths. This low growth may have been caused by the higher frequency of moisture stress under 0% shade. For example, seedlings grown under 0% shade had dried to -2.0 MPa approximately 6 to 8 times, while fully shaded seedlings were exposed to only 2 to 3 such cycles. It would appear that the more frequently stressed seedlings would have been exposed to greater moisture stress for cumulatively longer periods than seedlings that dried down relatively infrequently. In addition, daily internal water deficits probably occurred earlier and lasted longer due to higher temperatures in full sunlight.

No significant differences in total height were found between moisture treatments for seedlings growing under shade in the greenhouse. These findings are in agreement with Wood (1938) who found moisture to be of less importance than light intensity to oaks grown in the understory. Wood trenched around a field plot containing white oak, chestnut oak, and black oak seedlings growing under a canopy and found no difference in height growth due to root competition or to reduced soil moisture. In this study, differences in height due to moisture treatment only occurred under full sunlight. From 1 June until the end of the growing season,

cumulative height decreased in those seedlings growing under 0% shade and dried to -2.0 MPa.

During May, the height growth of 92% shaded seedlings in the greenhouse was significantly greater than seedlings under 0 or 63% shade. In order to "out-compete" other plants for limited amounts of light, seedlings grown under full shade accelerate stem elongation. This growth may be due to high levels of growth stimulators, such as gibberellins or auxins, in response to the quality or quantity of light that is available in the understory. Similarly, rapid height growth occurred under shade in the field. However, this rapid growth was not followed by dieback. In the field, full shade is not present year round; this may allow a seedling to utilize different light quantities and qualities during winter (Perry, personal communications). In addition, transplanting undoubtably contributed to dieback in the greenhouse under full shade. During the first year after underplanting, as much as 17% of the northern red oak exhibited net seasonal dieback in the field study (Biesteck, unpublished data) compared to 18% in the greenhouse experiment.

Survival

Although significant differences were not observed, red oak seedlings allowed to dry to -2.0 MPa in the greenhouse

had lower survival than those dried to no more than -0.3 MPa, regardless of light treatment. Generally, survival was 90% or better except for seedlings dried to -2.0 MPa under 0% shade (Table 13). Only 73% of the seedlings exposed to this treatment combination survived. As previously mentioned, these seedlings were exposed to greater solar radiation, which enhances transpiration and soil water evaporation. Consequently, seedlings grown in 0% shade and dried to -2.0 MPa were exposed to more frequent moisture stresses. If these trends continue, greater stem dieback and resulting height losses will undoubtably be associated with moisture-stressed seedlings.

Field-grown seedlings also did not display mortality differences due to shading. As in the greenhouse, survival of field-grown seedlings was approximately 90%. However, in no field treatment was survival as low as 73%. This probably was due to seedlings in the field experiment being better established than those in the greenhouse experiment. In addition, field soil water potential may not have reached -2.0 MPa.

Leaf Area, TNC, and Secondary Growth

Often seedlings grown under full shade will increase leaf area and invest biomass in stems and leaves at the expense of roots (Grime 1979). In the present study, such

expressions of morphogenetic plasticity were not found. In general, root and shoot dry weight and leaf area and leaf number were unaffected by light or moisture treatments. These results agree with Loach (1970) who found that more tolerant species such as red oak do not display the plasticity of less tolerant species such as yellow-poplar. These characteristics coincide with those of a stress tolerator as outlined by Grime (1979).

Although leaf areas or plant dry weights were not significantly affected, root non-structural carbohydrates were markedly diminished by increasing shade in greenhouse-grown seedlings. Throughout the study, stem TNC varied little and remained between 5 and 8% of dry weight, regardless of treatment. The greatest root TNC was found at season's end in seedlings grown under 0% shade and allowed to dry no lower than -0.3 MPa. With reduced moisture, root percent TNC was significantly reduced. Apparently, as soil moisture dried from -0.3 to -2.0 MPa, moisture can limit a seedling's photosynthetic ability. Yocum (1935) reported that stomata of oak seedlings under moisture stress were closed all day, as measured by transpiration. He suggested that carbon dioxide could not enter the leaf, and photosynthesis would be retarded. He did find reduced starch concentrations in leaves of moisture-stressed seedlings. In this study, even

seedlings dried to -2.0 MPa under 0% shade had higher TNC than well-watered but shaded seedlings. Under 63% or greater shade, it is probable that red oak cannot photosynthesize to its full capacity. It is also possible that, under 63% shade, red oak is respiring or growing more rapidly than under other light treatments. Therefore, it is also possible that photosynthate is utilized more rapidly under shade than under full sunlight.

Seedlings grown under shade generally had less root TNC in October than when they first began growth in May. It is therefore possible that future red oak growth in the understory may be reduced by lower photosynthesis and reduced TNC reserves. Although significant differences were not found, field-grown seedlings in a clearcut had higher root TNC than those grown in shade. Shaded wildlings had much lower TNC than non-shaded seedlings. Judging by ring counts, wildlings had existed at least 7 years in the understory. Thus, there is additional evidence that continued existence in the understory will lead to reduced TNC reserves in red oak.

Annual ring growth was rapid, occurring primarily within a two week period from early to mid June. Most ring-porous species display such rapid growth. However, rapid xylem genesis ordinarily occurs before or concurrently

with bud break (Zimmermann and Jeje 1981). In this study, the surge of xylem growth lagged nearly two weeks behind leaf expansion. In agreement with Zimmerman and Jeje (1981), water must have been transported through functional xylem cells from the previous year in order to support new shoot growth. Apparently, the small amount of secondary growth and previous year's functioning xylem supplied sufficient water to drive leaf expansion. However, later xylem enlargement may have been necessary to facilitate rapid transpiration. The delay in new xylem growth may have been due to, or one symptom of, "transplanting shock" and a contributing cause of dieback.

Species and Clipping Treatment Comparisons

In the field experiment, one-half of the underplanted white oak seedlings were clipped at the root collar to simulate dieback and to compare the sprout growth with that of intact seedlings. With increasing sunlight, early growth of clipped white oak was less than shaded seedlings. This decrease may be due to an inhibition by light of shoot growth. Full sunlight can reduce diffusible auxin and possibly gibberellins (Salisbury and Ross 1978). The reduced growth corresponding to increased sunlight also occurred in white pine and intact oaks. However, in clipped white oak, growth increased later in the growing season so that total

seasonal height growth was greater in the clearcut than in the shade.

Although clipped white oaks had greater seasonal growth than unclipped, they did not regain the height of unclipped seedlings during the growing season. In general, clipped seedlings were just under half the 35 cm height of intact seedlings. The growth rate might be expected to continue to be slightly faster for clipped white oak until the original root/shoot balance is restored. Along with shoot growth, root growth of shaded seedlings was low. An examination of root systems of several oak seedlings indicated little new root growth. Apparently, oaks were surviving but not aggressively establishing themselves in the understory.

In contrast to oaks, white pine grew well in the understory. With increasing exposure to direct sunlight, white pine height growth declined. Evidently, white pine was better adapted to growth in the understory than red or white oak. Greatest height growth of pine was obtained under a 90% canopy (9.5 cm). White pine may be able to compete for limited resources better than oaks.

Under full sunlight, white pine grew least (3.4 cm). Attack by white pine weevil caused considerable mean height loss and some mortality. In addition, considerable time elapsed following the killing of the terminal shoot and

establishment of a new leader from the lateral branches (Kramer and Kozlowski 1979). However, seedlings that apparently were not attacked also grew poorly in the clearcut. Understory white pine may have adapted to conditions in the understory (eg. lower temperatures and diffuse sunlight) and could not immediately take advantage of increased sunlight and moisture in a clearcut. Leaves of white pine that are developed in the shade are less xerophytic than those which develop in full sunlight (Kramer and Kozlowski 1979). Hence, the shade leaves of white pine may be susceptible to desiccation. It is possible that higher temperatures adversely affected white pine growth in the clearcut. Therefore, a series of light cuttings may be necessary to ensure adequate growth while harvesting the remaining canopy.

Growth Strategies

Oaks grew less than white pine regardless of light treatment. Both oaks and white pine are intermediate in tolerance (Powells 1965). They also have characteristics typical of the competitor plant strategy as outlined by Grime (1979). For example, they can perenniate in dormant buds. However, other competitive characteristics include a rapid and plastic response to changes in the environment and pronounced ability to capture resources--traits that are not

well-developed in the oaks or pine studied. In agreement with Loach (1979), I found red oak to undergo little morphological change in response to environmental variation. For example, leaf area did not increase with increasing shade. Thus, trees that had been growing under a dense canopy displayed characteristics of Grime's stress tolerator (Grime 1979). Stress tolerators undergo small morphogenetic response to environmental changes and conserve resources. As evidenced by this study, oaks can survive and appear to be able to tolerate shade conditions. Beneath dense tree canopies, vertical gradients in light intensity are not as great near the ground as in clearings. Consequently, the abiltiy to tolerate shade conditions is more important than the ability to compete for light by outgrowing potential competitors. Oak regenerants thus display stress tolerator characteristics by changing crown shape to the broad, flat-topped form which maximizes the capacity for light interception. Also, the dieback phenomenon common to understory oaks may also be a stress tolerance strategy. Gradual top dieback may enable the plant to export nutrients from senescing parts to the root. New shoots can then resprout. Under favorable conditions (e.g. canopy removal) these new sprouts may then be able to more effectively compete for light than seedlings that had not undergone die-

back. In this study, it was apparent that sprout growth is much more rapid than that of intact seedlings.

Northern red oak is generally classified as intermediate in tolerance (Baker 1950, Fowells 1965). Thus, under heavy shade, it cannot continuously grow for long periods as do beech and sugar maple. Nor, in this study, did it grow as rapidly as white pine. White pine apparently was more capable of capturing and utilizing limited resources. Perhaps white pine can better utilize diffuse radiation or sunflecks. There may also be a greater investment of biomass into shoot in white pine than oak. Teleologically, white pine may be opting for a strategy of striving into the canopy for light; while oaks are more conservative, accumulating a larger root system and food reserves and waiting for an opportune moment to take their place in the canopy.

Environmental Conditions Conducive to Growing Northern Red Oak

Correct site selection for oak planting cannot be over-emphasized. Red oaks will likely perform best on sites of intermediate site quality. Loftis (1979) found poor initial growth on dry sites. However, on excellent cove sites, red oak succumbed to competition from species such as yellow-poplar. The importance of selection of the correct site for oak regeneration has been demonstrated in this study.

In the field, oaks grew slowly and faced the danger of being overtaken on wetter sites by stump sprouts of maple and yellow-poplar in clearcuts. On dry sites in clearcuts, growth is so poor (as demonstrated by dieback in the greenhouse under 0% shade) that more stress tolerant species may overtake them. Carvell and Tryon (1961) also found that northern red oak did not survive well on poor or good sites. As in this study, they found red oak could not adequately compete on cove sites and were not stress tolerant enough for dry ridges. Best growth and survival of red oak was found on intermediate slopes where cove species, such as yellow-poplar, or dry site species, such as chestnut oak, could not compete as well as red oak.

This study is also in agreement with Carvell and Tryon (1961) in recommending that, as sites become dryer, canopy releases should be smaller. With too large an opening, poor growth and dieback will result in net height losses. On the other hand, with heavy canopy coverage (approximately 90% shade) carbohydrate reserves may be gradually reduced and weaken the seedling.

SUMMARY AND CONCLUSIONS

In this study, greatest dieback of northern red oak was observed under full or no shade. In both cases, dieback was probably induced by external factors such as drought. However, in full sunlight, dieback was caused by the severe disturbance of complete canopy removal in the field or by transplanting shock in the greenhouse. Dieback in full shade is likely induced by chronic light and moisture stress, rather than by severe disturbance.

In the field experiment, seedlings that had died back in clearcuts grew rapidly as sprouts and, as a result, average cumulative heights did not differ significantly among harvest treatments. If severe drought or competition do not occur on clearcut sites, red oak seedlings will probably grow taller than shaded seedlings. However, with extended deprivation of water, seedlings in a clearcut will dieback and grow poorly. In the greenhouse experiment, seedlings allowed to dry to -2.0 MPa under no shade had a significant loss in cumulative height and, also, high mortality.

Light intensity at the forest floor will significantly affect TNC accumulation in northern red oak seedlings. As available sunlight increased, TNC concentrations also increased. In addition, as available soil water decreases within a given amount of sunlight, carbohydrate accumulation

will decrease. Thus, it is not likely that red oak failures in clearcuts are due to diminished carbohydrate reserves. However, with extended suppression in the understory, TNC reserves could drop to levels that may adversely affect future northern red oak growth and survival.

Based on these results, northern red oak seedlings can be planted under a full canopy. However, if kept in a suppressed position too long, resulting slow growth and reduced TNC levels can adversely affect the seedling's competitive ability. A large but not complete canopy removal within 3 years of planting may be best suited to northern red oak survival. This cutting should be designed to control competition and herbicides may have to be used on certain sites. Based on the field and greenhouse experiments, the residual canopy cover should be less than 60%. Thus, regeneration of northern red oak appears viable using a combination of underplanting and shelterwood cutting. However, additional research is necessary to confirm the recommended timing of canopy removal. If root systems of planted oaks continue to enlarge while in the understory, then canopy removal should be delayed.

In the field experiment, results of white oak growth closely paralleled those of northern red oak. Although white oak is considered more tolerant than red oak, no differences in performance were observed under shade.

As the amount of canopy removal increased, white pine was more severely attacked by white pine weevil. In addition, white pine grew more slowly in clearcuts than in shade. It seems likely that the pine was physiologically adapted to shade conditions. For example, the chlorophyll a/b ratio may be lower in shade than sun to more efficiently utilize the available diffuse sunlight. Thus, an increase in direct sunlight may not immediately improve the photosynthetic capacity. With time, white pine will adapt to full sunlight and grow better in clearcuts than shade. If white pine weevil can be controlled, underplanting followed by clearcutting seems a viable regeneration system for eastern white pine in the Piedmont of Virginia.

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APPENDIX A

Table A1. Analysis of variance tables of net changes in height during April, May and June resulting from light and moisture treatments.

Source	df	SS	F	P>F
		APR		
LIGHT	2	148.18	3.13	0.04
BLOCK (LIGHT)	6	81.36	0.57	0.75
WATER	1	11.65	0.49	0.48
LIGHT*WATER	2	18.33	0.39	0.68
WATER*BLOCK (LIGHT)	6	75.78	0.53	0.78
ERROR	939	22249.93		
TOTAL	956	22590.84		
		MAY		
LIGHT	2	676.08	10.50	0.01
BLOCK (LIGHT)	6	562.17	2.91	0.01
WATER	1	1.53	0.05	0.83
LIGHT*WATER	2	25.45	0.40	0.67
WATER*BLOCK (LIGHT)	6	218.09	1.13	0.34
ERROR	894	28776.80		
TOTAL	911	30255.79		
		JUNE		
LIGHT	2	546.08	8.52	0.01
BLOCK (LIGHT)	6	53.82	0.28	0.95
WATER	1	163.39	5.07	0.02
LIGHT*WATER	2	349.71	5.46	0.01
WATER*BLOCK (LIGHT)	6	276.51	1.44	0.20
ERROR	714	22870.59		
TOTAL	731	24261.55		

Table A2. Analysis of variance tables of net changes in height during July, August and September resulting from light and moisture treatments.

Source	df	SS	F	P>F
		JULY		
LIGHT	2	121.67	1.64	0.20
BLOCK (LIGHT)	6	372.73	1.67	0.13
WATER	1	223.08	6.00	0.01
LIGHT*WATER	2	105.00	1.41	0.24
WATER*BLOCK (LIGHT)	6	163.98	0.73	0.62
ERROR	624	23216.13		
TOTAL	641	24216.44		
		AUGUST		
LIGHT	2	2.61	0.53	0.59
BLOCK (LIGHT)	6	21.97	1.48	0.18
WATER	1	0.72	0.29	0.59
LIGHT*WATER	2	5.53	1.12	0.33
WATER*BLOCK (LIGHT)	6	13.70	0.93	0.48
ERROR	534	1318.21		
TOTAL	551	1363.25		
		SEPTEMBER		
LIGHT	2	0.23	0.20	0.82
BLOCK (LIGHT)	6	4.38	1.26	0.27
WATER	1	1.99	3.43	0.06
LIGHT*WATER	2	3.49	3.01	0.05
WATER*BLOCK (LIGHT)	6	1.97	0.57	0.76
ERROR	424	245.40		
TOTAL	441	257.16		

Table A3. Analysis of variance tables of net changes in diameter during Spring, Summer and the entire growing season resulting from light and moisture treatments.

Source	df	SS	F	P>F
		SPRING		
LIGHT	2	0.03	4.57	0.01
BLOCK(LIGHT)	6	0.06	2.63	0.02
WATER	1	0.01	1.57	0.21
LIGHT*WATER	2	0.01	0.79	0.45
WATER*BLOCK(LIGHT)	6	0.03	1.20	0.31
ERROR	714	2.60		
TOTAL	731	2.72		
		SUMMER		
LIGHT	2	0.03	2.96	0.05
BLOCK(LIGHT)	6	0.05	1.64	0.13
WATER	1	0.01	2.06	0.15
LIGHT*WATER	2	0.02	1.66	0.19
WATER*BLOCK(LIGHT)	6	0.03	1.15	0.33
ERROR	425	2.03		
TOTAL	442	2.17		
		ENTIRE SEASON		
LIGHT	2	0.01	0.32	0.72
BLOCK(LIGHT)	6	0.06	2.08	0.05
WATER	1	0.03	6.05	0.01
LIGHT*WATER	2	0.01	0.32	0.73
WATER*BLOCK(LIGHT)	6	0.05	1.60	0.14
ERROR	425	1.99		
TOTAL	442	2.13		

Table A4. Analysis of variance tables of percent survival for the entire growing season resulting from light and moisture treatments.

Source	df	SS	F	P>F
LIGHT	2	0.14	3.17	0.08
WATER	1	0.08	3.57	0.08
LIGHT*WATER	2	0.04	0.97	0.41
ERROR	12	0.26		
TOTAL	17	0.51		

Table A5. Analysis of variance tables of changes in total non-structural carbohydrates during the entire growing season resulting from light and moisture treatments.

Source	df	SS	F	P>F
		SHOOT TNC (% dry weight)		
LIGHT	2	25.95	3.29	0.04
BLOCK(LIGHT)	6	1.76	0.07	0.99
WATER	1	5.56	1.41	0.24
LIGHT*WATER	2	0.43	0.06	0.95
WATER*BLOCK(LIGHT)	6	1.17	0.05	0.99
ERROR	90	354.68		
TOTAL	107	389.55		
		ROOT TNC (% dry weight)		
LIGHT	2	621.81	12.17	0.01
BLOCK(LIGHT)	6	180.14	1.18	0.33
WATER	1	9.48	0.37	0.54
LIGHT*WATER	2	85.49	1.67	0.19
WATER*BLOCK(LIGHT)	6	77.63	0.51	0.80
ERROR	90	2299.27		
TOTAL	107	3273.81		
		TOTAL TNC (g)		
LIGHT	2	892810.10	4.86	0.01
BLOCK(LIGHT)	6	478753.95	0.87	0.52
WATER	1	1413.32	0.02	0.91
LIGHT*WATER	2	160901.19	0.88	0.42
WATER*BLOCK(LIGHT)	6	227270.44	0.41	0.87
ERROR	90	8264010.04		
TOTAL	107	10025159.04		

Table A6. Analysis of variance tables of changes in total non-structural carbohydrates from July to the end of the growing season as a result of light and moisture treatments.

Source	df	SS	F	P>F
		SHOOT TNC (% dry weight)		
LIGHT	2	26.76	3.54	0.04
BLOCK(LIGHT)	6	1.15	0.05	0.99
WATER	1	34.15	9.04	0.01
LIGHT*WATER	2	1.90	0.25	0.77
WATER*BLOCK(LIGHT)	6	1.75	0.08	0.99
ERROR	72	271.85		
TOTAL	89	336.16		
		ROOT TNC (% dry weight)		
LIGHT	2	766.80	15.92	0.01
BLOCK(LIGHT)	6	243.13	1.68	0.22
WATER	1	0.01	0.00	0.99
LIGHT*WATER	2	36.02	0.75	0.48
WATER*BLOCK(LIGHT)	6	126.43	0.87	0.52
ERROR	72	1733.91		
TOTAL	89	2923.91		
		TOTAL TNC (g)		
LIGHT	2	1122716.62	5.82	0.01
BLOCK(LIGHT)	6	467183.08	0.81	0.64
WATER	1	42758.09	0.44	0.51
LIGHT*WATER	2	295486.59	1.53	0.22
WATER*BLOCK(LIGHT)	6	394397.47	0.68	0.66
ERROR	72	6939031.53		
TOTAL	89	9193544.06		

Table A7. Analysis of variance tables of changes in leaf number and area during the entire growing season resulting from light and moisture treatments.

Source	df	SS	F	P>F
		LEAF AREA (sq. cm.)		
LIGHT	2	1008247.68	2.02	0.14
BLOCK(LIGHT)	6	1269045.91	0.85	0.54
WATER	1	23632.65	0.09	0.76
LIGHT*WATER	2	990338.03	1.98	0.14
WATER*BLOCK(LIGHT)	6	1218376.89	0.81	0.56
ERROR	90	22463538.09		
TOTAL	107	26973179.26		
		LEAF NUMBER		
LIGHT	2	492.72	2.15	0.12
BLOCK(LIGHT)	6	355.61	0.52	0.79
WATER	1	10.70	0.09	0.76
LIGHT*WATER	2	216.24	0.94	0.39
WATER*BLOCK(LIGHT)	6	352.39	0.51	0.80
ERROR	90	10305.00		
TOTAL	107	11732.67		
		AVERAGE AREA PER LEAF (sq. cm.)		
LIGHT	2	3026.87	10.70	0.01
BLOCK(LIGHT)	6	1599.73	1.88	0.11
WATER	1	431.93	3.05	0.07
LIGHT*WATER	2	368.93	1.30	0.30
WATER*BLOCK(LIGHT)	6	1093.63	1.29	0.27
ERROR	89	12591.99		
TOTAL	106	18963.94		

Table A8. Soil analysis of potting media used in the greenhouse experiment, which analyzed growth of northern red oak under various light and moisture combinations.

SAMPLE	pH	P	K	Ca	Mg	NO3-N	Organic matter
		ppm					--%--
1	5.5	1	40	288	86	583	3.9
2	5.5	1	42	252	69	598	5.0
3	5.6	1	44	372	83	598	3.4

Table A9. Average April heights of each species under each harvest treatment in the field experiment.[1]

Species	Treatment		
	NC	PC	CC
	height (cm)		
White Oak (clipped)	0	0	0
White Oak (intact)	41.5	35.1	37.6
Red Oak	38.5	32.7	38.0
White Pine	44.3	42.4	41.8

[1] NC=no canopy removal; PC=partial canopy removal; CC= entire canopy removal.

Table A10. Percent of clipped white oak, white pine, white oak, and northern red oak seedlings within each harvest treatment of the field experiment in Patrick County, Virginia that had died back during any one month.

Treatment	Species	Month						
		Apr	May	Jun	Jul	Aug	Sep	Oct
		(% of seedlings which died back)						
NC[1]	RO	9	9	0	2	4	1	0
	WC	-	0	0	0	0	2	2
	WO	17	0	0	5	0	2	0
	WP	1	3	0	0	0	0	1
PC	RO	13	9	5	0	0	2	2
	WC	-	0	0	3	0	3	0
	WO	16	9	0	2	2	0	2
	WP	4	1	4	8	4	0	1
CC	RO	24	6	3	3	2	0	5
	WC	-	0	0	0	0	0	5
	WO	26	2	0	0	0	2	10
	WP	11	35	52	20	9	0	4

[1]NC=no canopy removal; PC=partial canopy removal; CC=complete canopy removal; RO=red oak; WC=clipped white oak; WO=unclipped white oak; WP=white pine.

Table A11. Percent of potted northern red oak seedlings within each light and moisture combination in the greenhouse that had died back during any one month.

Month	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	% of seedlings which died back					
Apr	17	22	19	13	14	18
May	14	12	14	16	8	8
Jun	12	20	7	6	6	7
Jul	5	6	2	6	5	14
Aug	1	2	2	0	1	1
Sep	3	3	0	1	3	3

Table A12. Root/shoot ratios of potted northern red oak seedlings within each light and moisture combination in the greenhouse.

Month	Shade (%)					
	0		63		92	
	limit of moisture stress (-MPa)					
	0.3	2.0	0.3	2.0	0.3	2.0
	Root/Shoot Ratios					
Apr	2.4		2.1		2.1	
May	1.9		1.4		1.7	
Jun	1.5		1.5		1.7	
Jul	1.5	1.5	1.6	1.6	1.6	1.6
Aug	1.7	1.4	1.3	1.6	1.4	1.8
Sep	1.6	1.7	1.5	1.4	1.5	1.4
Oct	1.7	2.0	1.7	1.7	1.5	1.9

APPENDIX B

AN INVESTIGATION OF VARIOUS TECHNIQUES USED IN AUXIN ANALYSIS

INTRODUCTION

Stem dieback in oak generally begins distally and proceeds basipetally, sometimes to the root collar. Loss of apical dominance frequently precedes oak dieback. Because auxin strongly influences apical dominance and cambial activity, the auxin economy of a shoot may influence dieback. Knowledge of how environment affects auxin levels and dieback in oak stems may yield insights into the physiology of dieback. Stem tips are generally considered the primary site of auxin production (Salisbury and Ross 1978) and were selected for auxin assay in this experiment. However, limited amounts of stem tip tissue were available for analysis (2 g or less from each sample). Therefore, partitioning techniques that would remove interfering substances while assuring high auxin recovery were investigated. I examined several different procedures for consistently producing auxin derivatives that would permit qualitative and quantitative assay of the hormone via gas liquid chromatography.

All of the partitioning and derivatization techniques examined in this study were found to be inappropriate for the experimental oak samples. Auxin analysis of oak stem tips proved to be impossible due apparently to low auxin

quantities in the small samples, high losses during extraction, and incomplete separation from interfering materials. During this experiment, several problems associated with auxin analysis were discovered that are underemphasized or ignored in the literature. This chapter discusses several difficulties with auxin isolation and derivatization. Although this work was done in attempts to assay oak tissue, the caveats and refinements suggested here should also be helpful in work with other materials.

The objectives of this study were to: (1) study three procedures for extraction of auxin from oak tissue; (2) quantify the percent recovery of auxin from several isolation methods; and (3) examine two techniques that can be used for the derivatization of auxin prior to assay with gas chromatography.

MATERIALS AND METHODS

Chemicals

Indole-3-acetic acid, B-[2-^{14}C] with a specific activity of 56.3 mCi/mole and a total of .05 mCi in 0.5 ml was purchased from New England Nuclear. This isotope-labeled auxin was used to examine efficiency of the extraction/isolation methods. One-hundred ul of the concentrated solution was dissolved in 10 ml phosphate buffer (20mM, pH 6.2) or in 10 ml 100% methanol. One ml of the diluted radioactive stock solutions was added at various points during the extraction or isolation process to determine recovery efficiency. Percent recovery of isotope was determined on a Beckman LS 100-C scintillation counter equipped with automatic internal standard (a cpm:dpm efficiency of 93.4% was determined). Organic and aqueous scintillation fluids utilizing PPO and POPOP in toluene were prepared according to Chase and Rabinowitz (1967).

Excessive auxin losses can result from oxidation by contaminants in solvents (Mann and Jaworski 1970). Consequently, efforts were made to minimize such contaminants. All solvents were distilled in glass. Ethyl acetate was partitioned against and simultaneously saturated with distilled water to remove oxidizing agents (Mann and Jaworski 1970). Additionally, the saturated ethyl acetate and the

methanol were passed over cotton plugs to remove other impurities (Iino et al. 1980). An antioxidant (sodium diethyldithiocarbamate) added to the 80% methanol extractant at a ratio of 1/5,000 (w/v) helped to minimize IAA losses (Mann and Jaworski 1970).

Extraction Procedures

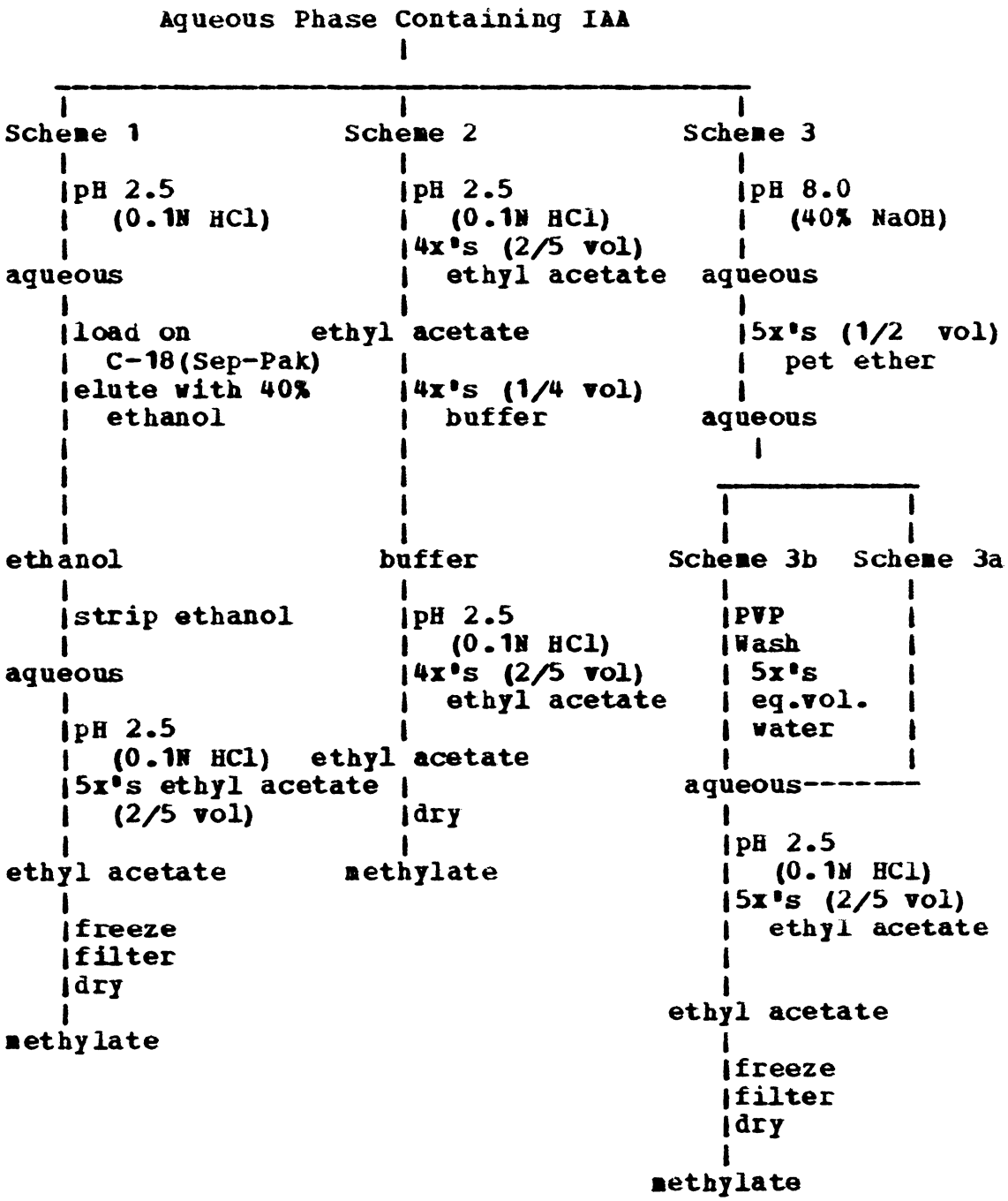
Three extraction procedures were examined for their relative efficiency in recovery of a known IAA standard added to 0.5 g (d.w.) of the pulverized, dried oak tissue. In this way, estimates could be made of losses due to interferring or IAA-destroying substances in the tissue. The first procedure included re-grinding the tissue in 80% methanol with an Omni Mixer for 3 min at -6 C to 0 C (maintained by an acetone ice bath) followed by filtration and reextraction of the filter cake twice more with grinding (20, 25, 35 ml extractant, respectively) (Shilling 1980). In the second procedure, pulverized, dried tissue was extracted in 80% methanol overnight at 3 C with agitation followed by two reextractions with agitation for 30 min (30, 35, 40 ml extractant, respectively) (modification of Dunlap et al. 1981). Tissue, in the third procedure, was extracted in 80% methanol for 2 min with sonication, centrifuged, and the pellet extracted twice more with sonication for 2 min (10 ml extractant each time) (modification of Caruso et al. 1978).

An 80% methanol solution was used for all extraction procedures, as it has proven to be a quick and efficient extracting solvent of auxin (McDougall and Hillman 1978, Hemburg and Tillberg 1980). Following each extraction series, the pooled supernatants were dried under nitrogen gas to an aqueous phase and the pH was adjusted to 2.5 with 0.1N HCl. The acidified aqueous phase was partitioned against ethyl acetate to facilitate solvent evaporation to complete dryness. The ethyl acetate was then dried under nitrogen gas leaving a residue containing IAA. The dry residue was then methylated and quantified with GLC-FID.

Isolation Procedures

In addition to extraction, various IAA isolation or purification techniques were investigated (Table B1). The procedures examined were selected for their general expediency and frequency of use by other researchers (McDougall and Hillman 1978). The most efficacious technique was to be used in oak analysis. Scheme 1 utilized an octadecyl-silane (C-18), reverse-phase column (Sep-Pak, Waters, Inc.), which allowed highly polar contaminants to pass through. Less polar compounds, such as IAA, could then be selectively eluted. Solvent/solvent partitioning was used in Scheme 2 to separate IAA from impurities. Ethyl acetate and phosphate buffer (1.0 M, pH 8.0) were the primary solvents used.

Table B1. Four partitioning schemes investigated to isolate indole-3-acetic acid from oak stem tips.



In Scheme 3, an alkaline solution was partitioned against petroleum ether and poly-N-vinylpyrolidone (PVP) (Polyclar AT; GAF Corp.) was tested for its ability to remove impurities, particularly compounds that contribute color (e.g. chlorophyll and phenolics). Aqueous extract solutions were passed through a Buchner funnel containing approximately 1 g of PVP. The PVP "cake" was then rinsed five times with distilled water. Rinse volumes were of approximately the same volume as the PVP "cake". Each purification step examined was replicated at least five times with radioactive IAA standards, both on pure IAA solutions and solutions extracted from oak tissue.

Solvents containing auxin had to be evaporated at several points during the various isolation procedures. Utilizing radioisotope techniques, several drying methods were investigated to determine percent auxin recovery in the residue. Solvents were evaporated at either 40 C or 25 C under vacuum with a rotary flash evaporator (Bucher) or in a vacuum oven. Evaporation was also investigated at atmospheric pressure (at either 40 C or 25 C) under a flow of nitrogen gas.

Derivatization Procedures

Because IAA has a high melting point, it must be converted to a more volatile compound for GLC analysis. Two

widely used volatile derivatives of IAA were tested for repeatability of peak area and peak retention time (rt). Trimethylsilyl (TMS) esters of IAA, in which the silyl esters are formed with both the carboxyl and amino groups of IAA, were investigated using bis -trimethysilylacetamide (BSA) (Supelco) as the esterifying agent (McDougall and Hillman 1978). The second esterification method involved formation of the methyl ester of IAA at the carboxyl group by reacting IAA with an ethereal solution of diazomethane. Diazomethane was prepared as outlined in Fieser and Fieser (1976).

A stock solution of 10 mg IAA/100 ml ethyl acetate stored at 0 C was used in all derivative experiments. One ml of the stock solution (100 ug IAA) was evaporated to dryness in a reaction vial under nitrogen gas at 70 C using a Meyer N-Evap Analytical Evaporator (Organomation Associates Inc.). Vials were then capped with Teflon tops (Supelco). These leak-proof caps permitted sample removal by syringe and minimized atmospheric exposure. For silylation, 100 ul BSA was added to each vial while working within a nitrogen-filled glove bag. Vials were recapped and removed from the glove bag. TMS derivatives formed during the subsequent incubation. Several incubation times and temperatures were tested. Methyl esters were produced by adding 100 ul ethe-

real diazomethane to 100 ug dry IAA in a vial. After 15 min, ether was evaporated by passing nitrogen over the sample. Methyl esters of IAA were then dissolved in 100 ul acetonitrile for GLC analysis. The derivatizing processes were repeated at least 10 times for both TMS-IAA and Me-IAA.

In addition to derivatizing esters from the direct dry down of known IAA, several samples of stock IAA were carried through part or all of the extraction/isolation procedure prior to derivatization. These experiments tested the effect of contaminants and solvents on derivative products. In both the direct dry down and the isolation experiments, TMS- and Me-derivatives of IAA were compared for repeatabiltiy of retention times and peak areas after samples were heated (for esterification) or stored for various lengths of time.

Detection/Identification Procedures

Auxin was quantified with a Varian 3700 gas-liquid chromatograph (GLC) equipped with a flame-ionization detector (FID). The following chromatographic conditions permitted unequivocal separation of properly-derivatized methyl esters of known IAA (modification of Shilling 1980): oven temperature, 120 C (isothermal); injection temperature, 280 C; detector temperature, 300 C; nitrogen carrier flow rate, 12 cc/min; attenuator, 16x10-11; 3 foot glass column packed

with 3% SE 30 (Supelco). Indole-3-acetic acid (IAA) (Sigma) was used as the standard in this study.

Detection of the methyl ester of IAA was also by gas chromatography-mass spectrometry (GC-MS). The GC-MS employed a continuous scanning Varian Mat 112 mass spectrometer equipped with an electron impact ion source. The MS parameters were as follows: accelerating voltage, 70 eV; emission current, 0.7 mA; temperature, 250 C; and pressure $5x10^{-5}$ torr.

To determine minimum amounts of tissue necessary for detection of IAA, stored and newly-harvested red oak stem tips were examined. Stored tissue had been quickly frozen in liquid nitrogen, lyophilized, and kept at 0 C for one month. Five replications of the extraction, using both dry and fresh tissue were performed. Prior to extraction, tissue was ground to pass a 10 mesh screen. Samples were then extracted by sonication, and solvent/solvent partitioning (Scheme 2, Table 1) was used for isolation.

RESULTS AND DISCUSSION

Extraction Procedures

No differences were observed among extraction techniques. Compared to a blank control with no tissue but equal amounts of known IAA, all three procedures recovered better than 90% of the IAA added to tissue samples. Because no differences in percent recovery were observed, the most expedient procedure, sonication, was used to extract tissue prior to purification.

Isolation Procedures

In this study, neither petroleum ether nor PVP removed contaminants from oak extracts sufficiently to allow auxin peak identification. A large number of unidentified and overlapping peaks masked the presence of auxin. It is possible that PVP in a larger column, coupled with a slower solvent flow rate may be more effective. But Scheme 3 without modification was considered inappropriate for isolation of IAA from oak tissue.

In Scheme 1, C-18 reverse phase column chromatography was used. Ethanol was chosen as the least polar solvent that could be used for elution of IAA from a C-18 column. A mixture of ethanol and phosphate buffer (2mM, pH 3.5) was used. In a 2:3 (ethanol:buffer) mixture, this solution brought about high IAA recovery with low amounts of eluate.

However, at least 10% of the IAA consistently came off the column in the aqueous (polar) phase while loading the sample (Table B2). In addition, it was difficult to elute IAA selectively from the column. Nearly 20 ml of 40% ethanol were needed for 80% IAA recovery from the column. This volume also resulted in co-elution of impurities. A C-18 column would probably be more effective as a purification step if it were used after the sample had already been cleaned considerably. In Scheme 1, high IAA recovery must be sacrificed for purity or vice versa. Because small amounts of IAA were present, Scheme 1 was considered inappropriate in this experiment.

The best overall IAA recovery and purification were found with Scheme 2 (Table B3). In general, at least 10% IAA was lost with each purification step. Although final recoveries were low (2-40%), they are within the range reported by Dunlap et al. (1981) and by Mann and Jaworski (1970). However, Dunlap et al. (1981) reported that IAA recovery as great as 65% was possible. Mean final recoveries without tissue tended to be 10 to 20 % higher than when tissue was present suggesting possible IAA destruction by oak metabolites. To avoid IAA destruction by peroxidase, ethyl acetate was used in place of diethyl ether (Davies, personal communications). In addition, phosphate buffer was

Table B2. Recovery of ^{14}C-IAA loaded on a C-18 column (Sep-Pak). Each value is the mean of five replications.

Fraction	Percent Recovery
aqueous waste (loading loss)	16
first 5 ml elution	54
second 5 ml elution	13
third 5 ml elution	8
fourth 5 ml elution	6

Table B3. Mean recovery percentage for IAA at various steps in the partitioning procedure of Scheme 2, based on trials with radioactive IAA.

		Range[1]	Mean Total Recovery
Stock IAA in 80% MeOH			
| sonicate 2 min			
| centrifuge 5000 rpm			
reextract 2x's			
pellet	supernatants	79-100	92
	| dry under N (40 C)		
	aqueous	66-100	80
	| pH 2.5		
	| 4x's (5 ml) ethyl acetate		
	ethyl acetate	30-100	69
	| 4x's (5 ml) phosphate buffer		
	buffer	39-81	57
	| pH 2.5		
	| 5x's (6 ml) ethyl acetate		
	ethyl acetate	28-62	32
	| freeze; filter		
	| dry under N (60 C)		
	| methylate		
	residue in acetonitrile	2-40	17

[1] Range values are based on trials with tissue present (low values) or absent (high values) during purification. Mean recoveries are based on purification trials when tissue was present.

selected for aqueous phase partitioning between ethyl acetate phases.

Although Scheme 2 was found to be the best of the procedures investigated, additional purification will be necessary in future work using larger samples. Reverse phase C-18 columns may prove useful when reducing a large aqueous volume to a smaller alcoholic volume. Also, highly polar and non-polar compounds may be eliminated in such a step. Generally, small C-18 columns, such as SepPak, would only be useful where small samples containing high IAA concentrations are being purified. Thin layer chromatography (TLC) may be another useful purification step. TLC could also be beneficial in separating water molecules from the auxin fraction if silylation is to be used. However, because of high IAA volatility, extreme caution must be used to reduce losses during separation on TLC.

Significant IAA losses due to volatilization were observed repeatedly during any drying step with rotary flash evaporation (Table B4). These results are based on more than 10 trials utilizing radioactive IAA. An average of 30% of the auxin present was lost when placed under vacuum, even at ambient temperatures. In this experiment, methanol could not be stripped at 250 mm Hg and 40 C as reported by Dunlap et al. (1981). A vacuum of 580 mm Hg was necessary to dis-

Table B4. Mean recovery percentage of ^{14}C-IAA in methanol after evaporation to dryness under 580 mm Hg vacuum or under a nitrogen stream at atmospheric pressure.

Evaporation Technique	% Recovery	
	Range	Mean Total Recovery
Under Vacuum	56-83	69
With N at standard pressure	94-100	97

till methanol from water at 40 C. Even with large aqueous residues, auxin recoveries were only 70%. Similar losses were observed when samples were placed in a vacuum oven at 40 C for 18 hours under 375 mm Hg vacuum. Thus, with 3 vacuum evaporation steps during extraction and purification, as much as two-thirds of the original auxin may be lost to sublimation or codistillation. Losses were considerably reduced if atmospheric pressures were used and nitrogen gas was passed over the solution to be dried while maintaining a temperature of 38 C. When nitrogen was used to evaporate a sample at 40 C, nearly 96% of the auxin was recovered. As much as 30 ml 80% methanol or ethyl acetate could be reduced to aqueous phase or dryness in 2 hr or less. IAA losses due to drying under these conditions never exceeded 10% and usually were closer to 5%. These data suggest that volatization of an IAA oxidation product may occur under aerobic vacuum.

The large amounts of auxin sublimed when organic solutions were rotary flash evaporated create an unexpected flaw in many published procedures. Any combination of temperature and vacuum needed to strip the organic solvent also resulted in 30% IAA losses. Many procedures used by researchers incorporate RFE to strip organic phases to a residual volume (McDougall and Hillman 1978). This experi-

ment demonstrated appreciable IAA loss, even when residual volumes are large. Mann and Jaworski (1970) and Shilling (1980) also found large IAA losses when samples were exposed to vacuum. Mann and Jaworski did not suggest, however, that excellent IAA recoveries could be obtained, as in the present study, from samples dried under a nitrogen stream at room temperature. It is possible that IAA co-distills readily in ether, the solvent used in their study. This experiment repeatedly obtained 95 percent or higher IAA recoveries when methanol or ethyl acetate was evaporated under nitrogen.

Results from GLC-FID and GC-MS analysis of oak stem tissue were inconclusive (Figure B1). although GC-MS indicated the possible presence of auxin, confirmation was not possible due to interfering ions from compounds of closely-associated peaks. Based on % IAA recoveries after purification and on the lowest detectable levels with GLC-FID, it appears that oak stem tissue contains less than 147 ng IAA/g f.w. or 0.8 uM.

The lowest detectable level of IAA using GLC-FID was 50 ug/peak. Assuming 17% recovery after purification, IAA concentration in oak stems must be less than 0.8 uM, as no auxin was found in a 6 g sample. Low endogenous IAA levels, losses during cleanup of extractions, and large amounts of

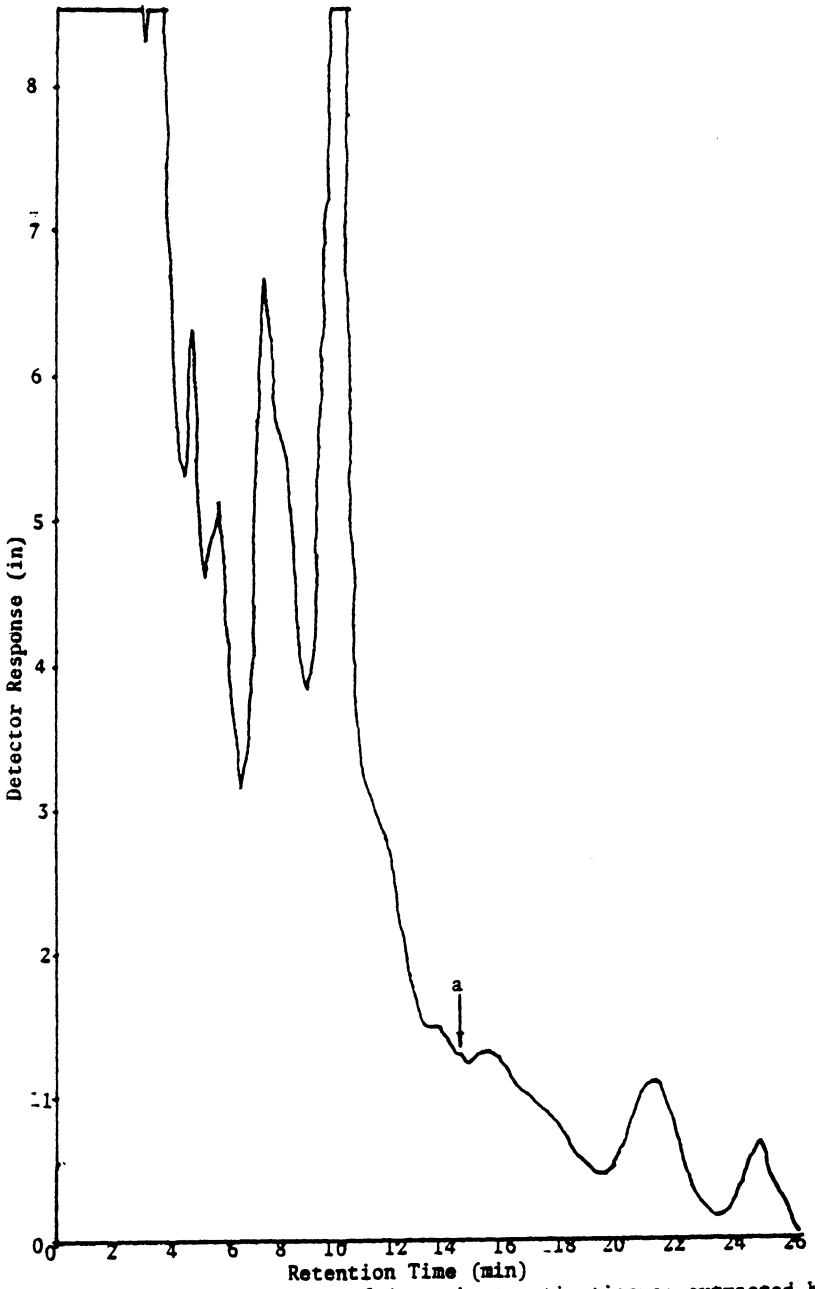


Figure B1. Chromatogram of 6 g oak stem tip tissue; extracted by sonication, partitioned using Scheme 2, and methylated. Position of IAA methyl ester is designated by a.

impurities require a large sample size (60 g or more) of oak to permit auxin analysis. Perhaps assay of stem sap would alleviate the need for extensive purification. However, auxin concentration calculated for the sap may not reflect the intra- and intercellular auxin status of the plant.

Derivatization Procedures

Experiments with IAA standards not carried through the extraction or purification steps indicated that trimethylsilyl esters of IAA had three peaks and predictable retention times (Table B5) but their peak areas were variable (Figures B2 and B3). The three peaks associated with TMS derivatives had retention times of 12.8, 22.0, or 31.3 min. Peak 1 (the fastest moving) was small (0.2 cm^2) and relatively constant over incubation time. In contrast, peak 2 was large (13.5 cm^2) shortly after BSA was added and then decreased with time. This decrease in peak 2 was accompanied by an increase in peak 3. After incubating the same sample for 10 hours, peak 2 was negligable while peak 3 was dominant (16.3 cm^2). The shift in dominance between peaks indicated that silylation reactions were still proceeding.

Chromatographic results were inconsistent when known IAA was carried through any part of a purification or extraction (with or without oak tissue) prior to TMS derivatization. Over a period of hours, peak 2 continued to

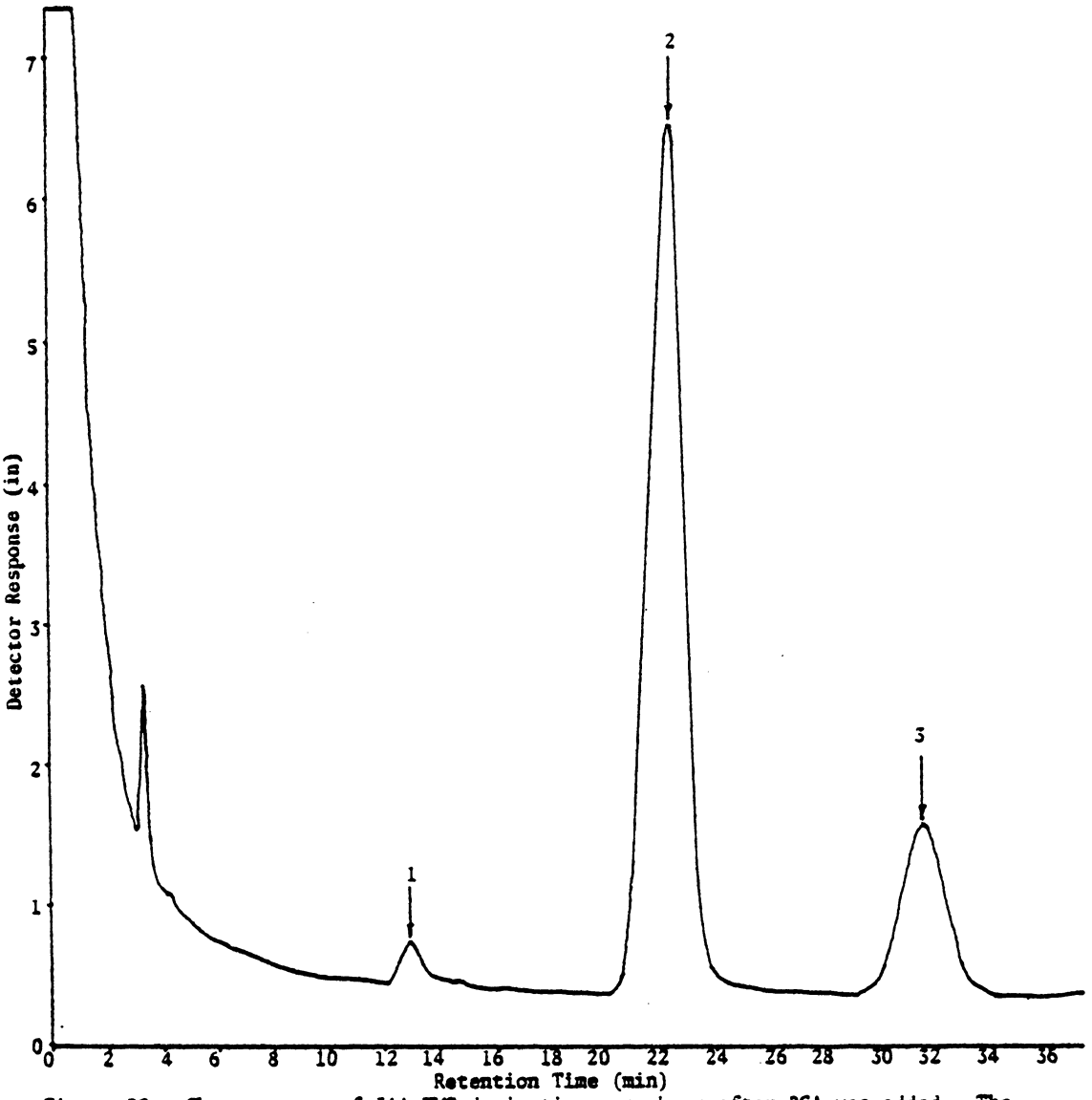


Figure B2. Chromatogram of IAA-TMS derivatives one hour after BSA was added. The resulting peaks are designated 1, 2, and 3.

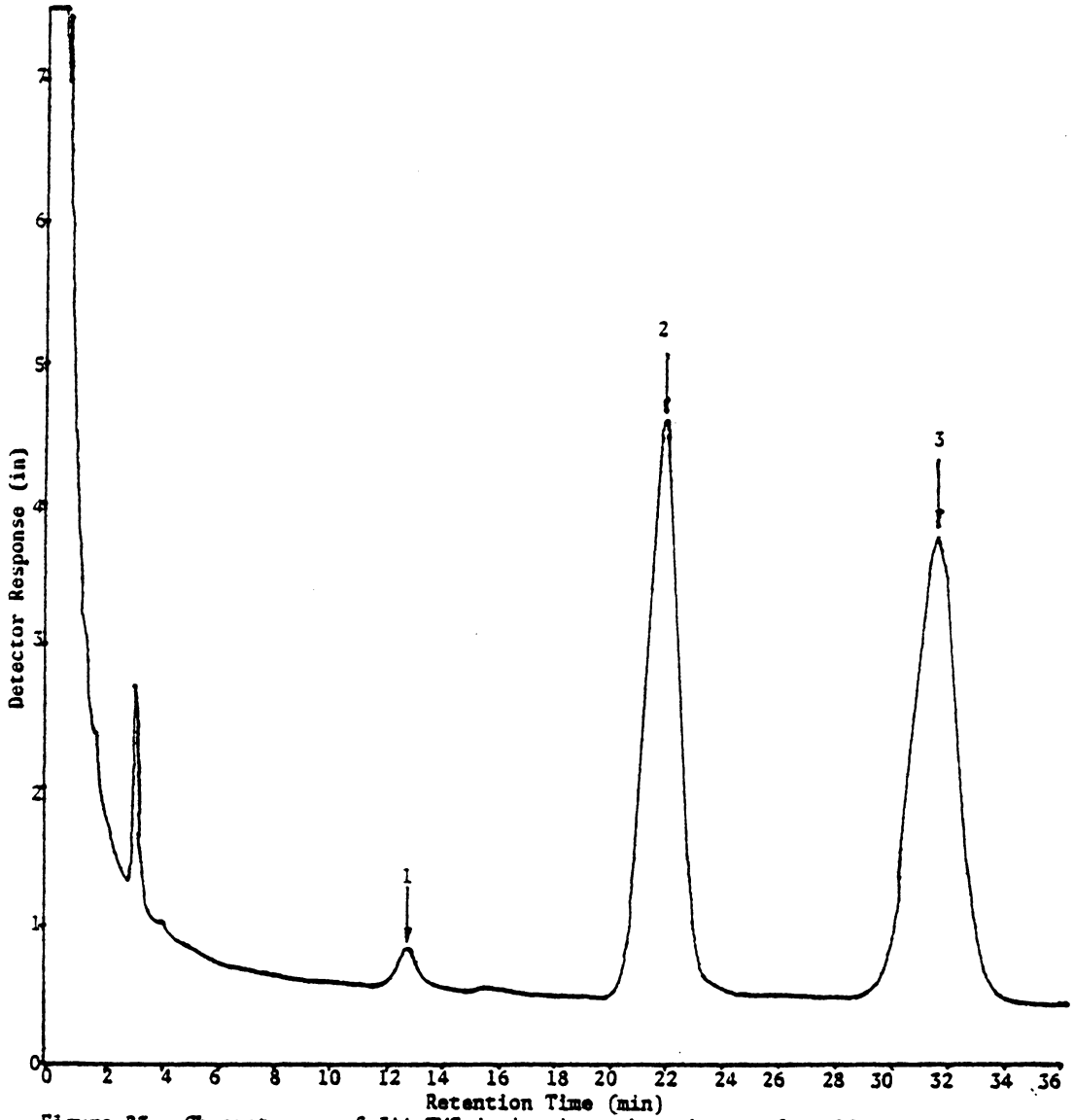


Figure 83. Chromatogram of LAA-TMS derivatives three hours after BSA was added. The resulting peaks are designated 1, 2, and 3.

Table B5. Peak areas of derivatives of IAA for various reaction times after the addition of BSA or diazomethane.[1]

Treatment	Time	TMS-IAA[2]			Me-IAA
	(min)	Peak 1	Peak 2	Peak 3	Peak 1
		(sq cm)			
No Purification	10	0.1	13.5	0.1	6.6
	30	0.2	11.1	1.8	6.2
	60	0.2	10.1	2.6	---
	90	0.2	9.8	3.9	---
	180	0.2	8.0	5.5	6.2
	225	0.2	6.7	7.4	---
	600	0.3	0.1	16.3	6.2
	3600	---	---	---	5.4
Purification	10	0.2	6.2	1.3	6.6
Water added	10	0.2	6.1	4.3	6.5

[1] Values are means for at least 5 replications.
[2] TMS-IAA samples were heated to 70 C in a heating block prior to the first injection and were heated for the length of time given. Me-IAA samples were held at room temperature.

decrease as peak 3 increased (Figures B2 and B3). However, within any given hour this trend could reverse, indicating that the derivatizing reactions also could be reversed. In addition, retention times changed by as much as 1 min. Apparently, contaminants associated with the purification solvents interfered with the derivatization process. Water may influence TMS derivatization (McDougall and Hillman 1978). When water was deliberately added prior to BSA, peaks 2 and 3 were both large compared to the usual large peak 2 and small peak 3. The unpredictability of peak formation lessened the desirability of TMS derivatives.

When a sample has been scrupulously dried, TMS derivatives can be formed with relative ease (McDougall and Hillman 1978). However, with trace amounts of water, the derivative may be destroyed. In this experiment, numerous techniques were employed to dry samples: freezing and filtering ice from water-saturated ethyl acetate; addition of a drying agent such as calcium sulfate; combinations of heating and vacuum in presence of drying agents. Some amount of vacuum was necessary to dry samples within a reasonable amount of time. As found by Mann and Jaworski (1970) and confirmed in this study, reduced pressure results in IAA losses due to sublimation. Therefore, total elimination of water from oak samples is incompatable with high IAA recov-

ery. It is likely that residual moisture or high auxin losses were responsible for the unpredictable results when BSA was used to derivatize purified samples.

Exposure of samples to atmospheric moisture was also controlled in an effort to eliminate this reported source of error (McDougall and Hillman 1978). BSA was added to dried IAA standards in a glove bag filled with nitrogen gas that had been passed through a calcium sulfate column. Aliquots of samples to be GLC-analyzed were removed from a reaction vial with a syringe through teflon caps. However, peak areas or retention times were essentially the same when these precautions were eliminated. Therefore, moisture contributed to the sample from the atmosphere had no observable effect on derivatization.

If a dry sample can be obtained, a major and reliable GLC peak of TMS-IAA can be produced by heating at 70 C for either 10 min or 10 hr. After 10 min, the major peak (peak 2) corresponds to monosilyl IAA, where a TMS ester has formed on the carboxyl group (Marsh 1974). After 10 hr, the disilyl form (peak 3) predominates in which TMS is bound to N of the indole ring as well as to the carboxyl group. These results do not correspond with McDougall and Hillman (1978) who predict complete silylation after 3 hr at 60 C. However, Marsh's (1974) results are in agreement with the

present study: after 10 hr at 70 C, the reaction had proceeded to 99 percent completion. Between 10 min and 10 hr, the reaction to complete disilylation proceeds slowly, occasionally with a net reversion to the monosilyl form (Marsh 1974). These short-term shifts in peak areas are masked by the long-term trend to disilylation. However, an experimenter using BSA should be aware that such inconsistencies in peak areas and retention times do occur.

Unlike the TMS derivatives of IAA, Me-IAA had a single peak and its area and retention time were unaffected by extraction or purification (Table B4). Me-IAA also showed consistent peak retention time (14.5 min) regardless of reaction length (Figures B4 and B5). An unquestionable advantage of Me-IAA over TMS-IAA was the reliable production of only one peak at a particular rt. This single peak reduced uncertainty in identifying an IAA peak, particularly when there were several competing compounds with rt similar to IAA's as occurred in a tissue sample.

Also in Me-IAA ester's favor is the fact that rigorous removal of water was not necessary. Results were not altered when water was deliberately added to a sample prior to derivatization. The single peak was obtained because the methyl ester formed only at the carboxyl group (Marsh 1974), and the N of the indole ring remained underivatized.

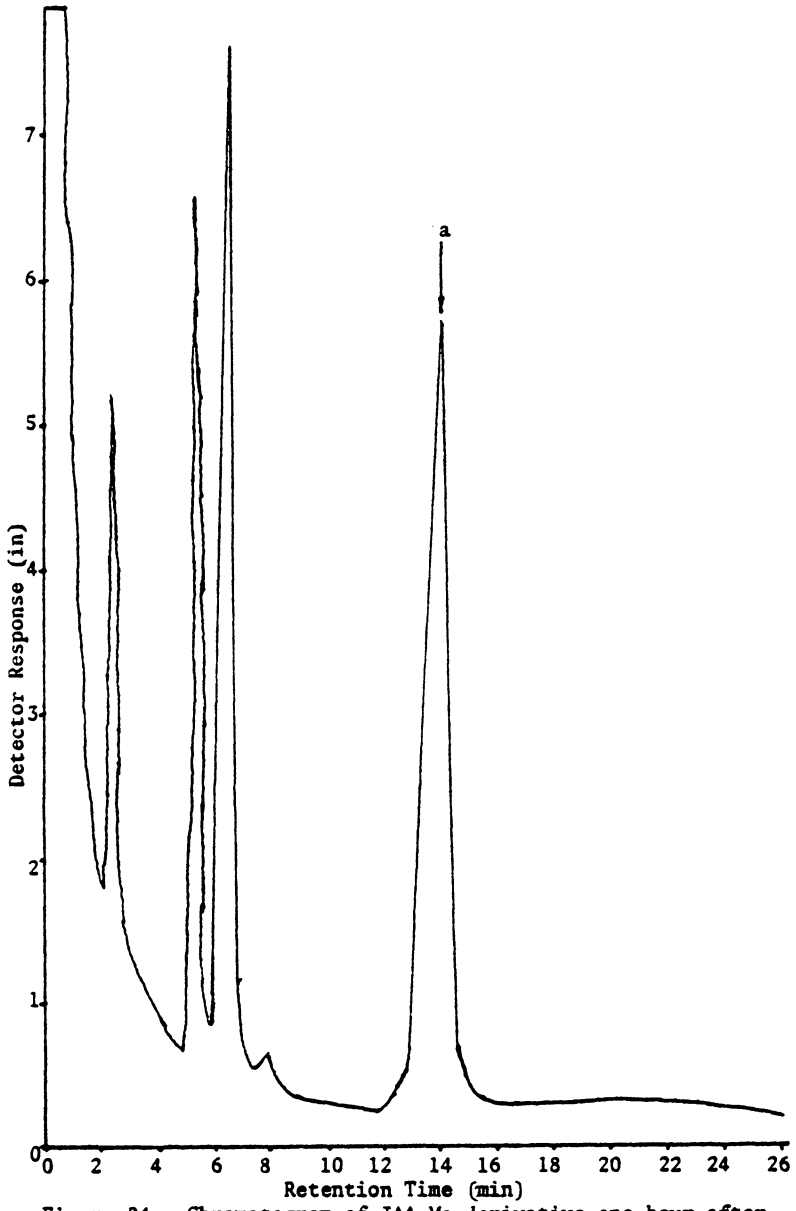


Figure B4. Chromatogram of IAA-Me derivative one hour after diazomethane was added. The resulting peak is designated a.

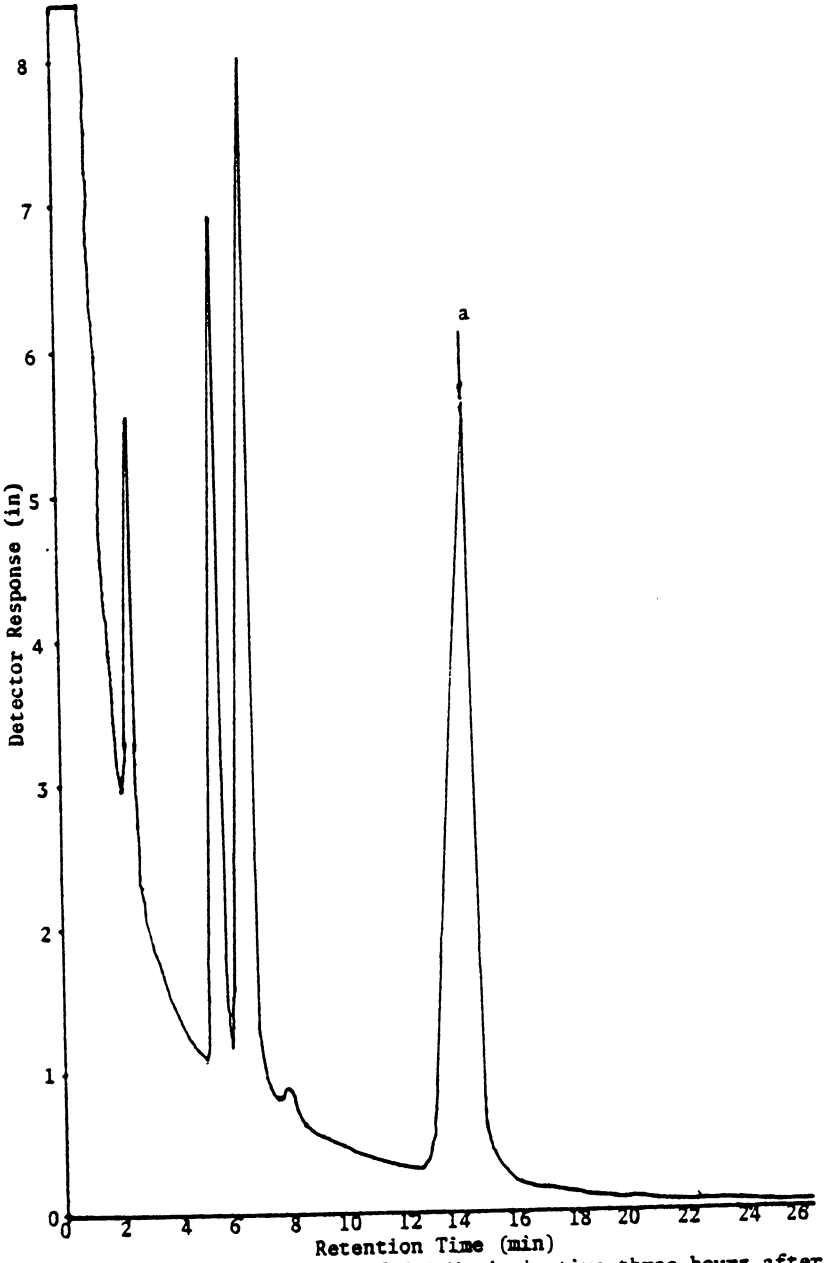


Figure BS. Chromatogram of IAA-Me derivative three hours after diazomethane was added. The resulting peak is designated a.

Elimination of multiple peaks increased the desirability of methyl derivatives. Multiple peaks associated with a single compound make conclusive identification difficult, particularly when closely related compounds are also present. Methylation has the disadvantage that methyl esters of IAA occur endogenously (McDougall and Hillman 1978). Such bound forms of IAA would therefore be assayed as free IAA. Also, ethereal solutions of diazomethane used in the reaction can be explosive (Merck Index 1976). However, the consistency of retention time and the presence of a single peak made methyl esters the preferred derivatives in this experiment.

CONCLUSION

When GLC-FID is used to analyze IAA from oak stems, methyl esters are superior to trimethylsilyl esters as derivatives of IAA. Water, other impurities, reaction temperatures, and reaction times affect formation of TMS-IAA derivatives, resulting in multiple IAA peaks. Methyl esters of IAA consistently appear as single peaks regardless of reaction time or the presence of water.

Because large amounts of IAA can be lost to sublimation, rotary flash evaporation should not be included in any step when auxin is to be assayed. An IAA solution in methanol or ethyl acetate can be concentrated by passing a stream of nitrogen over the solution maintained at room temperature.

IAA is present in oak stems at concentrations below 0.8 uM. This low amount of endogenous IAA coupled with a large amount of impurities, necessitate use of at least 60 g of oak stem tissue and extensive isolation procedures. However, caution must be used in selection of the plant part to be used for analysis. Tissue must be selected for physiological meaningfullness and not merely to supply adequate amounts of plant material.

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GROWTH AND DIEBACK OF UNDERPLANTED NORTHERN RED OAK SEEDLINGS UNDER VARIOUS LIGHT AND MOISTURE CONDITIONS

by

Thomas James Tworkoski

(ABSTRACT)

Survival, growth and dieback, endogenous auxin and total non-structural carbohydrate (TNC) relations of underplanted northern red oak (*Quercus rubra* L.) seedlings were investigated. Greenhouse-grown red oak seedlings were subjected to 0, 63, or 92% shade and soil water potential was permitted to reach -0.3 or -2.0 MPa before rewatering. In a field study, underplanted northern red oaks were exposed to three levels of canopy removal (0, 70, or 90% residual canopy). White oak (*Q. alba* L.) and eastern white pine (*Pinus strobus* L.) were included in the field experiment for comparative purposes.

In the greenhouse, dieback was observed under full sunlight and 92% shade but not in intermediate shading. In the field, dieback was greatest following complete canopy removal; but dieback was usually followed by rapid sprout growth and, as a result, average seasonal height increases generally were unaffected by light availability. Seasonal height increases were small (1-4 cm) and survival usually exceeded 90% in both the field and greenhouse. Field-grown white

oaks displayed similar growth and dieback as red oaks. White pines grew 3 cm and 10 cm under 0 and 90% residual canopy, respectively.

Final TNC levels from red oaks grown in drier soils and full sunlight were greater than seedlings grown under full shade and higher soil moistures (27 and 17% of total dry weight, respectively).

Under full sunlight, as occur with complete canopy removal, dieback was probably related to internal moisture/temperature stress. Dieback under 92% shade was likely induced by carbohydrate depletion and weak, succulent growth resulting from the chronically low light levels. Thus, oak dieback appears to be a survival mechanism in which an advantageous root/shoot ratio is maintained and nutrients can be conserved.

High or low TNC was not strongly related to dieback or sprout growth in this study. However, decreased TNC concentrations associated with increased shade indicated that long-term low light intensities may adversely affect growth and survival of red oak.

Quantification of endogenous auxin in oak stems with gas chromatography was impossible due to low IAA levels (less than 0.8 uM) and small amounts of available tissue. Large amounts of IAA were found to be lost to sublimation when IAA was subjected to vacuum.