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I am submitting herewith a thesis written by Geraldine Collier Hall entitled "A Comparison of Growth Rate Between Field Cultivated Trees and Callus in Sterile Culture of Wild Black Cherry." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.

James D. Caponetti

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A COMPARISON OF GROWTH RATE BETWEEN FIELD CULTIVATED TREES AND CALLUS IN STERILE CULTURE OF WILD BLACK CHERRY

A Thesis

Presented for the

Master of Science

Degree

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Geraldine Collier Hall

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ABSTRACT

The purpose of this investigation was to determine if correlations exist between callus growth in sterile culture and growth of the whole plant under natural field conditions of wild black cherry (Prunus serotina, Ehrh). The experimental data were to provide sufficient information to determine particular similarities in growth between callus in sterile culture and whole plants which could be used in developing an early-screening method for genetic growth potential.

The data were obtained by using clonal material produced by rooted cuttings. Thirty-three clones were established using seedlings from tree sources representing three broad altitudinal provenances in east Tennessee. The rooted plants were planted in three replications near Oak Ridge, Tennessee, and height growth measurements were recorded over a two-year period. Stem segments were collected from each clone represented in the field test, and callus cultures were established from cambial explants in the laboratory. Standard methods as used in other plant tissue culture were used in this study.

Statistical analyses of the data showed wide variations in growth rate of both the whole plants and callus cultures. Comparison of the data from the two sources showed

no correlations in growth rate.

The results reported herein do not allow for valid predictions from callus growth performance of the genetic potential for growth of the whole plant in the field under natural field conditions.

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I. A BRIEF PROBLEM ANALYSIS

Introduction

In various research efforts with several high value woody plant species in the eastern United States, breeding programs are being conducted aimed at genetic tree improvement. The U. S. Forest Service technical bulletins, various reports of State and University Departments of Forestry, and of the Tennessee Valley Authority's Division of Forestry, Fisheries and Wildlife Development report current information on research efforts with a number of forest tree species. Significant among these studies are the efforts of U. S. Forest Service at Carbondale, Illinois, with black walnut (Juglans nigra); of the University of North Carolina with yellow poplar (Liriodendron tulipifera); of The University of Tennessee with American chestnut (Castanea dentata); and of TVA's efforts with black cherry (Prunus serotina).

Variation in the rate of growth, growth form and insect and disease resistance are important characteristics considered in these breeding programs. The breeding process may include the selection of outstanding individual parents, their vegetative propagation, and the subsequent evaluation of the plants (clones) produced from them. The process involves considerable expense and time and requires taking care of all of the progeny in orchards or arboreta for long periods of time before "superior" growth characteristics can be determined.

This period usually involves 15-20 years with black cherry. Because of the expense and time involved for identifying the genetic potential for growth, it would be desirable to determine superior growth potential in clonal breeding populations early in the breeding cycle. No reports were found in the literature of techniques for screening trees for the purpose of early determinations of growth rate potential. Plant tissue culture offers a possible mechanism for use in these problem areas. In vitro culture is being used increasingly in studies of plant metabolism, morphogenesis and growth of forest tree species. Callus cultures developed from cambial explants of stem segments might serve as early indicators of growth performance of the intact plant.

This study represents an attempt to develop a technique for identifying genetic growth potential in several clones of wild black cherry (Prunus serotina, Ehrh) with tissue culture methods. The study includes the development of clonal populations by vegetative propagation (rooted cuttings) of seedlings from four broad altitudinal provenances in east Tennessee; establishment of the field test and growth evaluations; and the establishment of callus cultures.

Review of Previous Work

Vegetative propagation. The most widely employed method of vegetative propagation of hardwood species is

grafting. Techniques for rooting cuttings for a number of hardwood species have not been reported. It would be desirable, however, to have material produced from rooted cuttings for many of these species for two reasons: (1) it is better to have orchards and breeding arboreta of selections growing on their own root systems, preventing problems of graft incompatibility later; and (2) it could provide a practical method of producing clonal stock of a desired genotype commercially.

Black cherry is one of several hardwood species currently included in TVA's forestry research program. Methods for propagating this species had not been reported prior to work completed and published as a part of this effort by Farmer and Hall (1969). It was determined that physiologically juvenile material could be rooted with relative ease using a system of intermittent mist. Rooting was more difficult with cuttings from mature trees. Mist propagation techniques had been reported for other named varieties of horticultural cherries (Graham, 1958; Hartmann and Brooks, 1958; and Roberts, 1963). Summarizing the several reports, bases of softwood apical cuttings were treated with an auxin (indole-butyric acid, IBA) and placed under a system of intermittent mist. In the study of black cherry, the cuttings rooted well during spring and early summer. A dramatic decline in rootability was noted after this period, suggesting that rooting trials for this species should begin as early as practicable in the spring.

Plant tissue culture. Collectively, plant tissue culture can include the cultivation of any isolated tissue in an artificial medium. Techniques range from organ culture (Romberger, Varnell, and Tabor, 1970) to cell cultures (Short, Brown, and Street, 1969) to the induction of complete plants from cell and callus cultures (Steward, et al., 1963; Winton, 1970). "In vitro" culture is being used increasingly in studies of the physiological processes involved in growth variation patterns, nutrition and morphogenesis of forest tree species. Further, "in vitro" cell and tissue culture offer a convenient and uniform method of investigating tree species throughout the year without the confounding factors of cyclic environmental and physiological changes associated with trees in the forest (Mathes, 1964). Results obtained from tissue culture, however, must relate back to the tree under natural conditions in order for the information to be meaningful for tree improvement studies (Mathes, Einspahr, and Winton, 1970).

Much of the literature concerned with plant tissue culture and the expression of its potentials refer to the classic paper of Gottlieb Haberlandt. It was this paper which clearly presented many of the problems concerned with tissue culture which confront botanists, plant physiologists and plant biologists today. Though Haberlandt was unsuccessful in growing isolated plant cells, he foresaw the use of isolated tissue cultures as a means of studying

physiological and morphological problems (Krikorian and Berquam, 1969).

The historical development of plant tissue culture as a useful method in botanical research was outlined by White as early as 1931. Recognition of the value of tissue culture methods as applicable to forest research has been more recent. Reviews by Geissbuhler and Skoog (1957), Mathes (1964), and Haissig (1965), cover generally the application of these techniques to forestry research programs. Applications covered in the reviews included such areas of study as clonal (genetic) variation, nutritional requirements (Gautheret, 1959; White, 1963; and Butenko, 1964), experimental morphogenesis (Wetmore and Wardlaw, 1951), vegetative propagation, juvenile-mature physiological relationships and others significant to forest tree improvement work.

Callus tissue culture. Tissue masses, grown in sterile culture from the cambial region of excised stem segments, are called callus tissues. The masses usually range from firm white to friable (crumbly) yellow in appearance.

In the report of Mathes, Einspahr, and Winton (1970) with aspen, they found that isolated, undifferentiated tissue culture cells did not differ significantly in chemical composition from newly formed cells in trees. Accordingly, callus

cells grown on agar medium probably are very close in their chemical content to meristematic cells in trees. Each cell theoretically carries the same genetic information as every other cell in the same plant. In tree species meristematic cells in the cambium are relatively undifferentiated and continue dividing throughout the growing season. The derivative cells differentiate into phloem or xylem cells and become bark or wood, respectively. The developmental potential of newly formed cells to produce bark or wood depends upon their position relative to the cambial region and to various regulatory gradients. Butenko in his 1964 review describes the callus formation process:

> Specialized cells of various organs and tissues have defined functions in the whole plant, and their metabolism and structure are strongly correlated with these. However, if growth conditions are changed, such specialized cells usually begin to grow and undergo differentiation. After further changes in growth conditions the cells may experience secondary differentiation. . . . The characteristic property of dedifferentiation of tissues from various organs is that as a result of the reappearance of embryonic activity they form callus tissue.

Differentiation has been demonstrated in callus of several plant herbaceous species; including tobacco, Nicotiana tabacum (Skoog and Miller, 1957), carrot, Daucus carota (Steward, Mapes, and Mears, 1958) and geranium (Chen and Galston, 1967). For a review of work covering the induction of complete plants from tissue and cell cultures of herbacious angiosperms see Pillai and Hildebrandt (1969).

Few studies have been reported of tissue culture

methods with tree species. Jacquiot (1959) reported the successful culture of Prunus avium, Moench, and Bychekova (1963) cultured primary explants of Prunus padus L. Both are European cherry species closely related to the American black cherry. Mathes and Einspahr (1965) used aspen (Populus tremuloides) callus tissue to study tree growth rate; Wolter and Skoog (1966) studied the nutritional requirements of Fraxinus callus and Brown and Lawrence (1968) successfully cultured pine callus on a defined medium. The reports of Jacquiot (1964, 1966), Mathes (1964a), Winton (1966), and Wolter (1968) are other examples of the extent of differentiation studies with forest tree species. Winton (1970) reports the only successful production of a whole tree plantlet from undifferentiated aspen callus material "in vitro."

Most tissue culture studies with woody plant species have concentrated on the growth of undifferentiated callus. Callus proliferation and subsequent growth and differentiation are influenced by many factors, either singly or in combination. Factors of the environment, physiological age and genetic constitution of the source material are important factors to consider. Caponetti, Hall and Farmer (1971) investigated several of these factors as they affected the growth of callus initiated from trees of three physiological age groups. Growth of nine clones was compared on three different nutrient media. The three media used were as described by Wetmore and Rier (1963) for angiosperm tissue,

Murashige and Skoog (1962) for tobacco tissue cultures and Wolter (1968) for Fraxinus callus culture. Highest mean growth was recorded at 27°-32° C in the dark on Wetmore and Rier medium. Winton (1968) studied growth of a single clone of aspen callus under different light regimes and noted a higher mean growth rate in continuous darkness.

Growth variation. Variation in the rate of growth between two members of the same species may be attributed to either environmental and/or genetic differences. If the variation is due to genetic factors, which can be determined by subjecting individual plant systems to identical controlled environments, we may assume that genetic factors control the rate of callus production and growth in a controlled artificial environment.

Some attention has been given the effect of physiological age of the source material used to establish tissue cultures on growth variation patterns. Studies of juvenile-mature relationships "in vitro" have mainly used nonwoody material. Stoutemyer and Britt (1965) reported that growth of callus initiated from juvenile ivy plants was consistently greater than that from mature tissue. The only reported work found on the study of juvenile-mature cultures of tree species is that of Trippi (1963a,b) who worked with Aesculus hippocastanum, Castanea vulgaris, and Robinia pseudoacacia. He noted that juvenile tissue grew more rapidly than the

mature material, but he only studied it through one subculture. In the laboratory study of callus from black cherry (Caponetti, Hall and Farmer, 1971), the differences in growth rate from several tests of the juvenile-mature relationship were not significant.

The use of clonal populations developed from seedling sources collected from several altitudinal provenances could present a possible source of variation in the field test. The effect of source altitude on growth performance of trees grown at a different altitude has not received much research attention. In studies of western conifers from high and low elevations, evidence was given of increasing growth rate going from high to low elevation sources when plantings are at low or medium elevation sites. At high elevations, the high altitude sources perform best (Squillace and Silen, 1962; Roche, 1969; Irgens-Möller, 1968).

Wide clonal (genetic) variation in growth rate and form was observed in the black cherry callus study mentioned earlier. If positive correlations exist between callus growth and growth of the whole plant under natural conditions, callus growth can be used as an early indicator of potential tree performance.

The only reference found in this limited area of research was that of Mathes and Einspahr (1965). In their study they used stem sections from fast- and slow-growing aspen trees and observed a positive correlation between

the rate of callus production "in vitro" and the rate of tree growth under natural conditions.

II. MATERIALS AND METHODS

Vegetative Propagation of Black Cherry Clones

Seeds were collected from open-pollinated trees in Anderson and Monroe Counties in east Tennessee. The seedlings obtained were designated by numbers corresponding to numbers assigned the parent tree in the field, i.e. 37, 44. . . . Seedlings from a single parent were identified as follows: 37-1, 37-2 . . . 37-4. The parent tree numbers and the topographical elevations at which they grew are listed in Table 1.

The seedlings were initially planted in 2-1/2 inch styrofoam cups filled with a loam:peat:sand (2:1:1) planting mixture and placed in the greenhouse. A month later, the surviving seedlings were transplanted into 8-inch nursery cans and allowed to continue growth in the greenhouse using the same composition for the planting medium as described above. Apical buds were removed by hand to promote lateral branching, providing an increased source of material available for making cuttings. Powdery mildew was a problem during the early part of the season, and all the seedling material was sprayed with a solution of phaltan, a commercial fungicide (active ingredient: N-[(trichloromethyl)thio]-4-cyclo-hexene-1,2-dicarboximide) at the rate of 1-1/2 tablespoons per gallon of water.

Table 1. Numbers Assigned Parent Tree Sources of Black Cherry Seedlings and the Topographic Elevations Represented

Source Number	Elevation, Ft.
37	2,800
44	3,100
52	2,000
61	1,100
65	900
67	900
73	2,000
74	2,100
80	2,500
81	2,500
96	4,200
102	3,300
X-1	--[a]
X-4	--
X-5	--
X-9	--
X-16	--
2109	--

[a]Random selections of nursery material. Elevation source unknown.

When shoots were 8-10 inches long and no longer succulent, cuttings measuring 4-6 inches in length were made. The cuttings were immediately submerged in a 3000 ppm solution of benomyl fungicide (Benomyl[Methyl 1-(butylcarbomyl)-2-benzimidazolecarbamate] for one minute to reduce the degree of basal rot during the initial rooting period as described in a previous study of black cherry rooting techniques (Farmer and Hall, 1969). Plastic planting flats were filled with a sand:peat (3:1) rooting medium and placed in the greenhouse misting bench. The bases of the wet stems were treated with 0.8 percent indolebutyric acid in talc. This mixture was prepared by dissolving 80 milligrams of IBA in alcohol and stirring it into 10 grams of talc. The mixture was allowed to stand until the alcohol was evaporated before use. After this treatment the cuttings were stuck in the moist rooting medium under cycles of intermittent aqueous mist. The misting cycles were controlled by a commercially available misting device with timing regulated by evaporation as sensed by a fine wire mesh sensor attachment of the device.

Rooting was evaluated usually after 20-25 days; rooting percent by clone, the number of roots and longest root per cutting were recorded. The rooted cuttings were potted in the soil planting mixture and allowed to become established under reduced misting cycles for 3-4 days before

removing them from the mist benches. Rooting trials continued as material was available from the seedlings until early July when a marked decline in rooting was observed.

Inventory of the material produced at the end of the 1970 trials indicated the need for further propagation to furnish sufficient material for the field test as planned. Early the next year the potted plants which had been heeled-in in nursery beds over winter were brought into the greenhouse to force early bud break. By March 18, 1971, sufficient material was available to begin rooting trials. Procedures were as described for the earlier trials.

Establishment of Field Planting

A planting site located on Jones Island in Melton Hill Lake near Oak Ridge, Tennessee (elevation 760 feet), was used for the field test. Site preparation included mowing and disking the area before planting in early 1972. The field planting design was a randomized complete block with two-tree plots of the 33 clones in three replications (Figure 1). In the planting scheme size classes of the rooted plants were confounded with replication (The plants were assigned to replications based on their size). After the trees were planted, Simazine (a herbicide, active ingredient-(2-chloro 4,6-bis [ethylamino]-S-triazine, 80%) was sifted around each tree to a diameter of three feet, to control the growth of weeds during the initial establishment period.

REPLICATION III			REPLICATION II			REPLICATION I		
73 - 1	81-1	X-5	74-4	81 - 1	X-16	74-1	61 - 1	44-2
67 - 4	73-3	X-9	96-3	61 - 1	X - 1	44-4	65-2	X - 4
44 - 3	52-3	96-2	2109	73-3	X - 9	65-1	44-1	2109
61 - 1	74-4	74-1	44-4	44-2	96-2	X - 1	X - 9	74-4
44 - 4	2109	61-4	81-4	X-5	52-4	61-6	73-2	X - 5
52 - 1	81-4	61-6	52-3	52-1	73-2	37-3	96-2	52-3
52 - 4	65-1	X-4	73-1	44-3	61-6	52-4	X-16	61-2
65-2	102-1	44-2	61-2	65-2	65-1	96-1	67-4	44-3
96 - 1	73-2	80-1	44-1	74-1	61-4	80-1	96-3	52-1
44 - 1	X-16	96-3	67-4	102-1	96-1	61-4	73-1	81-4
37-3	61-2	X-1	80-1	37-3	X-4	102-1	81-1	73-3

Figure 1. Diagram showing the randomized-complete block design of the black cherry field test on Jones Island Planting Site.

Other cultivation during the period consisted of two-way disking between each row in May, 1972, and again in June, 1973. During one period, weed competition required hand hoeing around each plant.

Individual height measurements were made in May and December, 1972, and in June, 1973.

Establishment of Callus Cultures

Preparation of nutrient medium. The nutrient medium used for initiation and subculture of callus was as described by Wetmore and Rier (1963) for angiosperm tissue cultures. In a previous study (Caponetti, Hall and Farmer, 1971) in which black cherry callus growth was compared on three different media, the material showed consistently high growth results on the above medium. The medium composition is shown in Table 2.

Stock solutions were prepared of the major and minor salt solutions and the vitamin mixture (Table 3). The coconut milk was extracted from fresh coconuts and stored in a deep freeze until used in the medium. The pH of the nutrient mixture was adjusted to 5.6 before adding the agar. An automatic pipette was used to transfer the medium to 25 x 150 pyrex glass test tubes. The tubes were standardly filled with 20 ml of the medium. The tubes were plugged with non-absorbent cotton, placed in racks and autoclaved for 20 minutes at 15 psi.

Table 2. Composition of Nutrient Medium Used for Initiation and Growth of Black Cherry Callus

Ingredient	Amount
Knop's salt solution	500 ml
Double distilled water	200 ml
Minor salt solution	1 ml
Ferric citrate	10 mg
Coconut milk	150 ml
Casein hydrolysate	100 mg
Vitamin mixture	1 ml
Cysteine HCl	10 mg
2,4-Dichlorophenoxyacetic acid	1 mg
Sucrose	40 gm
Agar	8 gm
Double distilled water, to make	1 liter

Source: R. H. Wetmore and J. P. Rier, 1963, Experimental induction of vascular tissues in callus of angiosperms, Amer. J. Bot. 50:418-430.

Table 3. Components of Stock Solutions Used in Nutrient Medium Used for Black Cherry Callus Culture

Ingredient	Amount
Knop's Salt Solution X2	
$Ca(NO_3)_2 \cdot 4H_2O$	1.0 gm
KNO_3	0.25 gm
KH_2PO_4	0.25 gm
$MgSO_4 \cdot 7H_2O$	0.25 gm
Double distilled water	1000 ml
Minor Salt Solution	
H_2SO_4	0.25 ml
$MnSO_4 \cdot 4H_2O$	1.5 gm
$ZnSO_4 \cdot 7H_2O$	250 mg
H_3BO_3	250 mg
$CuSO_4 \cdot 5H_2O$	12.5 mg
$Na_2MoO_4 \cdot 2H_2O$	12.5 mg
$CoCl_2$	12.5 mg
Double distilled water	500 ml
Vitamin Mixture	
Thiamine HCl	10 mg
Pyridoxine	10 mg
Nicotinic acid	50 mg
Riboflavin	5 mg
Calcium panthothenate	50 mg
Folic acid	1 mg
Biotin	0.1 mg
Choline chloride	10 mg
Para-aminobenzoic acid	5 mg
i-Inositol	100 mg
Double distilled water	10 ml

Source: R. H. Wetmore and J. P. Rier, 1963, Experimental induction of vascular tissues in callus of angiosperms, Amer. J. Bot. 50:418-430.

Collection and preparation of stem segments for culture. The initial collection of material for culture was made in August 1972. The establishment of the cultures was carried out in the TVA laboratory at Norris. Stem segments measuring at least one-quarter inch in diameter were collected from one of the two trees in a plot from each field replication. The stems were placed in polyethylene bags and each bag was marked with the clone number and replication before placing it in an ice-cooled styrofoam container for transport back to the laboratory.

In the laboratory, each stem was treated as follows: The outer bark was washed thoroughly with an Alconox (detergent) solution, rinsed in running tap water; swabbed again with a 30 percent, v/v clorox solution; rinsed several times in distilled water and placed in marked petri dishes before taking the material into the tissue transfer room.

Inoculation. All stem segments were rinsed in 70 percent alcohol just prior to making incisions. The explants were made after stripping portions of the outer bark to expose the cambial layer. Two lateral cuts were made approximately one-inch apart, then longitudinal cuts approximately three centimeters apart were made, producing three to five explants per stem segment. The explants were lifted

from the stem, inverted to position opposite that of intact growth and placed singly in the medium-filled tubes. The cotton plugs were then covered with squares of polyethylene to decrease evaporation of water during the growth period. All instruments were flame sterilized using an alcohol lamp.

Completed cultures were randomly placed in the racks before covering the entire racks with sheets of aluminum foil to provide a dark growth environment. The racks were then randomly placed in controlled environment chambers with temperatures at 27°-30° C. At the end of a six-week culture period, the callus was subcultured.

Subculture. The medium was prepared a day before the scheduled subculture. Callus was removed from the tubes and placed on sterile petri dishes for surgical subdivision.

The formal comparisons of field and callus growth were to be made from the growth data obtained from the subcultured material; therefore, it was important to have the inocula as uniform in size as possible. It was also important to eliminate any source of possible contamination of the cultures. The feasibility of obtaining uniformity based on weight of the inoculum was decreased because of the greater risk of contamination and the longer period off the medium required for making weight determinations. It was decided to use a standard area measurement as the relative uniform measure. This was accomplished by placing a small

piece of paper with 5 mm square grid markings under the petri dish. This allowed easy cutting of the callus to the size of the grid square for all the callus. Representative sample weights ranged from 20-25 mg. Three tubes were filled from each callus culture representing the three field replications of each clone (a total of nine cultures per clone). The culture environment was the same as for initial cultures. At the end of the six-week culture period, the callus was removed from the culture tubes and fresh and oven-dry weights were determined. Cultures were established again in December 1972, and in June 1973, following the same basic procedures described earlier.

III. RESULTS

Vegetative Propagation

In the several rooting trials conducted to produce sufficient material for the 33 clones used in the field study, wide variations in rootability were observed (Table 4). Cuttings from some seedlings did not root well during the entire period, irrespective of season (note seedlings 80-2, 80-5 in Table 5). Other seedlings showed definite patterns of seasonal effects on rooting during both seasons, ranging from 100 percent rooting in March and April to 0 percent by late June and July (note 81-1). As a direct result, the clones chosen to be used in the field planting phase of the study were the most prolific rooters over the two-year period.

Field Planting and Growth Evaluation

Field establishment of the 33 clones was considered successful because a low mortality rate of 10.6 percent over the two-year period was observed. Most of the losses was attributable to competition from weeds during the second year; no more than two plants were lost from a single clone; no losses occurred with 17 of the 33 clones during the period.

Mean height growth by clone is presented in Table 6. The two growth periods represent the height increases from

Table 4. Mean Rooting Percentage of Black Cherry Cuttings For Two Seasons

Propagation Period	Rooting Percent Mean	Range[a]
1970		
May	91.0	0-100
June	61.2	0-100
July	37.1	0-100
1971		
March	79.1	43-100
April	77.7	0-100
June	54.0	0-100

[a]Range of seedling means.

Table 5. Mean Rooting Percents and Rooting Characteristics of Black Cherry Clones

Clone	Rooting Percent				Mean Number Roots Per Cutting	Longest Root, mm Per Cutting
	Apr.	May	June	July		
37-3	100	100	59	0	18.5	37.5
37-4	77	-	-	-	5.0	42.4
44-1	75	82	33	0	14.0	79.0
44-2	93	91	75	50	11.5	65.5
44-3	100	61	63	22	6.2	89.6
44-4	86	85	66	33	9.3	37.5
52-1	74	93	71	43	15.0	100
52-3	100	56	52	30	9.0	23
61-1	100	85	67	42	20.5	12.0
61-2	33	0	-	-	-	-
61-4	100	100	100	55	26.3	19
61-6	33	0	0	-	4.6	8.0
65-1	100	85	58	60	-	
65-2	86	86	43	-	11.0	18.5
65-3	0	23	-	-	-	
67-4	93	94	85	60	24.4	16.8
73-1	77	53	21	0	11.0	19.0
73-2	44	89	75	50	9.5	16.3
73-3	90	77	70	30	13.5	34.4
74-1	100	100	100	90	22.5	41.6
74-4	88	45	67	50	19	22.7
80-1	100	93	67	0	24	28.5
80-2	25	33	10	0	-	
80-5	0	33	0	-	-	
81-1	100	95	33	0	21.6	16.4
81-4	66	51	67	-	6.1	44.0
96-2	75	92	63	33	24	37.5
96-3	57	87	0	33	-	
102-1	100	100	91	65	25	23.5
2109	100	100	51	0	19.3	46.3
x-1	-	50	66	-		
x-4	-	66	85	-		
x-5	-	94	33	-		
x-9	-	83	66	-		
x-16	-	100	33	-		

Table 6. Mean Initial and Field Height Growth Measurements of Rooted Black Cherry Clones Over Two Growth Periods

Clone	Initial Height (cm)	1st Period 5/72-12/72	2nd Period 12/72-6/73	Net Height Growth (cm)
X-16	96.5	30.0	47.7	77.7
X-5	95.5	32.0	54.0	86.0
X-9	92.0	34.0	63.0	97.0
52-1	82.0	59.7	50.3	110.0
73-1	80.8	38.0	55.0	93.0
73-3	79.5	46.3	56.7	103.0
52-3	75.9	45.0	61.7	106.7
X-1	69.8	60.0	69.7	129.7
37-3	61.6	66.7	51.3	118.0
74-4	56.5	57.7	61.0	118.7
44-3	54.5	53.0	47.0	110.0
73-2	49.3	37.0	43.7	80.7
52-4	43.8	50.7	58.3	109.0
65-2	43.6	58.3	59.3	117.6
44-2	42.0	34.3	35.7	70.0
X-4	39.6	59.3	46.7	106.0
81-4	38.6	66.3	51.3	117.6
2109	37.0	37.0	49.0	86.0
61-1	37.0	43.7	59.7	103.4
44-4	34.6	73.3	53.0	126.3
67-4	33.6	83.0	48.7	131.7
61-4	31.5	29.0	36.0	65.0
96-3	31.0	37.0	36.7	73.7
44-1	30.0	29.7	32.3	62.0
102-1	29.5	70.3	40.0	110.3
61-6	29.5	56.0	61.7	117.7
81-1	29.3	63.3	53.3	116.6
61-2	27.6	43.0	48.7	91.7
65-1	27.5	50.0	54.3	104.3
96-1	23.5	52.0	44.3	96.3
74-1	23.5	54.3	49.0	103.3
96-2	22.5	57.3	34.7	92.0
80-1	20.0	32.3	23.0	55.3

May to December 1972, and from December to June 1973. The clonal variation in height growth is shown in Figure 2.

An analysis of variance of the height growth (Appendix, Table 9) showed that the variation among clones was statistically significant at .05 level of probability. Source altitude had no significant effect on the growth performance at the end of the test period. An analysis of covariance (Appendix, Tables 10 and 11) was computed to determine the effect of initial height on the growth results. When adjusted for initial heights, the clonal variation was not significant.

Callus Initiation and Growth Evaluation

Initiation and maintenance of callus material from the cambial explants were relatively simple. Contamination was perhaps the greatest problem, particularly with the initial explants. Clone means of fresh and oven-dry weights of the several trials are presented in Table 7.

Variation among clones in callus growth was statistically significant (Appendix, Table 12). At the end of the growth period, some cultures had filled the medium surface while other clones showed only maintenance growth. Growth form was similar to patterns observed in an earlier study of black cherry callus (Caponetti, Hall and Farmer, 1971). Some clones were very firm, while others crumbled readily at the touch of the instrument. Representative growth

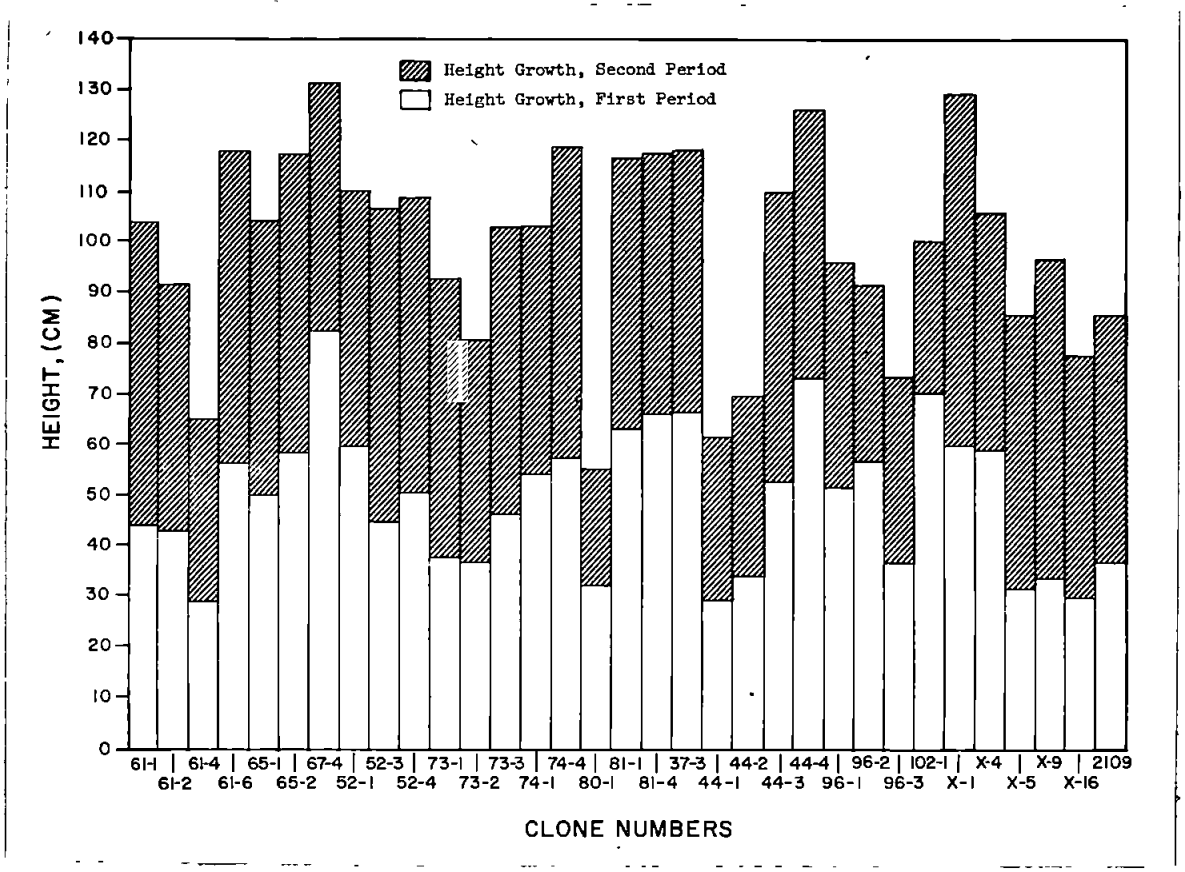


Figure 2. Variation in height growth of clones of black cherry for two growth years.

Table 7. Mean Fresh and Oven-Dry Weight of Black Cherry Callus After Six-Week Culture Periods

Clone	Fresh Wt. (gms)	Oven-Dry Wt. (gms)
67-4	1.476	0.210
73-1	1.349	0.191
73-2	1.199	0.189
73-3	1.084	0.213
96-2	1.064	0.147
52-1	1.045	0.176
96-1	1.038	0.104
X-4	1.036	0.130
X-9	1.015	0.160
2109	0.927	0.159
44-2	0.866	0.148
102-1	0.866	0.140
52-3	0.852	0.148
44-3	0.852	0.156
X-5	0.793	0.141
74-1	0.769	0.123
81-4	0.735	0.082
44-4	0.699	0.130
96-3	0.682	0.119
X-1	0.658	0.117
61-1	0.652	0.108
37-3	0.630	0.105
74-4	0.351	0.063
44-1	0.271	0.062
52-4	0.270	0.054
61-2	0.212	0.044
65-1	0.188	0.037

forms are shown in Figure 3. High contamination percentages resulted in the loss of six of the clones represented in the field test. Because of the high degree of contamination experienced throughout the several trials, an analysis of variance (Appendix, Table 13) was used to determine if any differences existed in the callus growth due to season of initiation. Ten clones which were represented in the December and June culture series were used for this analysis. The results showed no significant differences. The data allowed the grouping of the callus subculture data from the several trials for the formal comparison with the field growth measurements.

A second analysis (Appendix, Table 14) was performed to determine any influence of replication and source altitude on callus growth. Callus cultures for which source altitudes were known and clones which represented the three field replications of the planted material were used for this analysis. Source altitude and replication did not significantly affect callus growth. Clonal variation was significant at the 5 percent level of probability.

Comparisons of the field tree plantings and callus growth. The numerical rank of the clones are shown in Table 8. The correlation coefficient for rankings of the field growth and callus fresh weight was 0.062, for oven-dry weight 0.0215. An r value of 0.367 was required for significance at .05 level of probability.



Figure 3. Callus proliferation on black cherry cambial explants from two trees.

Table 8. Comparison of Clone Ranks for Field Height Growth and Callus Growth

Clone Number	Numerical Rank				
	Field Growth			Callus Growth	
	1st Period	2nd Period	Net Growth	Wet Weight	O.D. Weight
67-4	1	21	1	1	2
44-4	2	14	3	18	14
102-1	3	27	10	12	13
37-3	4	16	5	22	20
81-4	5	15	8	17	22
81-1	6	13	9	-	-
44-3	7	23	11	14	8
x-1	8	1	2	20	18
52-1	9	17	12	6	5
x-4	10	24	14	8	15
65-2	11	7	6	-	-
74-4	12	5	4	23	23
96-2	13	31	23	5	11
61-6	14	4	7	-	-
74-1	15	19	18	16	16
96-1	16	25	21	7	21
52-4	17	8	13	25	25
65-1	18	11	16	27	27
73-3	19	9	19	4	1
52-3	20	3	15	13	10
61-1	21	6	17	21	19
61-2	22	20	24	26	26
73-1	23	10	22	2	3
2109	24	18	25	10	7
96-3	25	28	29	19	17
73-2	26	26	27	3	4
44-2	27	30	30	11	9
x-9	28	2	20	9	6
80-1	29	33	33	-	-
x-5	30	12	26	15	12
x-16	31	22	28	-	-
44-1	32	32	32	24	24
61-4	33	29	31	-	-

IV. DISCUSSION

Considerable variation in rooting success was associated with genotype and season of propagation. While early spring trials resulted in higher percent rooting than later season trials, it is notable that the tree-to-tree range in rooting percent was 0 to 100 in March and April trials. The 33 clones used in the field growth phase of this study represented the more prolific rooters from the several trials. Survival and growth of the rooted cuttings were good under nursery conditions. The stem form of these plants was similar to that of seedlings. The establishment of the clonal population of black cherry by rooted cuttings is significant for this valuable timber species. This development offers much potential as a method of commercial production of genetically improved clonal stock because of the ease of establishment and relative inexpense.

The plant size was purposely confounded in the analysis of variance of the field height growth because of the differences in the initial plant size at planting. A longer growth period will be required for adequate determinations of the fast and slow growth producers. One possible alternative may be to cut all material back to a standard height for any further evaluations.

Clonal variation observed in the callus cultures originating from a number of ramets confirmed the results of Caponetti, Hall and Farmer (1971), with black cherry callus. Based on the analysis, no carry-over effects of field planting were observed on callus growth. The clones used in the callus culture phase of this study represent a random selection of the ramets in the field study and should be regarded as such when drawing conclusions regarding the genetic potential for growth of the clonal population.

The results of this study did not reveal that black cherry callus grown in sterile culture can be used in making early predictions of growth performance of the whole tree under natural conditions.

The field height growth and callus growth performance of a few clones showed some degree of numerical correlation. Outstanding among these was clone 67-4 which ranked high in both measures of growth. In other cases, however, some slower growing clones in the field showed consistently high rates of callus growth (note clones 73-2, 73-1 in Table 8, page 31).

In research efforts with aspen (Populus tremuloides) at the Paper Chemistry Institute, Appleton, Wisconsin, Lawson Winton and his associates (Winton, personal communication, 1972) have obtained similar results. Their attempts

were made to correlate callus growth from stem segments of seven-month-old aspen seedlings with the mature growth of the whole plant. They observed that the first several years of height measurements gave no statistical correlations. Their plans for future investigations were to cut all seedlings back to a uniform one-foot height and allow the plants to grow for at least five years to allow local site differences to smooth out.

My results are inconsistent with those reported by Mathes and Einspahr (1965) in which they report positive correlations between growth of aspen callus through one subculture and growth of the whole aspen plants under natural conditions.

Generally, it is felt that although these results reported here do not clearly determine that this technique can be used as an early indicator of whole tree growth performance, the merits of such a mechanism in forest tree improvement research would warrant further study. Critical factors adversely affecting these results could have been the planting of the field trees with inherent problems of establishment, lack of sufficient information on the effect of source altitudes on growth, differences in the initial height classes of the field material, and the effect competing vegetation had on the individual plant growth. Future studies should be designed which give full consideration to

these factors. Height measurements at the end of five years should be made, when identification of the fast- and slow-growing clones might be possible. The data should then be compared with the initial callus growth results reported here to determine if positive correlations exist at that time.

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APPENDIX

Table 9. Analysis of Variance of the Initial and Final Heights of 33 Clones of Black Cherry in Field Planting

Source of Variation	First Period				Second Period				Net Height Growth			
	df	SS	MS	F	df	SS	MS	F	df	SS	MS	F
3 Replications	2	115.899	57.950	<1	2	123.171	61.586	<1	2	17.535	8.768	<1
33 Clones	32	19,891.657	621.614	1.98*	32	10,141.171	316.912	4.36**	32	38,107.353	1,190.855	2.57**
R x C	64	20,121.434	314.397	-	63[a]	4,583.496	72.754		63[a]	29,159.132	462.843	
99 Total	98	40,128.990	-	-	97[a]	14,847.838	-	-	97[a]	67,284.020	-	-

[a]Loss of 1 df due to estimated missing value.

*Significant at .05 level of probability.

**Significant at .01 level of probability.

Table 10. Covariance Analysis of Black Cherry Height Growth Over Two Growth Seasons

Source of Variation	df	Sum of Products x^2	xy	y^2
Reps.	2	9,039.605	11,044.849	13,858.519
Altitude	2	11,961.800	10,423.847	11,969.469
Clones/Alt.	24	41,012.843	54,499.988	110,612.023
R x A	4	1,658.130	2,321.795	8,670.377
R x C	48	11,096.745	5,375.351	53,280.814
Error	63	6,255.370	600.705	28,421.124
Total	143	81,024.493	84,266.535	226,812.326
	x	6,301		20,623
	x^2	356,737	986,666	3,180,341
	Cor.	275,712.507	902,399.465	2,953,528.674
(A) Clones and Error (R x C)	72	52,109.588	59,875.339	163,892.837
(B)				
Clones	26	52,974.643	64,923.835	122,581.492
Error	52	12,754.875	7,697.146	61,951.191
Clones and Error	78	65,729.518	72,620.981	184,532.683

Source: R. G. Steele and J. H. Torrie, 1960, Principles and Procedures of Statistics, McGraw-Hill, N. Y.

Table 11. Analysis of Variance of Black Cherry Height (Adjusted)

Source of Variation	Initial Ht. (X) MS	F	2nd Period Ht. (y) MS	F	y Adjusted for X df	SS	MS	F
Reps								
Altitude	4,519.802	45.52**	6,929.260	15.36**				
Clones/Alt.	5,980.900	3.50*	5,984.735	1.30ns				
R x A	1,708.868	7.39***	4,608.834	4.15***				
R x C	414.532	4.17**	2,167.594	4.80**				
Error	231.182	2.32**	1,110.017	2.46**	47	50,676.952	1,078.233	
Total	99.292		451.129					
(A)								
Clones and Error (R x C)	Treatments Adjusted				71	95,094.438		
					24	44,417.486	1,850.728	1.72ns
(B)								
Clones					51	57,306.218	1,123.651	
Error					77	104,297.698		
Clones and Error					26	46,991.480	1,807.365	1.61ns

Source: R. G. Steele and J. H. Torrie, 1960, Principles and Procedures of Statistics, McGraw-Hill, N. Y.

Table 12. Analysis of Variance of Callus Growth of 27 Clones of Black Cherry

Source	df	Fresh Weight SS	Fresh Weight MS	Fresh Weight F	Dry Weight SS	Dry Weight MS	Dry Weight F
27 Clones	26	15.93941614	0.61305446	1.83*	0.34265434	0.01317901	1.84*
Error	150	50.22067070	.33480447		1.07531037	0.00716875	
177 Total	176	66.16008684			1,41796471		

*Significant at .05 level of probability.

Table 13. Analysis of Variance of 10 Clones of Black Cherry Callus to Determine if Differences in Season Of Initiation Affected Growth in Vitro

Source of Variation	df	Fresh Wt. g. SS	MS	F	Oven-Dry Wt. g. SS	MS	F
10 Clones	9	6.15295	0.68366	1.19 ns	0.160185	0.017798	1.95 ns
2 Dates	1	0.43556	0.43556	<1 ns	0.004661	0.004661	<1
C x D	9	5.17384	0.57487	1.70 ns	0.082022	0.009114	1.10 ns
Error	75	25.39608	0.33861		0.622032	0.008294	
95 Total	94	37.15843			0.868900		

Source: R. G. Steele and J. H. Torrie, 1960, Principles and Procedures of Statistics, McGraw-Hill, N. Y.

Table 14. Analysis of Variance of Callus Growth from Several Clones of Black Cherry to Determine any Effect of Replication and Source Altitude

Source of Variation	df	Fresh Weight g			Oven-Dry Weight g		
		SS	MS	F	SS	MS	F
Replication	2	0.07142613	0.03571306	1 ns	0.00200271	0.00100136	1 ns
Altitude	2	0.56558813	0.28279406	1 ns	0.01684436	0.00842218	1 ns
Clones/Alt.	7	3.60338039	0.51476862	3.49*	0.07692387	0.01098912	3.39*
R x C	18	2.65599545	0.14755530		0.05839450	0.00324413	
Total	29	6.89639010			0.15416544		

*Significant at .05 level of probability.

Source: R. G. Steele and J. H. Torrie, 1960, Principles and Procedures of Statistics, McGraw-Hill, N. Y.

VITA

Geraldine Collier Hall was born in Pike County, Georgia, on September 14, 1939. She attended elementary schools in Knoxville, Tennessee and was graduated from Austin High School in 1957. The following September she entered Knoxville College and chose a major in Biology. She married Clarence A. Hall at the completion of her sophomore year there. She resumed her training at Knoxville College in September 1962, and received her Bachelor of Science in Education degree in June, 1964.

She taught one year in the Knoxville City Schools before accepting a position with the Tennessee Valley Authority, Division of Forestry, Fisheries and Wildlife Development in Norris, Tennessee as a research assistant in 1967. While continuing her employment status, she entered the Graduate School of The University of Tennessee in January, 1969, and began part-time study toward a Master's degree in Botany. She received this degree in March, 1974.

She has two children, Clarence André and Katrina Darlene, ages 13 and 9, respectively.